REG. No. 24918 / 64
ISSN: 0022 - 1155
CODEN: JFSTAB

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (INDIA)

Vol. 36, No. 5

September–October 1999

ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA) MYSORE - 570 013

Telephone : 515557; 518670; Telex 0846-241 FTRI IN, Cable : FOODSEARCH, MYSORE.
Fax : 518670, 27697, 24475 (Country code : 91, Mysore code : 0821)

* Affiliated to the Institute of Food Technologists, Chicago, Illinois, U.S.A.
* The Association is a professional and educational organization of Food Scientists and Technologists, with its headquarters at Mysore.
* The chapters of the Association are located at Amritsar, Bangalore, Bhopal, Calcutta, Chennai, Delhi, Hisar, Hyderabad, Jabalpur, Jaipur, Jammu, Kanpur, Karnal, Kharagpur, Ludhiana, Manipur, Mumbai, Nagpur, Pantnagar, Parbhani, Pune, Solan, Thiruvananthapuram and Thrissur.

Objectives :

* Advancement of all the aspects of Science and Technology relating to production, processing and distribution of food, with the ultimate objective to serve humanity through better food.
* Promotion of research, development and training in the science, Technology and Engineering of Food.
* To provide a forum for exchange, discussion and dissemination of knowledge and current developments, especially among Food Scientists and Technologists as well as the Public and Society at large.

Major Activities :

* Publication of 'Journal of Food Science and Technology' (bi-monthly) and 'Indian Food Industry' (bi-monthly).
* Holding symposia/conventions on different aspects of Food Science, Technology and Engineering.
* Arranging Lectures and Seminars for the benefit of Members and the Public.

Membership :

* Membership is open to graduates and diploma holders in Food Science, Technology and Engineering as well as to those engaged in these professional activities.
* Types of membership include Life Member, Life Member (Resident Abroad), Corporate Members, Full Member, Member (Resident Abroad, Affiliate Member, Student Member and Student Member (Abroad).
* Each member will receive a free copy of the 'Journal of Food Science and Technology' or 'Indian Food Industry', as per the option exercised.

CENTRAL EXECUTIVE COUNCIL (CEC) OF THE AFST (I)

President :
G.S. Chauhan
G.B. Pant University of Agriculture & Technology,
Pantnagar

President-Designate :
M. Mahadeviah
Central Food Technological Research Institute
Mysore

Immediate Past-President :
S.S. Arya
Defence Food Research Laboratory
Mysore

Vice-President (H.Q.) :
T.R. Prabhu
Central Food Technological Research Institute, Mysore

Vice-Presidents (Chapters) :
M.M. Chitale
Protein Foods & Nutrition Development Association of India
Mumbai

R.R. Mohite
597, 10th B Cross, II stage
WCR, Bangalore-560 086

D.G. Rao
CFTRI Regional Centre
Hyderabad

S.M. Tyagi
G.B. Pant University of Agriculture and Technology, Pantnagar

Executive Secretary :
A.D. Semwal
Defence Food Research Laboratory
Mysore

Immediate Past-Secretary :
Rajesh S. Matche
Central Food Technological Research Institute, Mysore

Joint Secretary :
R. Naresh
Central Food Technological Research Institute
Mysore

Treasurer :
Akmal Pasha
Central Food Technological Research Institute
Mysore

Editor-in-Chief :
Journal of Food Science & Technology
B.R. Lokesh
Central Food Technological Research Institute,
Mysore

Chief Editor :
Indian Food Industry
Richard Joseph
Deputy Director (Retd)
Central Food Technological Research Institute
Mysore

For Membership, Subscription to the Journal and Other particulars, kindly contact :

The Honorary Executive Secretary
Association of Food Scientists and Technologists (India)
Central Food Technological Research Institute Campus, Mysore-570 013, India.

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

EDITOR-IN-CHIEF

Lokesh B.R.
Department of Biochemistry and Nutrition,
Central Food Technological Research Institute,
Mysore

EDITORS

Krishnamacharyulu A.G.
Central Food Technological Research Institute,
Mysore

Shamala T.R.
Central Food Technological Research Institute,
Mysore

ASSOCIATE EDITORS

Krishna Kantha T.P., Mysore
Narasimham P., Mysore
Sakariah K.K., Mysore
Sreemathi Hariprasad., Mysore
Sri Hari B.R., Mysore

ADVISORS

Arya S.S., Mysore
Asopa V.N., Ahmedabad
Chauhan. G.S., Pantnagar
Dubash P.J., Mumbai
Kumar Bhatia, New Delhi
Manju Sharma, New Delhi
Pandit S.D., Mumbai
Patil S.F., Jalgaon
Prakash V., Mysore
Thakre N.K., Pune
Thyagarajan G., Madras

EDITORIAL BOARD

INDIAN

Aravinda Prasad, Mysore
Bawa A.S., Amritsar
Guha Biswas N., New Delhi
Chincholkar S.B., Jalgaon
Jamuna Prakash, Mysore
Kochar G.K., Palampur
Krishnaprakash M.S., Mysore
Kulkarni S.D., Bhopal
Rajeshwar S. Matche, Mysore
Maheshwari P.N., Kanpur
Mital B.K., Pantnagar
Muktha Bai K., Mysore
Muralidhara, Mysore
Paul Thomas, Mumbai
Pillaiyar P., Thanjavur
Potty V.H., Mysore
Rajesh Kapoor, New Delhi
Rama Murthy M.K., Bangalore
Sattigeri V.D., Mysore
Sharma N., Izatnagar
Shekar Shetty, H., Mysore
Smita Lele., Mumbai
Srikar L.N., Mangalore
Srinivas P., Mysore
Upadhyay K.G., Anand
Varadaraj M.C., Mysore
Veerabasappa Gowda T., Mysore
Vijaya Rao D., Mysore

OVERSEAS

Brown C.M., Edinburgh, UK
Bulan Phithakpol, Bangkok, Thailand
Carlos Ricardo Soccol, Curtiba-PR, Brazil
Daniel Martinez-Carreara, Puebla, Mexico
Dietrich Knorr, Berlin, Germany
Gumbira-Said E., Bogor, Indonesia
Jacobsen M., Frederiksberg C., Denmark
Jan Bol, Zeist, The Netherlands
Jayatissa P.M., Colombo, Sri Lanka
Khalil I. Ereifej, Irbid, Jordan
Kwon T.W., Kyungsangnam-do, S. Korea
Lee Yuan Kun, Singapore, Rep. of Singapore
Leupold G., Freising-Weihenstephan, Germany
Lozano Y., Montpellier, France
Paolo Fantozzi, Perugia, Italy
Raimbault M., Montpellier, France
Ramaswamy H., Quebec, Canada
Rao M.A., New York, USA
Robin Y. -Y Chiou, Chiayi, Taiwan
Rolz C.A., Guatemala City, Guatemala
Roussos S., Reading, UK
Spencer J.F.T., Tucuman, Argentina
Viniegra-Gonzalez, Mexico City, Mexico
Zeki Berk, Haifa, Israel

Journal of Food Science and Technology accepts advertisements from manufacturers and dealers of chemicals, glassware, instruments, machinery as well as publishers/distributors of books/scientific journals. The Journal reaches research professionals, academicians, industrialists seeking information on the latest findings in all branches of science, technology and engineering of foods and food products. It has wide circulation at national and international levels.

Please refer this issue for more information on rates for colour and black & white advertisements. The advertisement matter may be in the form of art pulls, bromides, positives and blocks, which should be sent along with the relevant remittance in the form of DD favouring,

The Executive Secretary
AFST(I)
CFTRI Campus
Mysore - 570 013. India
☎ : 0821-515557, Fax: 0821-518670
e-mail : afsti@blr.vsnl.net.in

TO OUR SUBSCRIBERS

Consequent on the steep rise in the production costs to bring out our journals *Journal of Food Science and Technology & Indian Food Industry*, the Central Executive Committee of the Association of Food Scientists and Technologists (India) has taken a decision to revise the subscription rates as follows to be effective from **1st January 2000.**

REVISED RATES

	Journal of Food Science & Technology	*Indian Food Industry*
India :	Rs. 750/-	Rs. 450/-
Overseas :	US$ 225/-	US$ 135/-

Subscribers are requested to continue their patronage to the journals as in previous years. We, on our part, assure you to accelerate the process set in motion, to serve you better in the coming years.

Editor-in-Chief
Journal of Food Science and Technology

Chief Editor
Indian Food Industry

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

INDEXED AND SELECTIVELY ABSTRACTED IN

* *Current Contents-Agriculture, Biology and Environmental Sciences*
* *Indian Food Industry*
* *NCI Current Contents*
* *Chemical Abstracts*
* *Biological Abstracts*
* *Food Science and Technology Abstracts*
* *Food Technology Abstracts*
* *Dairy Science Abstracts*
* *Nutrition Abstracts and Reviews-Series A-Human and Experimentals*
* *International Packaging Abstracts*
* *PIRA CD-ROM-Paper, Printing and Packaging Database*
* *Online PIRA Databases-Data-Star, Dialog, Orbit Search Service, PFDS Online and STN*
* *Fisheries Review*
* *Cambridge Scientific Abstracts-Microbiology, Biotechnology, Health and Safety Science*
* *Food Adlibra Dialog File 79.*
* *Food Adlibra Alerting Bulletin*
* *Food Adlibra Current Awareness Supplements for Food Science and Seafood*
* *Food Adlibra Current Awareness Supplement for Snacks and Confectionery*
* *Biology Digest*
* *NAPRALERT-Online Access via Bitel, Interest Compuserve, Prodigy and Phone Modem*
* *NAPRALERT-Off-line Access*
* *AGRIS Database of FAO*
* *The Engineering Index Monthly*

Volume 36 Number 5 Sept./Oct. 1999

C O N T E N T S

REVIEW

Food Fortification in Developing Countries - Current Status and Strategies
G. Subbulakshmi and Mridula Naik **371**

RESEARCH PAPERS

Determination of Thermal Process Schedules for Canned Gourds
L. Saikia and S. Ranganna **396**

Flow Properties and Physico-chemical Characteristics of 'Perlette' Grape Juice Concentrates
S. Tejinder, S. Sukhvinder, K. Bhupinder, K. Harinder and S.P.S. Saini **402**

Crushing Characteristics of Mustard with Commercial Oil Expeller
R.T. Patil and Nawab Ali **408**

Effect of Hot Deboning and Polyphosphate on Refrigerated Storage Stability of Quail Sticks
R.P. Singh, S.K. Anand and S.S. Verma **413**

Nutritional Evaluation of Liver and Body Flesh Lipids of Ray Fish, *Dasyatis bleekeri.* (Blyth)
Debasish Pal, Tarun K. Patra, Dipankar Banerjee, Joydeep De and Amitabha Ghosh **418**

Diffusion of Sodium Chloride and Citric Acid in Raw and Fried *Paneer* at Different Temperatures
K. Jayaraj Rao and G.R. Patil **424**

Physico-chemical Changes During Storage of Papaya Fruit (*Carica Papaya* L.) Bar (*Thandra*)
K. Aruna, V. Vimala, K. Dhanalakshmi and Vinodini Reddy **428**

RESEARCH NOTES

Effect of Parboiling, Hand-pounding and Machine-milling on Chemical Composition of Rice
Sadhna Singh, Manoranjan Kalia and S.R. Malhotra **434**

Non-destructive Measurement of Vacuum in Canned Water
S.G. Patwardhan **436**

Journal of Food Science and Technology, devoted to original R & D contributions in all branches of Science, Technology and Engineering of Foods and Food Products, is a bimonthly publication of the Association of Food Scientists and Technologists (India), Mysore. Review papers are also published to a limited extent.

No part of the journal should be reproduced without written permission of the Editor.

Publication of paper in the journal automatically transfers the copy rights from the authors to the journal.

The Editor reserves the privilege of editing the manuscript and adding or deleting relevant parts, to make it suitable for publication in the Journal.

The Editor assumes no responsibility for the statements and opinions expressed by the contributors.

Manuscripts for publication, books for reviewing and matter concerned with advertisements in the Journal should be addressed to the Editor, Journal of Food Science and Technology, AFST (I), CFTRI Campus, Mysore-570 013, India.

The Instructions to Authors are published in the January-February issue of the journal. These are required to be followed *in toto*. No deviation is accepted.

New members of AFST (I) are entitled to receive 'Journal of Food Science and Technology'(JFST) or 'Indian Food Industry' (IFI).

Members opting for IFI can subscribe to 'Journal of Food Science and Technology' at a concessional rate.

Those members who were earlier getting or had opted for IFI, can now change the option (once in a year only), to get gratis copies of 'Journal of Food Science and Technology', if they so desire.

Advertisements from manufacturers and dealers of chemicals, glassware, instruments, machinery as well as publishers/distributors of books/periodicals are accepted.

ADVERTISEMENT (per insertion)

	Rs.
Full Page (B&W)	3,000/-
Half Page (B&W)	2,000/-
Inside Cover (B&W)	4,000/-
Inside Cover (4 colours)	8,000/-
First page facing inside front cover (B&W)	5,000/-
First page facing inside front cover - 4 colours	8,000/-
Back cover (4 colours)	10,000/-

Correspondence regarding subscription and membership rates should be addressed to the Executive Secretary, AFST(I), CFTRI Campus, Mysore-570 013, India.

All payments shall be made by Demand Draft or Money Order in favour of the Executive Secretary, AFST(I), CFTRI Campus, Mysore-570 013. India.

In case of Indian Institutions and nationals, the subscription through agents is not entertained.

Non-refrigerated Storage of Tomatoes - Effect of HDPE Film Wrapping
Ashok Kumar, B.S. Ghuman and A.K. Gupta — **438**

Effect of Spices on Urinary Calcium and Serum Vitamin A Levels in Adult Women
B. Praveena and G. Sarojini — **441**

Spectrophotometric Methods for the Determination of Di-t-Butylhydroquinone in Oils
U. Viplava Prasad, V. Srinivasulu, P.Y. Naidu and G. Venkateshwara Rao — **444**

Effect of Grinding Time and Moisture on Size Reduction of *Makhana*
S.N. Jha and B.B. Verma — **446**

Studies on Advanced Potato Hybrids for the Preparation of French Fries
R.S. Marwaha, Devendra Kumar and P.C. Gaur — **449**

Zinc, Calcium and Iron Availability Using Molar Ratios in Processed and Cooked Wheat Products
Harpreet K. Grewal, Charanjeet K. Hira and B.L. Kawatra — **453**

Nutritional Evaluation of Vegetable *Colocasia* Grown in Himachal Pradesh
R.A.K. Aggarwal, P.S. Arya and N.C. Mahajan — **457**

Physico-chemical, Microbiological and Sensory Characteristics of Washed Fish Mince Prepared from Some Selected Species of Fish
Femeena Hassan and Saleena Mathew — **459**

Comparison of Mutton, Rabbit and Their Combination of Meats for Sausage Processing
A.R. Sen — **463**

Post-harvest Biochemical Changes in Mature Oil Palm (*Elaeis guineensis*) Fruit Pericarp During Storage
Salini Bhasker, G.L. Minil Kumar and C. Mohankumar — **466**

Keeping Quality of Salt Cured Shark *Carcharhinus sorrah*
A. Tamil Selvi, P. Jeyachandran, G. Indra Jasmine, S.A. Shanmugam and S. Kannappan — **469**

Polyaromatic Hydrocarbons in Fresh Marine Fin and Shell Fishes
S. Kannappan, G. Indra Jasmine, P. Jeyachandran, A. Tamil Selvi — **472**

Development of Pulverised Starter for *Kinema* Production
Jyoti Prakash Tamang — **475**

Physico-chemical Changes in Insect Infested Wheat Stored in Different Storage Structures
R. Samuels and R. Modgil — **479**

BOOK REVIEWS — **483**

INDIAN FOOD INDUSTRY - CONTENTS — **Inside Cover**

Food Fortification in Developing Countries - Current Status and Strategies

G. SUBBULAKSHMI* AND MRIDULA NAIK
Department of Post-Graduate Studies and Research in Home Science,
S.N.D.T. Women's University, Juhu, Mumbai-400 049, India.

For the last two decades, micronutrient deficiencies specifically anaemia, iodine deficiency disorders (IDD) and vitamin A deficiency have been a subject of concern for nutritionists and health authorities in developing countries. Among the available interventions for micronutrient malnutrition, food-based approaches have been considered as most acceptable, safe and sustainable. These food-based strategies include nutrition education, dietary diversification and food fortification. Supplementation still remains an important therapeutic intervention for critical deficiency status. Dietary diversification and nutrition education are more long term but require lot of perseverance. In this contest, food fortification is a broad, effective and immediate way to target a widespread problem like micronutrient malnutrition. The present review is an attempt to appraise the current status of food fortification in developing countries with specific reference to its technological, nutritional, beneficial and economical aspects.

Keywords : Food fortification, Micronutrients, Vitamin A, Iodine, Iron, Strategies.

Less visible than protein-energy undernutrition are deficiencies of micronutrients, e.g., iron, iodine, vitamin A, and the other vitamins, minerals and trace elements. Until recently, micronutrients were low on the development and hunger agendas. As a result, very few programmes designed at remedying these forms of undernutrition were implemented, and little efforts were made at gathering reliable and comparative data. Starting at 1980s, considerable efforts have been made to alleviate this (Peter 1994).

The need for combating micronutrient malnutrition was emphasized in the World Declaration and Plan of action for Nutrition adopted at the International Conference on Nutrition (FAO/WHO 1992). The reasons for this increased interest in micronutrients include the recent understanding that micronutrient deficiencies affect more people and have more serious consequences than previously thought and that prevention or treatment of such deficiencies can be achieved at low cost (Peter 1994). According to Reutlinger (1993), a third reason is that measures to deal with these kinds of malnutrition have the capacity to reduce human suffering, but yet do not threaten the existing economic and political structures.

Vitamin A deficiency (VAD), iron deficiency anaemia (IDA) and iodine deficiency disorders (IDD) are among the most common forms of micronutrient malnutrition. Table 1 shows the estimates of people by regions at risk and affected by IDD, VAD and IDA. Other micronutrient deficiency problems do exist and are no less in their deleterious health hazards. Although the major malnutrition problems are found in underdeveloped and developing countries, people in developed countries also suffer from various forms of micronutrient malnutrition. Table 2 indicates prevalence of three major micronutrient deficiencies in 1995 and also the targeted figures with sustainable efforts to alleviate these problems.

Vitamin A Deficiency (VAD)

Vitamin A deficiency (sub-clinical) affects about 285 million (42%) children under five years of age globally and about 0.5% are severely affected (xeropthalmia). VAD affects as many as 256 million children in more than 75 countries in the developing world. Of 2.7 million pre-school children, who have eye damage resulting from this deficiency, an

TABLE 1. ESTIMATED NUMBERS OF PEOPLE (IN MILLIONS) AT RISK AND AFFECTED BY THE THREE MAIN FORMS OF MICRONUTRIENT MALNUTRITION

Region	IDD		VAD*		IDA
	At risk	Affected (goitre)	At risk	Affected (xerop-thalmia)	
Africa	181	86	52	1.0	206
America	168	63	16	0.1	94
South-East Asia	486	176	125	1.5	616
Europe	141	97	-	-	27
East Mediterranean	173	93	16	0.1	149
West Pacific	423	141	42	0.1	1058
Global (Total)	**1572**	**655**	**251**	**2.8**	**2150**

*Estimates for vitamin A deficiency are for children from birth to five years of age
Source : FAO/ILSI (1997)

* *Corresponding Author; Present address : 501, B-Wing, Brahma Towers, Sector-2, Charkop, Kandivali (W), Mumbai-400 067*

TABLE 2. MICRONUTRIENT MALNUTRITION IN DEVELOPING COUNTRIES, 1995 AND 2025

	Number of affected persons (millions)	
Disorder	1995	2025*
Goitre	834	350
Iron deficiencies	3580	2750
Vitamin A deficiencies	2.85	0.17

*Projected
Source : World Health Organization (1998)

estimated 3,50,000 go blind every year and up to 60% die within a few months of becoming blind (WHO 1998). Vitamin A deficiency is also linked with an increase in the severity of infections, particularly measles and diarrheal diseases. Through synergism with measles infection, vitamin A deficiency contributes to some extent to the estimated 9,60,000 childhood deaths from measles every year. Supplementation with vitamin A has been shown to be effective in reducing mortality by as much as 23% from these conditions in areas where this deficiency is common. (WHO 1998). It is the main cause of paediatric blindness with 5–10 million children developing xeropthalmia each year, of whom a half-million have potentially blinding corneal disease and half of these die. Mild xeropthalmia (i.e., night blindness and conjuctival xerosis) does not blind but is associated with higher risk of infectious morbidity and mortality in children. Community trials have shown that pre-school child mortality can be reduced by 25–30% in malnourished populations, when children over 5 months of age are supplemented with vitamin A directly or via vitamin A fortified foods, leading to estimates of up to 2.5 million preventable child deaths occurring each year due to underlying VAD (Keith 1997). In addition to the clinical VAD in children, sub-clinical deficiency is also very common in pregnant and lactating women. This has to be considered as the root cause of VAD in children as the conditioning of foetal stores and breast milk content of vitamin A are dependent on maternal vitamin A status. Clinical vitamin A deficiency could be eliminated by 2025 with sustained efforts.

Iodine Deficiency Disorders (IDD)

Iodine deficiency disorders affect about 15% of the world's population, 834 million having goitre and 16.5 million with cretinism (WHO 1998). Goitre was first observed in the Sub-Himalayan belt and has since spread throughout India. Several district level surveys have been done on the prevalence of goitre over the past 20–30 years in 29 of the 32 States and union territories of India. Of the 236 districts surveyed, 194 districts were found endemic (Ministry of Industry 1994). Since iodination started over 30 years ago, goitre has been reduced, though still remains relatively high. Lack of iodine in the diet is a major nutritional problem. It affects the normal development of about 250 million people in India, and accounts for an estimated 90,000 stillbirths and neonatal deaths every year. It has led to 2.2 million Indian children being afflicted with cretinism and another 6.5 million becoming mildly retarded. (Ministry of Industry 1994). Yet, the simple, inexpensive solution of iodizing all salt supplies has been available for decades. Iodine deficiency disorders are declining rapidly due possibly to near-universal salt iodization (USI). Sustainable elimination by 2000 or 2010 is possible.

Iron Deficiency Anaemia (IDA)

An estimated 2 billion people are anaemic, with nearly 3.6 billion iron-deficient. Prevalence of anaemia is highest (around 50%) in pregnant women and pre-school children in developing countries. In India, its prevalence is 60–70% in pre-school children, about 70–80% in pregnant women and about 50% in lactating women (Mathew et al. 1999). Iron deficiency anaemia leads to impaired work performance and deficits in learning ability. It is increasingly recognized that these impacts occur even in the absence of clinical anaemia, i.e., with a moderate deficiency. IDA is particularly widespread in pre-menopausal women and may increase susceptibility to illness and complications of pregnancy (Finch and Cook 1984).

Cost of micronutrient malnutrition

The cost of ill health due to micronutrient malnutrition is difficult to quantify, as it is a 'Hidden Hunger'. Only in recent years, sophisticated mathematical models have been developed to measure economic losses due to disability and premature death. One of them is the indicator created by the WHO and the World Bank for the 1993 World Development Report, measured in DALYs (Disability-Adjusted Life-Years). By counting the number of years of healthy life lost due to disability and premature death and adjusting for a variety of parameters (age, sex, demographic region, etc), experts were able to estimate the global burden of disease.

By this calculation, 1.36 billion DALYs were lost worldwide in 1990, the equivalent of 42 million

TABLE 3. DIRECT CONTRIBUTION OF MALNUTRITION TO THE GLOBAL BURDEN OF DISEASE

Region	Millions of disability-adjusted life-years (DALY) lost (1990)
Sub-saharan Africa	7.0
India	15.5
China	6.3
Other Asian countries	7.0
Latin America and Caribbean	3.9
Middle East	4.5
Former socialist economies	0.6
Established market economies	0.9
Worldwide	**45.7**

World Development Report (1993)

deaths of newborn children or of 80 million deaths at age 50. In the developing world, two thirds of DALY losses were due to premature death. More than a quarter of the global burden of disease resulted from infectious and parasitic diseases in children. The direct contribution of malnutrition (protein–energy malnutrition, vitamin A deficiency, iodine deficiency, iron deficiency anaemia) to the global burden of disease in 1990 was estimated at 45.7 million DALYs (Table 3). Indirect effects (mortality from other diseases attributed to underweight and vitamin A deficiency) accounted for a further 99.5 million DALYs worldwide (World Bank, World Development Reports 1993).

Causes of micronutrient malnutrition

In most developing countries, the adequacy of food supplies at the national level does not ensure that adequate food is available at the regional, household, or individual level. Factors that can influence the ability of an individual to acquire and utilize nutrients include: local food and water availability, food prices, a country's capacity to import food, income and purchasing power, women's workload and education level, local customs and food taboos, sanitary conditions and health status. Thus, because these social, political, and economical factors contribute to malnutrition, solutions require more than the provision of food and nutrients. There are interrelationships among malnutrition, poverty and economic development. Gillespie and Mason (1994) categorically evaluated causes of micronutrient malnutrition, which are listed in Table 4.

Strategies for management of micronutrient malnutrition

Strategies for the prevention of micronutrient deficiencies may vary among population groups, and will depend on detailed assessments of the prevalent deficiencies and their causes. To plan, implement and evaluate programmes cost effectively, it is essential to have information on food consumption patterns, as well as socio–cultural and economic factors that influence both intake and metabolic need. International organizations active in micronutrient projects (WHO, UNICEF, IVACG, INACG) recommended four key strategies to eradicate micronutrient malnutrition. These are (i) Nutrition education; (ii) Dietary diversification; (iii) Dietary supplementation and (iv) Food fortification.

TABLE 4. CAUSES OF MICRONUTRIENT MALNUTRITION

Immediate

- Low intake of foods rich in micronutrients
- Low intake of substances like vitamin C that enhance absorption
- High intake of factors like phytates and tannin that inhibit absorption
- High incidence of measles, diarrhoea and parasitic infections
- Maternal deficiencies

Underlying

- Inadequate breastfeeding practices
- Inadequate and/or incorrect complementary feeding practices
- Inadequate caring capacity: time, knowledge etc
- Low levels of family education, awareness, knowledge and motivation
- Intra-household maldistribution of access to food, health services and care
- Poor cooking, food preparation, storage, preservation and processing facilities at household levels
- Beliefs and practices that restrict access to certain foods for family members
- Poor health service and/or agricultural infrastructure
- Lack of institutional capacity in nutrition and/or personnel trained in the various components of micronutrient deficiency prevention programmes
- Low production of micronutrient-rich foods
- Lack of household-level gardening
- Insufficient marketing for key foods
- Poorly developed commercial food processing industry

Basic

- Lack of resources to produce micronutrient-rich foods
- Failure to consider micronutrient needs in agriculture and health policy making
- Poor economic or physical access to markets
- Little or no productive land
- Lack of access to seed and other inputs
- Lack of access to water for drinking, hygiene and/or irrigation
- Seasonality of food availability
- Low status of and lack of resource control by women
- High prevalence of certain endemic diseases

Source : Gillespie and Mason (1994)

During recent years, there has been considerable debate on which of the four strategies is most efficient, as each has its strengths and weaknesses. A deeper analysis of the nutritional problems in many parts of the world shows that for maximum impact and efficacy each strategy must be analyzed in the context of its cultural and socio-economic environment (Blum 1997).

Nutrition education is certainly the most sustainable one. Along with the knowledge, positive attitude and motivation to adapt to the new behaviour are essential demands for the successful implementation of the strategy. Activities directed at encouraging improved behaviours are termed as 'social marketing'. This relies on the use of mass media like radio, TV, newspaper advertising, competitions, gimmicks, etc. to sell an idea rather than a product. It has been used successfully to introduce home gardening and new agricultural production methods. However, it requires a long term effort and much perseverance and education. Dietary diversification is seen by many as the ultimate solution to eradicate malnutrition. It requires people to change their dietary habits. It may also involve the need to increase production, distribution and consumption of micronutrient-rich crops and animal products. Dietary supplementation is usually seen as a short term, emergent therapeutic intervention to deal with acute deficiencies causing clinical symptoms in high-risk groups. It has often proved effective but difficult to implement for logistic reasons. Food fortification is a convenient, inexpensive, safe and effective way to eliminate widespread micronutrient deficiencies. When fortification programmes are nationally co-ordinated and efficiently monitored, success is not dependent on changes in agricultural and nutritional practices (Nilson 1994).

Food-based strategies are best

The FAO/ILSI (1997) observed that food-based approaches were the most effective way to address existing micronutrient deficiencies. Except supplementation, dietary diversification, improved food availability, food preservation, nutrition education and food fortification are categorized under this heading. Existing constraints in many developing countries include agricultural, economic, environmental, socio-cultural, political, health-related and infrastructural facilities.

The cost-effectiveness of the different nutrition interventions show that all alternatives provide a high return on investment in terms of disability-adjusted life-years gained (Table 5). Comparatively, food fortification seems to be the most economical. In every case, the most appropriate combination of the above mentioned nutrition strategies must be employed to overcome local constraints and achieve the desired results. The long-term solution to micronutrient deficiencies rests in providing adequate quantities of all micronutrients from a well balanced diet (FAO 1995).

TABLE 5. RETURNS OF NUTRITION INVESTMENTS

Intervention	Target group	Cost per life saved ($)	Cost/DALY gained ($)
Vitamin A supplementation	Children under 5 years	325	9
Vitamin A fortification	Entire population	1000	29
Education on vitamin A	Mothers	238	Not applicable
Iron supplementation	Pregnant women	800	13
Iron fortification	Entire population	2000	4
Iodine supplementation	All under 60 years	1000	37
Iodine fortification	Entire population	1000	8

Source : World Bank (1994)

Since the early 1980s, an immense amount of effort and resources has been devoted to identifying the extent and severity of micronutrient deficiencies in developing countries. Programmes implemented to combat these deficiencies have met with varying levels of success. While nutrition education is recognized as an important component in combating nutritional deficiencies, it alone cannot eradicate the problem. Supplementation programmes, which depend on an effective health care system, have tended to end up as pharmaceutical programmes directed at specific age and physiological groups, rather than to sectors of society. Food fortification programmes, on the other hand, are better suited to combating micronutrient deficiencies because they depend on people's participation in the market economy (Nestel 1993).

Food fortification

Food fortification refers to the addition of essential micronutrient to food. It is also known as food "enrichments" (mainly used in the context of flour and bread) or "nutrification". The basic principles were defined by the Codex Alimentarius Commission in 1987. According to these guidelines, essential nutrients may be added: (i) to replace losses that occur during manufacture, storage and handling of food (restoration). For example, the

removal of cream from milk takes almost all of the natural vitamins A and D and therefore skimmed milk may be fortified with the same vitamins at levels as fluid whole milk; (ii) to ensure nutritional equivalence in imitation or substitute foods; (iii) to compensate for naturally occurring variations in nutrient levels (standardization). For instance, milk and butter are subject to seasonal variations in vitamins A and D contents. Some dairy products are fortified with vitamins A and D in order to maintain constant vitamin levels; (iv) to provide levels higher than those normally found in a food (fortification). For example, margarine is fortified with vitamins A and D (in Western countries) to render it nutritionally equivalent to butter and (v) to provide a balanced intake of micronutrients in special cases (dietetic foods) e.g., infant formulas, special foods for athletes, medical foods i.e., enteral nutritional products.

In addition to the above mentioned objectives, nutrients may be added to perform specific processing functions. Beta-carotene is added to products such as juices, pasta, margarine, cakes and processed cheese to impart colours. The use of beta-carotene has been tried for coloration and vitaminization of instant beverage powders (Nagy 1982). Vitamin E can be used as an antioxidant to stabilize pure oils and fats including margarine.

Of the names in current use in various parts of the world, the Joint FAO/WHO Expert Committee on Nutrition considered the term "fortification" to be the most appropriate to describe the process whereby nutrients are added to foods to maintain or improve the nutritional quality of individual foods of the total diet of a group, a community, or a population to correct specific nutritional deficiencies. Examples of food fortification include addition to vitamins and iron to breakfast foods, fortification of sugar with vitamin A and fortification of table salt with iodine. Foods fortified with vitamin A, iron, iodine and multinutrient mixes are given in Table 6.

TABLE 6. FOODS FORTIFIED WITH VITAMIN A, IRON, IODINE AND MULTINUTRIENT MIXES

	Ongoing	Experimental
Vitamin A	Sugar	Whole wheat, rice, tea, Oil*, salt*, MSG
Iron	Wheat flour, corn flour, infant formula, rice	Sugar, salt, milk, biscuit, water, fish sauce, curry powder, maize meal, 'kool-aid', MSG*, salt*
Iodine	Salt, tea, water, bread, milk	Sugar
Multi-nutrients	-	Wheat flour, corn meal, wheat flour noodles

* Laboratory stage only
Source : Nestel (1993)

History

One of the earliest recorded examples of fortification and therapeutic applications of trace elements was in 4000 BC, when the Persian physician Melamus, prescribed a diet including sweet wine laced with iron filings to strengthen the sailor's resistance to spears and arrows (Frazer 1935). Efficacy of this particular tonic was never proven, but the story goes on to describe how while in search of the Golden Fleece, Jason and his crew had many adventures and how they lingered too long with women of Lemnos. As has been reported subsequently, the nutritional rationale for this prescription was amazingly far-sighted and even today, none of the other trace elements attract more attention than iron.

The concept of food fortification with vitamins and trace elements was documented over 50 years ago to prevent overt and sub-clinical deficiencies of vitamins, minerals and trace elements. Earliest reported example is the iodization of table salt in Switzerland since 1923 to prevent goitre and cretinism, which were widespread throughout the alpine region until then. Rickets, caused by vitamin D deficiency, was once common in young children in the Northern Hemisphere because of the lack of sunshine in the winter months. It was prevented by the addition of vitamin D to infant formulas and by vitamin D fortification of milk and dairy products. Margarine was the first substitute or imitation food produced on a large industrial scale. Its introduction in Denmark in the 1930s led to widespread deficiency of vitamin A in children. It was soon recognized that in order to be of nutritional equivalence to butter, margarine had to be fortified with vitamin A. Vitamin D was also added later. Flour enrichment was first introduced in the USA during World War II to prevent pellagra and sub-clinical deficiencies of thiamine, riboflavin and niacin, then common in large segments of the less privileged population living in the southern States. Fortification of sugar with vitamin A, first introduced in Guatemala in 1974, is a more recent example of a large-scale nutritional intervention to prevent nutritional blindness and sub-clinical vitamin A deficiency (Blum 1997).

In India, possibilities of fortifying salt with lysine, iron and vitamin A were tried as early as

in 1970. Central Silk Marine Research Institute (CSMRI), Bhavnagar, developed a process where gypsum ($CaSO_4$) which is already present in seawater can be co-precipitated with NaCl in a single operation. Fortification at the level of 5% $CaSO_4$ proved successful at the production and sensory tests. The observations suggested that the utilization of calcium from $CaSO_4$ added to salt was similar to that of $CaCO_3$ and calcium lactate (NIN 1970).

Fortification as means of improving nutrition

Advances in food technology and new manufacturing processes have also created the possibilities for increased exposure to some essential nutrients through food fortification. Although the concerns about losses of specific nutrients in various processes have to be considered in the context of the diet as a whole, the addition of micronutrients can be used to maintain or enhance the nutritional value of commonly consumed foods. Before discussing the rationale for food fortification, it is important to note that food fortification is used for two purposes. First, it can restore nutrients lost during food processing by enriching a food with the depleted nutrient. Second, it can increase the level of specific nutrient in a food. In both the cases, fortification increases the intake of a specific nutrient previously identified as inadequate. Table 6 illustrates current status of food fotification.

Food fortification has the following advantages: (i) Food fortification does not require people to change their eating habits, thus it is socially acceptable. The "target" population continues to eat the food chosen as a "vehicle", which, once fortified, becomes a good source of nutrient; (ii) The effect of fortification is both fast and broad. Nutrient intakes of the targeted group improve immediately, and an impact on micronutrient status can be detected within one to three months; (iii) Furthermore, fortification does not affect organoleptic properties; (iv) Food fortification is the safest strategy as the added nutrient is provided in the diet in low but constant amounts, so the possibility of excess intake of micronutrient is less (v) Way to deliver necessary amounts of micronutrients; (vi) Compared with other interventions, fortification is the most cost-effective approach to prevent nutrient deficiencies; (vii) It can be introduced quickly through existing marketing and distribution system; (viii) Benefits of fortification are readily visible; (ix) Food fortification is sustainable as it is socially acceptable and (x) In addition, fortification will reach secondary target risk groups, such as the elderly, the ill and those who have an unbalanced diet.

Largely because of food fortification programmes, micronutrient deficiencies do not exist in developed countries. Food fortification is, however, technically easier to implement in developed countries because of the higher dependency on processed foods by all income groups. Consequently, a wide variety of processed foods can be fortified with different micronutrients.

The following criteria should be considered for selecting the vehicle for food fortification.

(i) Food fortification is appropriate when there is a demonstrated need for increasing the intake of essential nutrients in one or more population groups. This may be in the form of actual clinical or sub-clinical evidences of deficiency, estimated low intakes, or possible risks following changes in eating habits; (ii) The fortified food must be consumed by a large section of the population, especially those at greatest risk of deficiency; (iii) Relatively little inter- and intra- individual variation occurs in the amount of the fortified food consumed. This will ensure that nutrient intakes remain within a safe range; (iv) The essential nutrient(s) should be present in amounts that are neither excessive nor insignificant, taking into account intakes from other dietary sources; (v) The nutrients added should not adversely affect the metabolism of any other nutrients; (vi) The nutrient(s) added should be sufficiently stable in the food under customary conditions of packaging, storage, distribution and use; (vii) The nutrients added should be physiologically available from the food; (viii) The nutrient(s) added should not impart undesirable characteristics to the food (changes in colour, taste, smell, texture, cooking properties) and should not unduly shorten shelf-life; (ix) The fortified food must be through central processing in which nutrients can be added under controlled conditions and minimum cost; (x) The marketing and distribution channels must be such that the fortified food to consumers can be tracked and (xi) The additional cost of the fortification should be reasonable for the consumer.

Nilson (1997) has summarized the suitability of vehicles for specific nutrients (Table 7).

Limitations of food fortification

One has to be aware of the following limitations of food fortification before deciding on the food fortification as a strategy against micronutrient malnutrition :

TABLE 7. FOOD FORTIFICATION : VEHICLES AND INGREDIENTS

Food	B-carotene	Vit A	B_1	B_2	B_3	B_6	Folic acid	B_{12}	Fe	Ca
Milk										
Liquid	+	+	+	+	+	+	+	+	0	+
Powder	+	+	+	+	+	+	+	+	+	+
With cereal	0	+	+	+	+	+	+	+	+	+
Flours										
Wheat	0	+	+	+	+	+	+	+	+	+
Corn	0	+	+	+	+	+	+	+	+	+
Rice	0	+	+	+	+	+	+	+	+	–
Rice	0	+	+	+	+	+	+	+	+	+
Pasta	+	+	+	+	+	+	+	+	+	+
RTE cereals	+	+	+	+	+	+	+	+	+	+
Oil	+	+	–	–	–	–	–	–	x	0
Margarine	+	+	0	0	0	0	0	0	x	0
Juices	+	0	+	+	+	+	+	+	+	+
Sugar	–	+	–	–	–	–	–	–	–	–
Beverage powders	+	+	+	+	+	+	+	+	+	+

+ : Possible 0 : Trials needed x : Not possible
RTE : Ready-to-eat
* Vitamin B_2 at high level may produce unacceptable yellow coloring of rice Source : Nilson (1994)

(a) A thorough knowledge of dietary habits and nutrient intakes in the target group(s); (b) A complementary educational programme is required particularly when the fortification influences organoleptical characteristic of the food, when home preparation treatment may remove or destroy the added nutrient or when the fortification programme depends on the addition of a locally or a centrally prepared premix; (c) Food fortification is not the ultimate solution of a nutritional deficiency problem but it may be an essential part of a nutritional programme. Food fortification must never become a substitute for a planned nutrition programme designed to improve the food supply or food usage (CIH/EUSPH, 1992).

Fortification technology

Advancement in science and technology has enabled the technologist to fortify almost all foods. Foods can be fortified with nutrients either in powder or liquid form. Micro-encapsulation techniques enable liposoluble vitamins to be produced industrially in a hydro-dispersible powder form. The techniques of food fortification depends on the food processing technology used and are as follows:

(i) Dry mixing for foods like cereal flours and their products, powder milk beverage powders, etc.; (ii) Dissolution in water; For liquid milk, drinks, fruit juices, bread, pastas, cookies etc.; (iii) Spraying: For corn flakes and other processed foods requiring cooking or extrusion steps that would destroy vitamin activity; (iv) Dissolution in oil: For oily products such as margarine; (v) Adhesion: For sugar fortification. Vitamin A in powder form is absorbed onto the surface of the sugar crystals when used with a vegetable oil; (vi) Coating: For rice. The vitamins sprayed over the grain must be coated to avoid losses when the grains are washed before cooking and (vii) Pelleting: For rice. The vitamins are incorporated into pellets reconstituted from broken kernels.

Vitamins are very sensitive to external factors such as humidity, heat, oxygen, pH, light, oxidizing and reducing agents. Some losses must therefore be expected during food processing and storage. Apart from using a process that causes minimal losses and packaging material that gives maximum protection, the food industry must add extra amounts of the vitamins (so called overages) to compensate for those losses and to ensure that the finished product has at least the amounts declared on the label during its normal shelf life (Nilson 1994).

Misconceptions regarding food fortification

Mora (1995) quoted common myths about fortification that makes industry reluctant to adapt this technology. The main ones are listed below:

(i) Food fortification technology is not fully developed; (ii) Food fortification technology is complex; (iii) Food fortification is expensive;

(iv) Fortification is a problem of legislation and enforcement; and (v) Food fortification is an economic burden.

However, these apprehensions are not supported with any solid evidences. In fact, the present knowledge and experiences have proved that food fortification is a simple, easy to adapt with minor modifications in the existing production plant. Technology for fortification of staple foods with micronutrients is not only well developed but has been successfully applied in industrialized and developing countries over several decades. Existing industry equipment could be used or otherwise acquired at relatively low cost. Single nutrients and nutrient premixes for most of the fortificants are available locally. Percentage of added cost of fortification is relatively insignificant and could be passed on to the consumer. Appropriate legislation along with an industrial commitment can make food fortification successful.

Major components of a food regulatory system to enable food fortification

In FAO's view (FAO/ILSI 1997), the following should be the major components of legislation to enable food fortification :

(i) Authorization to fortify food to address nutritional deficiencies and improve health; (ii) Designing of the government agency (frequently the Ministry of Health) with responsibility for issuing specific enabling regulations for each fortification measure (this approach offers the ability to respond promptly to changing requirement) and (iii) Enforcement and penalties for non-compliance with regulations.

Enabling regulations should cover: (i) Foods for which fortification is mandatory or is permitted; (ii) The type, quality and amount of fortificant to be added, the minimum levels required at production/port of import, distribution and consumption; (iii) Labelling specifications and (iv) Quality control and monitoring with designed responsibility and regular mandatory reporting of results.

Fortification programme design

When the decision for food fortification as a suitable strategy against micronutrient malnutrition has been adopted, the following steps in the design of a fortification programme can be distinguished: (i) Identification of the target group; (ii) Identification of the nutrient to be added; (iii) Selection of foods to reach the vulnerable i.e., target group; (iv) Level of nutrient to be added and (v) Execution of test protocol: laboratory testing, bioavailability and pilot trials.

When the steps (i) and (iv) are to be decided upon by nutritionist, epidemiologist and national health authorities, steps (ii), (iii) and (v) are mainly in the focus of the food technologist.

Safety of food fortification

A preferred intervention in the management of micronutrient malnutrition is through a diet that provides a safe concentration of intakes. As a matter of safety, it is advisable to give a near physiological dose daily which does not require strict medical supervision. Except for vitamin A and other fat-soluble vitamins, other vitamins are non-toxic even if ingested at levels higher than the recommended intakes. Level of food fortification generally ranges between 15% and 25% per serving, which is much below the critical levels. For example, vitamin C may be consumed in amounts up to 100 times the recommended daily allowances (RDA) for very long periods without any undesirable side effects. This safety range is smaller for vitamin A being only 10 times the RDA (Marks 1989). Blum (1997), therefore, recommends that vitamin A fortification be restricted to commodities like milk, margarine, vegetable oil, flour and rice. For iron, it is estimated that the safety index is 5 i.e. 5 times the RDA (Hathock 1985).

Fortification with vitamin A

This intervention entails adding vitamin A to one or more widely consumed foods. Its application is justified when widespread or blanket coverage is desired. This implies that vitamin A deficiency is extensive and not limited to specific groups or isolated communities. Thus, evidence must exist that the magnitude of the deficiency is of public health significance (IVACG 1988a). Blanket coverage increases the likelihood of reaching all the groups at risk regardless of age, socio-economic status or geographic area. Coverage can include the whole country or a region.

When to consider food fortification with vitamin A an appropriate intervention?

National-level vitamin A fortification is indicated when at least two of the following three criteria are met: (Arroyave and Dary 1996).

(i) Twenty percent or more of preschool children have serum retinol levels below 20 mcg/dl; (ii) Twenty-five percent or more of lactating women

have breast milk retinol levels below 30 mcg/dl; and (iii) Twenty-five percent pre-school children consume less than 50% of their recommended daily allowance for vitamin A.

Table 8 shows various countries reporting commercial production of fortified and high retinol foods for distribution. Fortification of sugar with vitamin A has been successfully implemented in Guatemala, Costa Rica, Honduras, El Salvador and Panama (Arroyave 1972; Arroyave et al. 1979; Mejia and Arroyave 1982; McKingney 1983; 1991; McKingney 1992). Only Guatemala, Honduras, El Salvador are currently fortifying sugar.

Fortification of wheat with vitamin A has been considered in Bangladesh (Darton-Hill 1988; Crowley et al. 1989). Both the Dominican Republic (Emodi and Scialpi 1976) and the Philippines (Florentino and Pedro 1990; Murphy et al. 1992)

TABLE 8. COMMERCIAL PRODUCTION OF FORTIFIED AND HIGH RETINOL FOODS FOR DISTRIBUTION IN VARIOUS COUNTRIES

Region	Countries with programmes	Commodity fortified	Programme status	Legislation status
East and Southern Africa	Botswana	Weaning food (Tsabana)	Implemented	-
	Ethiopia	Weaning food	Implemented	-
	Kenya	Sugar and Oil	In preparation	-
	Malawi	Maize meal	Investigating feasibility	-
	Mozambique	Sugar	In preparation	-
	South Africa	Maize meal	In preparation	-
		Sugar	Investigating feasibility	
	Tanzania	Palm Oil*	Implemented	-
		Orange beverage	Experimental	
	Zambia	Palm Oil*	In preparation	-
		Maize meal	Investigating feasibility	
	Namibia	Wheat flour	In preparation	-
West and Central Africa	Senegal	Dried mango*	Implemented	
	Nigeria	Food flavouring cubes	Investigating feasibility	-
South Asia	Bangladesh	Sugar	Investigating feasibility	-
	India	Dairy & Veg. Fat	Implemented	-
		Sugar	Investigating feasibility	
	Pakistan	Vegetable fat	implemented	-
	Sri Lanka	Margarine & dairy	In preparation	-
East Asia and Pacific	Indonesia	MSG	In preparation	-
	Philippine	Wheat	In preparation	
		Margarine	Implemented	
		Sugar	Investigating feasibility	
	Thailand	Condensed milk	Implemented	
		Instant noodles	Experimental	
	China	Dairy & vegetables	Implemented	
	Papua New Guinea	Sugar	Investigating feasibility	
Latin America	Cuba	Milk & other basic foods	Proposed	
	Dominican Rep	Sugar	Proposed	Under discussion
	EL Salvador	Sugar	Implemented	Mandatory
	Guatemala	Sugar	In preparation	Mandatory
	Haiti	Dried mango*	Implemented	
	Honduras	Sugar	Implemented	Mandatory
	Bolivia	Sugar	Pilot scale	
	Venezuela	Enriched formula and whole milk	Implemented	
	Brazil	Sugar	Pilot scale	Under discussion
	Mexico	Chocolate drink mix	Implemented	
	Nicaragua	Sugar	Implemented	
	Ecuador	Sugar	Implemented	In preparation
	Columbia	Sugar	In preparation	Under discussion

* Unfortified
Source : UNICEF field office reports (1997)

have attempted to fortify rice with vitamin A (Rubin et al. 1977). Rice fortification, however, requires an approach that differs from other food vehicles. The reason is that rice is consumed as a whole grain that is picked over for irregular kernels and then washed before cooking. Initially, rice was fortified by applying a coating that included vitamin A to rice grains. The vitamin A coated rice grains constituted the premix. Technically viable process was unacceptable to Philippines authorities because washing losses ranged from 10–20%. More recently, a synthetic rice grain comprising vitamin A has been developed as the fortificant and is undergoing field trials (Nestel 1993).

In both India (Brooke and Cort 1972) and Pakistan (Fuller et al. 1974; Crowley et al. 1989) fortification of tea was considered. Both tea dust and tea leaves were successfully fortified in India. Pakistan showed interest in fortifying tea leaves. Fortification of soybean oil is currently under trial in Brazil (Favaro et al. 1990). Fortification of salt with vitamin A has been tried under laboratory conditions (Bauernfeind and Arroyave 1986; Crowley et al. 1989; Bauernfeind 1991). Monosodium glutamate (MSG) has also been tried for fortification with vitamin A 'white' in Indonesia (Murphy et al. 1987).

An overview of various food vehicles for vitamin A fortification is given in Table 9. Foods that have been successfully fortified with vitamin A include margarine, fats and oils, milk, sugar, cereals and instant noodles with spice mix. Moisture contents in excess of about 7–8% in a food are known to adversely affect the stability of vitamin A. Beyond the critical moisture content, there is a rapid increase in water activity, which permits various deteriorative reactions to occur (FAO 1995). Repeated heating, as may be experienced with vegetable oils used for frying, is known to significantly degrade vitamin A. The hygroscopic nature of salt has prevented its use as a vehicle for vitamin A fortification in countries of high humidity. In trying to overcome this problem, a new vitamin A fortificant, encapsulated to provide an additional moisture barrier, was evaluated with limited success. The cost of using highly protected fortificants can be prohibitive in many cases.

Carotenoids can be used as a source of vitamin A. However, the conversion and bioavailability of carotenoids are known to be affected by the vitamin A status of the individual and by dietary composition. A conversion factor of 6.1 (six milligrams of beta-carotene equivalent to one milligram of retinol) is currently being applied in calculating intake (ICMR 1994). The cost factor in using carotenoids as the source of vitamin A activity in fortification is generally considered prohibitive.

Results of the field trials conducted for fortification programmes established for sugar (Arroyave et al. 1981), MSG (Muhilal et al. 1988 a,b) and salt were found to be effective in improving the vitamin A status. Improvement in the iron nutriture was also observed in the trials conducted for fortified sugar and MSG. The parameters of evaluation included average daily intake of Retinol Equivalent (RE), serum retinol levels, clinical eye signs and child growth and retinol levels in breast milk (Nestel 1993).

Safety in vitamin A fortification

There is essentially no risk of vitamin A deficiency or toxicity, when dietary intake from food totals 10.000 IU vitamin A/day. The safety range

TABLE 9. FORTIFICATION WITH VITAMIN A

Vehicle	Fortificant	Potency	Dilution	Stability
Sugar	Retinol palmitate	Premix : 50,000–55,000 IU vitamin A/g	1:1000 premix to white sugar	91% at RT after 9 months
MSG	Vitamin A palmitate The fortificant known as 'white'	'white' : 1,75,000 IU vitamin A/g	17.1kg 'white in 982.9 kg MSG. 3000 IU vitamin A/g MSG	50% after 18 months at 25°C
Whole wheat	Vitamin A	Not stated	1:400 of premix to wheat	Not stated
Rice	Retinol palmitate	Premix : 2000–2500 IU vitamin A/g	1:199 premix to rice	100% after washing
Tea	Vitamin palmitate and acetate	125 IU vitamin A/g fortified dry tea	–	85% after a year storage at RT
Oil	Retinol palmitate	1,700,000 IU/g	200 IU/g fortified soybean oil	98.5% after 9 months in dark, poor light after 18 months
Salt	Vitamin A palmitate	–	–	Protection of vitamin A activity upto moisture level 2%

is 10 times the RDA (Nilson 1994). Under normal physiological conditions, however, significantly higher levels of retinol intake just before or soon after conception may have teratogenic consequences. For this reason, WHO (1982) and IVACG (1988b) recommended that daily supplements of 3000 RE (10,000 IU) vitamin A can be given safely anytime during pregnancy.

Given that the diet usually includes some vitamin A, fortification is generally used as a means of making up the deficit between intakes and requirements. A food fortified with vitamin A should not be considered the sole source. Often, however, the information available on vitamin A intake is inadequate. In these situations, the fortified food should provide the critical intake level or at least one–half of the RDI (IVACG 1988b). The limiting factor is the RDI for the groups most vulnerable to deficiency and/or toxicity (young children and/or pregnant women). Thus, for fortification to be effective and yet remain within the upper safe limit, it must meet the RDIs for children aged 3–5 years.

The concentration of fortificant to be used in the food vehicle should be based on the per capita consumption of the food vehicle by different social and economic groups in the different ecological regions. These data are important because delivery of too little vitamin A results is an ineffective programme, while too much vitamin A is not only wasteful but also can exceed the upper safe limit. Once the minimum and maximum intake levels of the food vehicle have been ascertained, a simple mathematical calculation (Table 10) determines the safe range of vitamin A fortification where C, the midpoint of A, minimum requirement and B, maximum permissible intake, will be more than basal needs of pre-school children from the lowest socio-economic group and at the same time will remain below maximum acceptable limit with highest consumption of food vehicle. It will also account for losses during distribution and storage

TABLE 10. DETERMINING SAFE LEVEL OF VITAMIN A FORTIFICATION

Mean intake - of food vehicle	RDI*, IU	Estimated level of fortification, IU/1g	Fortificaiton level, IU/g
Minimum = X g	Min (670)	670/X = A	$\frac{A+B}{2} = C$
Minimum = Yg	Max (10,000)	10,000/Y=B	

* RDI : Recommended Daily Intake
Modified from Nestel (1993)

over a period of time, keeping the final intakes at acceptable range.

It is recommended, therefore, that other potential food vehicles for vitamin A and suitability of currently available technologies for these new vehicles need to be identified and evaluated. New and effective vitamin A forms, if necessary could be tested and use of beta-carotene as a fortificant need to be explored.

Fortification with iron

Fortification with iron has been successfully adopted for wheat flour (Cook and Reusser 1983; Arroyave 1992), rice (Florentino and Pedro 1990), sugar (Zoller et al. 1980; Cook and Reusser 1983; Molina 1991), salt (Zoller et al. 1980, Anon 1983; Mannar 1990; Bauernfeind 1991), milk (Walter 1990), fish sauce (Cook and Reusser 1983) and curry powder (Lamparelli et al. 1987). Other foods like wheat biscuits, wheat flour noodles and maize meal have also been tried (Cook and Reusser 1983). Table 11 shows various food vehicles for iron fortification. Iron fortification practices should be based on feedback from comprehensive surveillance programmes monitoring their impact on iron status. It should be noted that iron overload is not a concern in growing children and menstruating women, since the iron requirement of these groups is much higher than for adult male populations. But there is a need to evaluate the long–term risks of excessive iron intake in the latter group as consumption of the fortified foods is generally not restricted to the group at the greatest risk of deficiency. A combination of nutrition intervention strategies should be used to meet the high iron requirements of these population groups. It is important to assess the contribution of iron fortification in combination with other strategies for the control of iron deficiency in specific age/sex groups, so as to establish optimal goals for iron fortification of foods.

Surveillance data on iron status should be interpreted with caution because of the known alteration of serum ferritin levels and other indicators of iron status in the presence of recent infection. Serum transferrin receptor determination appears promising in the evaluation of iron status in the presence of infection. Furthermore, it should be stressed that not all types of anaemias are due to iron deficiency, although the latter is most common. Several aspects of the interaction between iron and other nutrients, such as vitamin A, iodine, zinc and calcium, need to be investigated in

สำนักหอสมุดและศูนย์สารสนเทศวิทยาศาสตร์และเทคโนโลยี

TABLE 11. FORTIFICATION WITH IRON OF DIFFERENT FOOD VEHICLES

Vehicle	Fortificant	Potency	Dilution	Stability
Wheat flour	Reduced iron	–	–	Good
Rice	Anhydrous ferrous sulphate	14 g anhydrous ferrous sulphate yields 448 mg iron/100 g premix	1:200 premix to rice premix gives 2 mg Fe/ 100 g rice after loss during preparation	–
Sugar	Ferric sodium EDTA	–	13 mg of Fe as ferric Na-EDTA/100 g sugar	good
Salt	Ferrous sulphate +Na-pyro-PO_4	1 mg Fe/salt	3.5 g fortificant/kg salt 10–15 mg Fe/adult/day	–

relation to the possibility of multiple fortification. The stability and the cost of fortificant compounds as well as the bioavailability of the nutrients need to be considered. Meal composition and dietary variety are important factors in optimizing iron status.

Given the wide variability of iron bioavailability, influenced by physical and chemical properties of the fortificant as well as the presence of substances, which either inhibit or improve iron absorption, there is a need to develop convenient models for the evaluation of iron bioavailability. The latest information on the bioavailability of iron in fortified foods should be used to assess the necessity of revising levels of iron fortification in some products. This is particularly important as an increasing range of iron fortificants with different physico-chemical properties, is being developed and marketed.

Benefits derived from added iron depend on the form of salts employed, uniformity of the food fortified and composition of the meal and total diet in which the food article is eaten. In general, iron compounds, which exhibit the best availability, tend to cause adverse technological and nutritional effects in products. These effects may hinder consumer acceptability. Iron sources currently used in food fortification and their Relative Biological Values (RBV) are shown in Table 12.

The major chemical characteristics of iron sources that determine their behaviour in foods are: (i) Their solubility e.g., ferrous salts are more soluble than ferric salts; (ii) Their oxidative state: e.g., ferrous salts are more effectively utilized than ferric salts (in water) and tend to be more reactive in food system; (iii) Their ability to form complexes, which are not bio-available: generally ferric iron has a greater tendency to form chelates than ferrous iron. The ferric form (Fe3+) and its reduced form (Fe2+) are the only states, which occur naturally in the foods. Elemental iron is found rarely in biological systems, but is used widely as an added nutrient; (iv) The presence of metal ions such as iron can speed up vitamin degradation and loss of nutritional value of a food product, particularly for vitamin C, thiamine and retinol; (v) Catalyze the oxidative rancidity of oils and fats and (vi) Produce undesirable colours, colour fading off-flavours and undesirable precipitates.

Safety in iron fortification

Young children aged six to 18 months, pre-school age children, women and adolescent girls are most vulnerable to iron deficiency anaemia (INACG 1990). Determining measures of "safe intake" for minerals is problematic because of the different reactivities of the substances in various food vehicles (Clydesdale 1991). Nevertheless, a mineral safety index (MSI) has been proposed. MSI

TABLE 12. SELECTED IRON SOURCES CURRENTLY USED IN FORTIFICAITON

Compound	Other common name	Fe content, g/kg	RBV
Ferric phosphate	Ferric orthophosphate	280	3–46
Ferric pyrophoshate	Iron pyrophosphate	250	45
Ferric sodium pyrophosphate	Sodium Fe-pyrophosphate	150	14
Ferric ammonium citrate	–	170	107
Ferrous fumarate	–	330	95
Ferrous gluconate	–	120	97
Ferrous lactate	–	380	-
Ferrous sulphate	–	320	100
Fe	Elemental Fe, Ferrous reductum, Metallic Fe	1000	-
Reduced Fe, H_2 or CO process	–	960	34
Reduced Fe, electrolytic	–	970	50
Reduced Fe, carbonyl process	–	980	67

Source : Richardson (1983)

= MTD/RDI where, MTD is the minimum toxic dose as obtained from the literature and the RDI is the highest value for a non-pregnant and non-lactating woman. The estimated adult oral MTD for iron is 100 mg. Thus, the MSI would be 2 or 4 depending on whether the iron availability in the diet was low or intermediate, respectively (Nestel 1993). Nilson (1994) quoted the safe intake for iron as 5 i.e., 5 times the RDA.

Iron EDTA is not currently approved for use in foods in US because total EDTA levels in the diet from other types of EDTA used in processed foods approach allowable limits. Iron EDTA is more bio-available form of iron compared to some other forms. Nevertheless, in developing countries where people consume much less total dietary EDTA, it should be seriously considered as an attractive iron fortificant.

Evidences from various field trials suggest that iron fortified foods helped in reduction and prevention of anaemia in different age groups, the results being best among people with worst anaemia. Field trials with iron-fortified milk showed significant but inadequate reduction in anaemia (27-10%) among children aged three to 15 months. The low bioavailability of iron was considered to have limited the decline in anaemia levels. However, when ascorbate was added (100 mg/l) to enhance iron absorption, anaemia in the children was virtually eradicated. But the process significantly increased the cost of production. Hematocrit values were used as an indicator for improvement in iron status in feeding trials with iron fortified fish sauce. Significant improvement of 25-35% in Hematocrit values among those receiving the supplementation was observed (Garby and Areekul 1973). Field trials with iron fortified curry powder after two years revealed a significant improvement in the iron status of women. No excessive accumulation was observed among individuals with normal iron status at the beginning of the trials (Lamparelli et al. 1987) Table 13 compares various iron fortificants and their costs, bioavailability and stability. However, over a period of time, economics have undergone tremendous changes and we lack current data with this respect which needs to be updated.

Strategies

The suggested strategies are the following :- (i) Methodologies that are accurate and reliable in the presence or absence of recent infection to define iron status in different population groups could be tried; (ii) Convenient models of iron bioavailability (non-human or *in vitro* models) need to be developed as current methodologies for the evaluation are problematic; (iii) The necessity to revise levels of iron fortification in some products because of improved iron bioavailability of new fortificants can be determined; (iv) The use of new iron fortificants such as liposome micro-encapsulated iron compounds and amino acid chelates need exploration; (v) Their bioavailability, long-term safety, regulation of absorption and retention need to be studied; (vi) long-term risks of iron intake for adult male populations should be evaluated as their iron requirements are lower than those for adult females and children and iron fortification of foods generally does not allow for preferential targeting among these groups; (vii) The contribution of iron fortification, in combination with other strategies, for the control of iron deficiency in specific age/sex groups should be assessed and (viii) Issues of multiple fortification (iron/vitamin A; iron/iodine; iron/zinc; iron/calcium, etc) in terms of their practicality, nutrient interaction, stability and bioavailability also need attention (FAO 1995).

Fortification with iodine

Salt is one of the most suitable vehicles for iodine fortification for the general population. It has been successfully and, in general, safely used for over 70 years in programmes around the world to prevent iodine deficiency problems. Two chemical

TABLE 13. COSTS OF IRON SOURCES USED IN FORTIFICATION

	Fe %	1990 Cost $/kg	Cost $/kg Fe	Bioavailability	Colour	Stability
Hydrogen reduced Fe	98	1.94	2.00	Fair-	Black	G
Electrolytic Fe	98	4.71	4.80	Fair+	Black	G
Ferrous sulphate	32	2.35	7.30	Good	Tan	F
Ferric orthophosphate	28	2.73	9.80	Poor	White	Excellent
Ferrous fumarate	33	2.94	8.90	Good	Red	F?
Fe - EDTA	13	2.40	18.50	Excellent	Off white	G?

Source : INACG (1990); G = Good; F = Fair

forms of iodine are currently used for iodination, these are iodates and iodides. The Codex Alimentarius standard for food grade salt permits the use of the sodium and potassium salts of iodides and iodates in the iodination of salt. The level of fortification that has been used ranges from 30–200 ppm. These two forms of iodine, however, need to be harmonized in terms of equivalence in order to avoid trade barriers (FAO 1995).

A major consideration in the choice of the two compounds is the purity of the salt. The iodides are more readily degraded in the presence of impurities, whereas the iodates remain stable in salt of lower quality. Of the two compounds, potassium iodide is more soluble and is needed in smaller quantities. It is unstable under conditions of moisture, high temperature, sunlight, excessive aeration and in the presence of salt impurities. But it is also less expensive.

Universal Salt Iodization (USI) - The Mid-Decade Goal (IDD Newsletter 1994)

The UNICEF–WHO Joint Committee on Health Policy met in Geneva in January 1994. It briefly reviewed the progress in IDD, and sharpened the focus on iodized salt. The report summarizes a number of positions and recommendations that have been formulated by ICCIDD jointly with WHO and UNICEF.

Highlights of the committee's report

The highlights of the committee's report are as follows : (i) The current intermediate goal is now to iodize all salts for human and animal consumption (including salt for food processing) ("universal salt iodization") in all countries where iodine deficiency disorders (IDD) are a public health problem; where full salt iodization is not possible in areas where IDD are a severe public health problem, supplementation with oral or injected iodized oil would be recommended as a temporary measure; (ii) IDD assessment is necessary to know if the problem exists, but great detail is not necessary in order to act towards salt iodization; (iii) The levels of iodine in salt should be adjusted after the system is in operation and monitored by urinary iodine levels of people living in the main risk areas; (iv) The steps towards salt iodization include: (a) identifying major salt sources; (b) setting standards, regulations and legislation; (c) establishing advocacy and mobilization plans; (d) conducting feasibility studies of universal salt iodization; (e) establishing a procurement and installation plan; (f) assuring adequate supplies of iodine; (g) establishing internal and external quality control procedures; (h) developing an education campaign for iodized salt; and (i) mobilizing necessary resources to achieve effective iodization. Oral iodized oil may be necessary in areas of severe IDD where iodized salt will not be available reasonably soon. The Committee recommends that provision of iodized oil supplements should be supported on the condition that the government agrees to take necessary steps to ensure that iodized salt will be made widely available by the end of the supplementation period; (v) Monitoring is recognized as essential to a sustainable programme (WHO/UNICEF/ICCIDD 1994).

Strategies

These are as follows :- (i) Iodine stability in traditional food processing needs to be further probed as very little information on this is currently available; (ii) Research is needed on the efficacy of iodine resins added to water as an iodine source, as findings regarding the effectiveness of this practice are inconclusive; (iii) Research is needed on iodine interaction with various goitrogens so as to better understand the influence of diet on the effectiveness of iodine fortification and (iv) Losses of iodine during storage and distribution and at household levels have to be minimized.

IDD prevalence and control programme data: Indian scenario

Ministry of Health and Family Welfare in collaboration with the salt commissioner in the Ministry of Industry ensures the production, distribution and quality control of iodinated salt. There is a Central IDD Cell at the Directorate-General of Health Services (DHGS) and each State has its own IDD Control Cell. State Health Departments are responsible for quality control of salt within State, creating consumer demand, monitoring iodized salt consumption, training, information, education and communication. India is self-sufficient in its salt production.

It is now estimated that over 70% of salt consumed in India is iodized. The scale of salt iodization activities underway in the major salt producing regions of the country is very impressive. In addition to the commitment of the Government of India to this initiative, the existence of a separate entity-the Salt Commission's office to organize and coordinate the development of the salt industry,

improving manufacturing methods and overseeing the distribution of salt throughout the country deserves mention. The co-operation and collaboration of the salt producers, who understand the importance of eliminating IDD and of their role in improving public health has resulted in rapid progress towards USI. The production of large crystal salt, which posed a problem for the iodization programme, has been largely discontinued. Although the large crystals could be iodized by spraying with iodate solution, they were difficult to pack and the bags often get torn. During storage and transport, if dust and dirt accumulated on the crystals, consumers would end up washing the salt, thereby washing away the iodine on the surface of the crystal and thus very little iodine would actually reach the consumer. Table 14 lists the availability of iodized salt in relation to their requirements of different States in the descending order.

A combination of consumer demand and the Salt Commissioner's efforts has made producers, to modify their production techniques to produce smaller crystals. This development may have important implications for USI in Nepal, where large crystal salt, produced in India, is widely used and where it is reported that crushed, packed salt would be unacceptable due to consumer resistance and high prices. There is also a rapidly increasing demand and supply of salt packed in 1 kg-polythene bags. It is reported that currently about

TABLE 14. A STATE WISE AVAILABILITY OF IODIZED SALT AS % REQUIREMENTS

State	Availability of iodized salt as % requirements
Assam	126
West Bengal	119
Bihar	119
Uttar Pradesh	83
Madhya Pradesh	37
Punjab	26
Maharashtra	20
Karnataka	19
Himachal Pradesh	15
Gujarat	12
Kerala	8
Rajasthan	7
Tamil Nadu	7
Haryana	7
Orissa	5
Andhra Pradesh	4
INDIA	**65**

Source : Salt Commissioner's Report (1994)

20% of all edible salt consumed in India is packed in such bags - up from virtually none five years ago. An increase in the proportion of salt sold in labelled 1 kg polythene bags will help to sustain and improve the iodization programme for the following reasons - consumer brand loyalty and quality control. Furthermore, the widespread consumption of packed refined salt will be a pre-requisite for fortifying salt with other minerals such as iron. The Indian food industry is using iodized salt in products such as pickled vegetables, fruit juice concentrates, chocolate, carbonated drinks (Pepsi and Seven Up), and western style bread. No negative reports have been received so far about the effects of iodized salt on these products. (UNICEF 1995b).

Before 1983, iodized salt was confined to the Public Sector. Presently, there are approximately 9000 common salt producers in India, mainly in the private sector (90%), who, until recently were partially subsidized by the government (potassium iodate). There are around 500 iodination plants for commercial production of iodized salt. Nine control laboratories have been established by the National Goitre Control Programme (now the National Iodine Deficiency Disorders Control Programme) to ensure the quality of iodized salt and to make sure that all edible salt is iodized at both production and consumption levels (required in 18 States/ Union Territories and only partially in 6). The primary private producers are all found in Nawa. Salt is produced using sea brine, sub-soil brine and lake brine. Less than 10% is produced from mines. Salt is sprayed with potassium iodate solution and packaged on site. Recent estimates are that 60% of all salt is iodized. Public producers like Sambhar Salt Lake and Hindustan Salt Limited produce salt from lake and sub-soil water. Together, they produce salt having a content of 98% sodium chloride. They use spray technique with potassium iodate, batch processing and submersion. Salt is fortified with iodine and iron separately and packaged in low-density polyethylene. Salt varies in quality from coarse to fine white. In total, 2.6 million tonnes are available for human consumption per year, which is well below the 5 million-tonne goal for 1992. The estimated daily per salt consumption being 15 g per day KIO_3 at a concentration of 30 ppm has been used in spraying the common salt. Ministry of Health and Family Welfare, Government of India (1994) in its Report on Advocacy Policy Meeting on IDD held in collaboration with UNICEF

emphasized USI as the most cost-effective measure for alleviating the problem of IDD. The cost-benefit ratio being reported to be 1:8 (UNICEF 1995 a).

Issuing ban on sale of non-iodized edible salt: Report on Advocacy Policy Meeting on IDD also expressed its concern over the fact that the target for USI was not achieved by 1992 mainly because of not banning the non-iodized salt by 9 states/ Union Territories. Moreover, a partial ban, as notified by a few States, can not be effective in ensuring availability of iodized salt to the identified pockets. Non-issue of the ban by major non-producing States adversely affects the supply of iodized salt. It is now recommended that States should ban the sale of loose salt.

The Government of India (The Salt Commissioner's Office) with support from UNICEF and CIDA, has prepared an IDD information kit in English which includes a Handbook for Traders, a pamphlet on 20 questions on IDD and an update on the state of bans on edible non-iodized salt. Other information booklets produced are a 'State wise profile of the production and availability of iodized salt' and a quarterly newsflash called 'USI Newsletter' (UNICEF 1995a).

Indian experiences in food fortification

Interesting aspect of food fortification in India in earlier days was that the fortified foods were for prevention rather than cure. Thus, foods tried were wholesome foods e.g. Indian Multi Purpose Food, synthetic rice, bread etc. Lysine supplementation of cereal diets dominated initial phases of fortification in India (NIN 1969, 1970). Later on, the focus has been shifted to single nutrient fortification as a curative measure for specific nutrient deficiency. Now with the realization that single nutrient deficiency is inter-linked with other nutrient deficiencies, reversal of earlier concept of multi-nutrient fortification has surfaced.

Indian multi purpose food : One method of fortification of the common dietaries is to provide a supplement that the consumer can add to his food to provide the required extra vitamins and proteins. In some way, this is a more elegant approach than broadcast fortification of the staple grains. It is selective in its approach and involves consumer participation. This approach was tried through Indian Multi purpose Food (IMPF). IMPF is a blend of 75% of edible peanut flour and 25% of *Bengalgram (Cicer arietinum)*. It provided 42% of fairly well balanced protein with added vitamins, calcium, and iron (Subramanyan et al. 1957). It was recommended for use along with other foods and provided a fair amount of the daily requirements. Several studies on its nutritive value both with rats and children were significantly in its favour. Consumer acceptance of IMPF was well established and guidelines for recipes were prepared (Subrahmanyan et al. 1957). The large volume of sales that it enjoyed during the project proved its popularity. However, the project was not continued due to lack of marketing techniques and poor governmental support (Orr 1972).

Synthetic rice : Fortification of a starchy material with a high protein source like edible peanut flour was also tried by the scientists at Central Food Technological Research Institute (CFTRI) (Subrahmanyan 1954). The technique adopted was to prepare a dough with tapioca flour and extrude it through a macaroni cutter. The grains formed were treated in such a way that they did not disintegrate while cooking but were not tough for digestion. The grains were fortified with minerals and vitamins by mixing the fortificants with the dough. The project was successful both nutritionally and technologically but the programme of establishing a plant at Kerala, which is the major tapioca-growing area did not succeed. A similar trial under the sponsorship of USAID was reported in 1971 in Thailand, using extruded imitation rice kernels containing lysine, threonine, vitamins and minerals (Borenstein 1974).

Fortification of wheat flour : Fortification of whole wheat flour with edible peanut flour was recommended by the CFTRI to raise the protein content of wheat flour (normally about 11 to 13.5%) by adding 5% edible peanut flour. Since peanut flour protein is poor in both lysine and sulphur containing amino acids, addition of peanut flour to wheat flour did not upgrade the protein quality of wheat very much. Also, it introduced problems of acceptability as the *chapati* (unleavened bread) made using this flour was dark in colour and not soft. A consumer acceptance trial at Mumbai, organized by the Protein Foods and Nutrition Development Association of India, confirmed these findings.

Fortification of bread : Modern Bakeries, a public sector organization of Government of India, opted for fortification of its bread with lysine, vitamins and a few minerals. The objective was obviously to correct the lysine deficiency in wheat flour and thereby that of bread. Significant improvements in the weights and heights of children fed *chapati* using lysine - fortified wheat flour has

been reported by Prabha and Bagchi (1978). Similar findings using bread from lysine-fortified wheat flour has been reported in Haitian children by King et al (1963). Lysine in bread does not alter the taste or texture of bread but may become unavailable after baking, if reducing sugars are present. The fortification of modern bread with lysine has now been given up by the producers. Occasionally, they use soy flour as a wheat supplement, particularly for bread used in school feeding programmes.

Fortification of salt with iron : Iron deficiency anaemia is so widespread that only a broadcast method like fortification of a basic food item consumed by all age groups irrespective of age, economic status and rural or urban setting can meet the requirements. Such a vehicle for fortification in the Indian milieu is salt. A project for salt fortification was undertaken by the Food and Nutrition Board of the Government of India in collaboration with the National Institute of Nutrition. To overcome the problems of bioavailability, storage and utilization in Indian culinary practices, the fortification of salt with iron has been intensively studied by the NIN (NIN 1977). Bioavailability of different combinations of labelled ferrous or ferric salts with orthophosphoric acid or its salts and sodium sulphate have been studied (Rao and Vijayasarathy 1975; Rao 1978; Nadiger et al. 1980). The final formula recommended by NIN is fortification of salt with combination of ferric orthophosphate and sodium acid sulphate, at a concentration that supplies 1 mg iron per g of salt. The cost of such fortification was estimated to be 17 paise per kg. A large quantity of this fortified salt was produced by the Food and Nutrition Board and the consumer acceptance and the nutritional effect on consumers was tested at four centers – All India Institute of Hygiene and Public health, Calcutta, the All India Institute of Medical Sciences, New Delhi, the National Institute of Nutrition, Hyderabad, and the Institute of Child Health, Chennai. The first three were tested under rural conditions and the last under urban conditions. The results have confirmed that salt fortified with iron is acceptable and that the consumption of such salt was associated with an improvement in haemoglobin status and a reduction in prevalence of anaemia even in the presence of hookworm.

Iron fortification of other foods : Fortification of sugar has been studied in detail by Disler et al (1975) and Layrisse et al (1976) and proved its effectiveness as a suitable vehicle for iron fortification. However, sugar may not be a very useful medium for fortification in India as many of the lower income groups use very little sugar. Fortification of tea was seriously considered in India by the Food and Nutrition Board as a vehicle for iron fortification. Unlike coffee, tea is consumed throughout the country and even by children. However, the bioavailability of iron in tea needs testing.

Multiple nutrient fortification

It is well known that nutrient deficiencies do not occur in isolation. The inter-relationship of various nutrients points to the fact that a single nutrient bullet is not going to alleviate the widespread micronutrient deficiencies. Considering the complexity of the problem of malnutrition, a multi-pronged approach is needed. In this regard, it makes sense to add more than one nutrient, while opting for staple food fortification. It may marginally increase the total cost of fortification but the advantages of doing so are far more beneficial.

White flour and corn meal have been tried in developing countries for fortification with vitamins and minerals (Cort et al. 1976). Organoleptic properties of products made from these flours were known to be on par with their conventional counterparts. Addition of magnesium and calcium in these flours has to be technically improved upon due to their ability to impart off-flavour and colour.

Bioavailability of nutrients from fortified foods

Nutrients specifically vitamins indigenous to food are not efficiently absorbed because of poor solubility of specific vitamins, destruction in the gastrointestinal tract, poor availability of bound vitamin forms, slow digestibility of specific foods, and the presence of meal components, which inhibit absorption (Borenstein et al. 1988). In almost all cases, commercial synthetic vitamins are chemically identical to naturally occurring vitamins and may be viewed as "natural identical". The bioavailability of vitamins in fortification are thus a matter of absorption from the gastro-intestinal tract rather than the bioavailability of the compounds *per se*.

Folic acid added to lactose-casein liquid model food system was biologically available to chicks after the food was thermally processed. The high bioavailability indicates that no complexes were formed during the processing, which could inhibit folate utilization (Ristow et al. 1982). Pelletier and Keith (1974) showed the equivalent utilization of

synthetic ascorbic acid and that in orange juice. In this study, relative bioavailability in men was determined by measuring ascorbic acid in serum, leukocytes, and urine. The authors concluded that the bioavailability of synthetic ascorbic acid is slightly superior to that of natural ascorbic acid provided by orange juice.

The concern that added water-soluble vitamins may become bound to food components and therefore, be poorly absorbed, can be dispelled in many cases. Extraction of vitamins from fortified foods with water or simulated gastric juice demonstrates the absence of vitamin binding. The extraction procedure used in analysis of vitamins in foods is an evidence for the lack of significant binding. Mild extraction techniques can not be used in the case of many coated water-soluble and fat-soluble vitamins available commercially. These specialized products are commercially available to solve specific fortification technology problems such as poor water solubility, poor stability and unsatisfactory flavour and odour. The coatings used include high-melting-point fats, monoglycerides, gums and gelatin (Borenstein et al. 1988). Vitamin manufacturers, who produce these modified vitamin products, have an obvious obligation to monitor the absorption and/or bioavailability of these products. This should not be the responsibility of the food processor.

Vitamin A available in market used in fortifying low-fat foods such as beverages and cereal products are almost always stabilized in liquid or dehydrated emulsions. These vitamin products would be expected to be well absorbed, since the vitamin A is in the form of emulsified droplets. In a comprehensive study on vitamin-mineral-fortified flour, Lan-Ing and Parrish (1979) reported a 95% or higher vitamin A biopotency even after extended flour storage (Table 15). The study was done with rats, using vitamin A liver storage as the index of absorption. The vitamin A source used was a commercial stabilized, spray-dried product, identified as vitamin A palmitate, 250 SD Scialpi (1987) showed that the dehydrated, stabilized vitamin A product had excellent bioavailability, even though the matrix was difficult to dissolve in boiling water.

TABLE 15. BIOPOTENCY OF VITAMIN A IN STORED, FORTIFIED FLOUR

Storage conditions	Biopotency, %	
	Corn flour	White bread flour
3 m at 40°C	99	98
3 m at RT	98	99
3 m at 45°C	97	95
9 m at 45°C + 3 m at RT	-	95

m : months
Source : Lan-Ing and Parrish (1979)

Table 16 presents data on the efficacy of various fortification programmes. Thus, the literature demonstrates that the bioavailability of vitamins added to foods is at least equivalent to that of indigenous vitamins ins foods. When specialized formulations are properly developed, there is no loss in bioavailability of the vitamins, when they are added to foods (Borenstein et al. 1988).

TABLE 16. EFFICACY OF FORTIFICATION PROGRAMMES IN VARIOUS DEVELOPING COUNTRIES

Fortification	Vehicle	Country	Field trial results
Vitamin A	Sugar	Guatemala, Costa Rica	A three fold increase in the mean daily intake of RE
		Panama	Increase in serum retinol levels in children
		Honduras	Improved iron nutriture
		El Salvador	Increased retinol levels in breast milk
	MSG	Philippines, Indonesia	Improved serum retinol in children <5 years
	Rice	Philippines	Not determined
	Tea	India, Pakistan	Not disclosed
	Oil	Brazil	Not undertaken
	Salt	Lab stage	Improved vitamin A status in pre-school children
Iron	Wheat flour		Reduced incidences of anaemia
	Rice	Philippines	Not stated
	Sugar	Guatemala	Substantial reduction in incidences of anaemia
	Salt	India	Improvement in haemoglobin
			Reduction in incidences of anaemia
	Milk	Chile	Virtual eradication of anaemia with iron and ascorbate fortification
	Fish sauce	Thailand	Improvement in Hematocrit value among 25-35% of recipient
	Curry powder	Asia, South Africa	Significant improvement in iron status of women
Iodine	salt	India	Significant reduction in goitre and cretinism

Quality assurance and control

The development and implementation of a quality assurance system should guarantee that the food is properly fortified at the production level and reaches the consumer with the required levels of potency and quality. Fortification may take place within a country or, if the food is imported, in the country of origin. Inevitable, some fortificant is lost between the points of production and consumption. These losses are higher in countries where packaging quality, storage and transportation conditions are inadequate.

In foods, the stability of vitamins is more precarious than that of minerals because vitamins are sensitive to heat, oxidizing and reducing agents, susceptible to light and other kinds of physical and chemical stress. Although vitamins in wheat flour for e.g. are stable as such, high humidity and temperature together do adversely affect vitamin A. The use of encapsulated forms of vitamin A will help to overcome this problem. There is some evidence of minor losses of other vitamins during flour storage (Table 17).

Countries should establish expected micronutrient levels at different phases in the distribution system, taking into account climate, type of packaging and per capita food consumption. Micronutrient concentration must be regularly monitored. At the national level, overall responsibility for quality control of food fortification programmes should be clearly identified. Responsibility is frequently vested in Ministry of Health, Food or Agriculture. Criteria for assessing the adequacy of salt iodization programmes have recently been established (WHO/UNICEF/ICCIDD 1994). Similar guidelines can be established for other fortification programmes.

Failure to implement and sustain effective quality assurance system is likely to result in the failure of food fortification programmes. In Guatemala, poor monitoring and regulation of the sugar fortification programme resulted in low levels of vitamin A in sugar for several years after the programme was initiated. Slackening of monitoring of the salt iodization programme led to a recurrence of IDD (FAO/ILSI 1997).

TABLE 17. NUTRIENT RETENTION IN FLOUR WITH HUMIDITY AT ROOM TEMPERATURE

Nutrient	Label claim	Content per kg			
		Initial	2 m	4 m	6 m
Vitamin A IU	16534	18,078	18,078	17,681	17,526
Vitamin B_6, mg	4.41	5.18	4.85	5.07	4.85
Vitamin E, IU	33.07	35.05	35.05	35.05	35.05
Folic acid, mg	0.66	0.82	0.66	0.77	0.66
Vitamin B_1, mg	6.39	7.50	NR	NR	7.50

m : months
Source : OMNI/Roche/USAID (1997)

Walter (1990) has given guidelines for the factors to be kept in mind by the food analyst in quality aspect of the fortification.

The analytical method to be adopted : This will depend on the resources in terms of money, time and labour. Although, sophisticated and fast techniques like HPLC are available, methods based on Spectrophotometric/Colorimetric principles also offer satisfactory results.

Micronutrients to be analyzed : Food analyst has to decide whether the total micronutrient content in food is to be estimated or only the added nutrient, the latter being more easier and accurate.

Validity, specificity, precision and sensitivity of the method adopted : The effective quality control system to regulate the production of a fortified food is an essential component of the sustainability of the programme.

Implementation of food fortification quality assurance programme

Wilson (1988) identified the following steps in the implementation of quality assurance programme for a fortified food product :

Product specifications : All specifications for the fortificant, vehicle and any other ingredients must be documented, including particle size, colour, potency, level of fortification and other requirements considered necessary. Acceptable deviations from the specifications must also be documented.

Product safety assessment : Microbiological, chemical and physical hazards of all ingredients, as well as of the finished product, must be assessed.

Product analysis : Procedures for sampling and testing all ingredients, as well as the finished product must be documented.

Determination of critical and quality control points : The entire production process (including the plant facility, equipment and environment) must be examined to identify stages (control points) at which inadequate quality control could adversely affect product quality or lead to unacceptable health risk. Controls and actions to be taken at each control point must be documented.

Recall system : A mechanism must be in place to recall products if necessary.

Quality assurance audit : Periodic checks must be conducted to verify that the quality assurance system is effective and that the product quality is maintained upto and including the time the consumer receives the product.

Feedback mechanism : A mechanism must be in place to correct any product deficiencies identified by consumers or other relevant groups.

Documentation : All steps in the quality assurance system must be documented and readily available to relevant individuals and organizations.

A well-controlled quality assurance system ensures satisfactory food fortification.

Monitoring and evaluation

The goal of food fortification is to increase the intake of a micro-nutrient(s) status of population preventing irreversible functional damage. The purpose of evaluation is to monitor the progress of programme to ensure that the prestated objectives are met. Progress towards objectives can not be measured unless programme goals are clearly stated and the necessary baseline biochemical, clinical and dietary data are collected and made available.

IVACG (1988) has suggested that the goal of vitamin A food fortification programmes should be to reduce : (i) the proportion of the population with intakes below the critical level (i.e. one-half the safe RDI level) to less than 10%; (ii) the prevalence of serum retinol levels under 20 mcg/100 ml and under 10mcg/100ml to less than 15% and 5% respectively; and (iii) the prevalence of clinical eye signs to the extent that Bitot's spots with conjunctival xerosis do not exceed 2%, corneal xerosis/ulceration and/or keratomalacia are less than 0.01% and corneal scarring is less than 0.1%.

INACG (1977) has suggested that intervention goals may be expressed as a proportion of the population with haemoglobin concentration below a certain level or as an accepted frequency distribution of the haemoglobin concentration within the physiological group. Each country must set its own minimum standard because not all anaemias are the result of iron deficiency.

ICCIDD (Pandav et al. 1987) states that the goal of iodine fortification should be to reduce : (i) the prevalence of goitre to less than 10%; and (ii) the incidence of neo-natal hypothyroidism to less than 0.05% by a defined time period, by introducing programmes that will ensure the availability of minimum 150 mcg of iodine per person per day.

Because the monitoring and evaluation process tracks the extent to which goals are being met, periodic biochemical, clinical and dietary intake assays need to be undertaken and compared with the specific intermediate targets as set out by the programme designers. It is important to note the influence of externalities, which could affect the programme so that the interpretation of cause-effect relationship could be evaluated. As part of the monitoring and evaluation process, the critical point in the fortification process need to be renewed to ensure that, among other things, the fortified food contains (i) sufficient micro-nutrients to increase intake level and improve biological status; (ii) is an effective means of increasing intake; (iii) flows effectively through the proper marketing and distribution channels and (iv) benefits those most in need. Any ineffective or malfunctioning component needs to be identified and corrected (Nestel 1993).

Few fortification programmes implemented to date have undergone extensive monitoring and evaluation. Exceptions include the Guatemala sugar fortification programme (Arroyave et al. 1979; Mckingney 1992), the Philippines MSG programme (Popkin and Bisgrove 1980) and iron programmes in Kenya, Mexico and Indonesia (Levin 1986).

Mannar (1988) pointed out that most IDD programmes suffer from serious shortcomings in their effort to evaluate their progress impact. The main difficulties include poor definitions of methods for IDD control and surveillance, defective sampling in prevalence surveys, unspecified frequency for repeated evaluations, different classification systems for goitre, inadequate manpower and training and insufficient resources for carrying out control and evaluation activities. Furthermore, many programmes lack laboratory facilities and funds for checking the iodine levels in salt and evaluating the resultant impact on populations' health status. Similar shortcomings could no doubt be found in other fortification programmes.

Sustainability of any food fortification programme needs political, social, technical, financial and managerial efficiency.

Economic aspects of food fortification

Because of the lack of data in developing countries, it is difficult to measure and quantify the benefits of food fortification. Eliminating the

deficiencies can have major yet subtle social and economic benefits. The benefit of food fortification in this regard needs to be analyzed from multiple angles. Deficiencies of iodine and vitamin A strike at an early age and have direct impact on an individual's adult life, whereas iron deficiencies affect larger sections of population at later stage of life having an indirect impact. The low cost of fortification with vitamins, iron and iodine (as little as \$5–\$20 per DALY gained) and large potential health gain in populations where deficiencies of these three micronutrients are prevalent, food fortification may be one of the most cost-effective health interventions known. Many other interventions yield health improvements only at a cost of \$100 or more per DALY (Jamison 1993). The World Bank recommends that micronutrient interventions, including fortification, be included in an 'essential package' of public health and clinical services, which governments should ensure are available to the whole population and should subsidise for the poor. (World Bank 1993). In Zaire, a World Bank assisted project combats iodine deficiency through a national salt fortification programme. The World bank also assists the governments of China and Mali with iodine fortification. The Mali project is experimenting with a new technique that fortifies the rural water supply. Iodine cylinder is attached to the community water pump and released as the water flows. Tests have shown that iodine deficiency is eliminated in six months with no side effects and at a cost of US 10-20 cents per person (World Bank 1992).

Musgroev (1995) has stated that fortification is a significant response to the problems of poverty and ignorance. Poverty is most clearly relevant where it keeps people from eating micronutrient-rich foods, if they are expensive. In this case, fortification of cheaper foods is equivalent to a price reduction for the micronutrient-rich foods. Thus, iodized salt is, so far as iodine deficiency is concerned, equivalent to a reduction in price of seafood and iron fortification of flour is equivalent, with respect to iron deficiency anaemia, to a reduction in the price of meat. Fortification programmes almost invariably assume that people should be educated about the benefits of the fortified foods, since the creation of demand is the indispensable factor for success (World Bank 1994). Such education will counteract the risk of reduced demand because of incomplete or erroneous beliefs. Thus, food fortification is intimately linked to ignorance as a cause of malnutrition and it is often used as a means to reduce ignorance.

Fortification is not only cost-effective, but also cheap. Blum (1997) stated that the annual cost per person for some successful programmes on a commercial scale to be (i) <0.3 US\$ to fortify sugar with vitamin A in Guatemala; (ii) <0.1 US\$ to fortify cornflour/wheat flour with iron, vitamin A, thiamine, riboflavin and niacin in Venezuela; (iii) 0.2 US\$ to fortify margarine with vitamin A in the Philippines and (iv) <0.2 US\$ to fortify salt with iodine and iron in India.

Among the micronutrients, vitamin A, in particular, is expensive. While the cost of vitamin A, compounded with the cost of other nutrients as well as other programme components, may be considered expensive, the overall cost of vitamin A fortification can be relatively inexpensive, especially when compared with the consequence of vitamin A deficiency and the cost of other interventions. The cost of iron fortification varies depending upon the source of the iron, the percent of iron in the fortificant, its bioavailability and its functionality (colour and stability). It was later noted at XII INACG (1990) meeting that the costs of iron fortificants are significant and increasing rapidly. However, no data were presented as to how much iron fortificants contribute to overall fortification costs. Table 18 illustrates cost factors for various fortification programmes. As stated earlier, the present literature lacks the updated information and we need to re-evaluate the issues of economics pertaining to food fortification for more recent data.

General recommendations

FAO (1995) in its report of a technical meeting on Food fortification agreed upon the following general recommendations :

(i) Where foodstuffs cannot provide naturally occurring essential nutrients to population groups, the use of fortification should be given serious consideration as a means of achieving nutrition and health goals; (ii) The general population should be made aware of the benefits of food fortification and should be encouraged to pay for the same; (iii) A multi-sectoral approach must be adopted in the establishment of any food fortification programme, encompassing participation of relevant governmental organizations, food industry, trade organizations, consumers, academic and research facilities, marketing specialists and any involved international organizations and agencies; (iv) Efforts should

TABLE 18. COSTS OF SELECTED FORTIFICANTS

Vehicle	Fortificant	Dosage	Cost Range US$/caput/year	Country
Salt	Potassium iodate	50–80 ppm	0.03 - 0.05 (1990)	Several
Salt	Potassium iodate	20 ppm	0.12 - 0.15 (1990)	India
Sugar	Vitamin A	50,000 IU/kg	0.30 (1991)	Guatemala
Cooking fat	Vitamin A	50,000 IU/kg	0.30 - 0.40 (1990)	India
Wheat flour	Ferrous sulphate	29–44 mg/kg	1.5 (based on 430 g flour/person/day)	Egypt

Retinol palmitate (250 000 IU/g), potassium iodate and ascorbate, cost US$ 33/kg, US$ 30/kg and US$ 16/kg, respectively
Source : FAO/ILSI (1995)

continue to harmonize national legislation concerning fortified foods, with the international standards of the Codex Alimentarius; (v) International guidelines to advise food aid donors on acceptable and safe fortification practices should be developed; guidelines should not be so restrictive as to impede the provision of high quality food aid commodities or hinder communication on fortification between relevant parties; (vi) There should be appropriate fortification of foods used in food aid programmes, with donors being required to provide relevant nutritional information particularly through adequate labelling; (vii) Levels of fortification should be evaluated and adjusted according to bioavailability of the nutrient(s) in the diets of target populations; (viii) It is important to evaluate the potential of local food industries to become involved in the production of high quality fortified food products, including those destined for use in food aid programmes, in areas where problems of micronutrient deficiency are likely to occur; (ix) Food control systems based on HACCP principles, risk-based inspection procedures and internationally accepted analytical methods should be developed in support of fortification programmes; and (x) The impact of food fortification on the nutritional status of target populations should be monitored so that appropriate corrective action can take place as required.

Conclusion

The science and technological research thus far has not reached the weaker sections of society, who should be the real beneficiaries as far as food fortification is concerned. The concept of technology is enhanced only when it has the involvement of a common man and it is an effective tool to uplift his standard of living in every aspect of his life.

A problem as complex as micronutrient deficiency, the most prevalent nutritional disorders in the world, demands more comprehensive and long term strategies involving multi-sectoral participation. Yet, while programmes such as fortification of food can prevent these deficiencies in substantial segments of population and are feasible, they are rarely initiated in developing countries due to various barriers to technological applications. Instead, control programmes that tend to focus on short-term therapeutic strategies are favoured.

"No other technology offers as large an opportunity to improve lives... at such low cost and in such a short time" is the basis for World Bank's policy on "overcoming vitamin and mineral malnutrition in developing countries". The time is now ripe to use our knowledge, abilities and experiences to restructure our action plans towards our efforts in eliminating micronutrient malnutrition. This is possible only when there is a convergence of nutritionists, technologists, and policy makers in the struggle to conquer the battle of micronutrient malnutrition.

References

Anon (1983) Use of iron fortified salt to combat anaemia: The Indian experience. Nutr Rev 41(10): 302-303

Arroyave G (1972) Distribution of vitamin A to population groups. In: Proceedings of the Western Hemisphere Nutrition Congress III. Futura, New York

Arroyave G, Dary O (1996) Manual for sugar fortification with vitamin A. Part 1. USAID/INCAP

Arroyave G, Aguilar JR, Flores M, Guzman MA (1979) Evaluation of sugar fortification with vitamin A at the national level. PAHO, Washington DC

Arroyave, G, Mejia LA, Aguilar JR (1981) The effect of vitamin A fortification of sugar on the serum levels of preschool Guatemalan children : A longitudinal evaluation. Am J Clin Nutr 34: 41-49

Arroyave G (1992) Food nutrition with emphasis on the addition of micronutrients to wheat flour. VITAL/ISTI, Washington DC

Bauernfeind, JC (1991) Foods considered for nutrient addition: Condiments. In : Bauernfeind JC, Lachance PA (eds) Nutrient Additions to Foods. Food and Nutrition Press, Trumbell CT

Bauernfeind, JC, Arroyave G (1986) Control of vitamin A deficiency by the nutrification approach In: Bauernfeind JC (ed) Nutrient Additions to Foods. Academic Press, London

Blum M (1997) Status paper: Food fortification - a key strategy to end micronutrient malnutrition. Nutriview/special issue

Borenstein B (1974) Enrichment of wheat products. In: Inglett GE (ed) Wheat Production and Utilization, AVI Publication, Westport, Conn

Borenstein B, Bendich A, Waysek EH (1988) Vitamin bioavailability in fortified foods. Food Technol 42(10): 226-228

Brook CL, Cort WM (1972) Vitamin A fortification of tea. Food Technol 26(6): 50-52

CIH/EUSPH (1992) PAMM country training in food fortification, social marketing/communication laboratory management. Republic of the Philippines, Center for International Health/ Emory University School of Public Health, Atlanta, Georgia, USA

Clydesdale FM (1991) Mineral additives. In: Bauernfeind JC, Lanchance PA (eds) Nutrient Additions to Food. Food and Nutrition Press, Trumbell CT

Cook JD, Reussr ME (1983) Iron fortification: an update. Am J Clin Nut 38: 648-659

Cort WM, Borenstein B, Harley JH, Oscada M, Scheiner J (1976) Nutrient stability of fortified cereal products. Food Technol 30(4): 52-56

Crowley PR, Barrett FF, Weil RP, Fellers DA, Blocker NM (1989) Final Report : Food technology for development project. OICD/USDA, Washington DC

Darton-Hill I (1988) Vitamin A fortification of wheat : Bangladesh experiences. Helen Kellen International, New York

Dary M (1991) Sugar fortification with vitamin A in Guatemala. XXIII IVACG Meeting, Ecuador

Disler PB, Lynch SR, Charlton RW, Bothwell TH, Walter RB, Mayet F (1975) Studies on the fortification of cane sugar with iron and ascorbic acid. Brit J Nutr 14: 141-152

Emodi A, Scialpi L (1976) Rice fortification for the Dominican Republic. In: Nestel P (ed) Food Fortification in Developing Countries. USAID/VITAL. Unpublished report. Hoffman-La Roche, New Jersey

FAO (1995) Food Fortification : Technology and Quality Control. Report on an FAO Technical Meeting, Rome, Italy. Food and Nutrition Paper 60

FAO/ILSI (1997) Preventing micronutrient malnutrition : A guide to food-based approaches : A manual for policy makers and program planners. International Life Sciences Institute Press, Washington DC

FAO/WHO (1992) International Conference on Nutrition, World Declaration and Plan of Action (December). Food and Agriculture Organization, Rome

Favaro R, Ferreira JF, Desai ID, Dutra de Oloveira JE (1990) Studies on the fortification of refined soybean oil with all-trans-retinyl palmitate in Brazil : stability during cooking and storage. University of San Paulo Medical School and University of British Columbia

Finch CA, Cook JD (1984) Iron deficiency. Am J Clin Nut 39: 471-477

Florentino RF, Pedro MR (1990) Rice fortification in the Philippines. In : Combating Iron Deficiency Anaemia through Food Fortification. INACG, Washington DC

Frazer JC (1935) The golden bough : The magic art and the evolution of King's. MacMillan, New York

Fuller CE, Nelson JH, Smith DE, Crowley PR, Swanson JL, Wells FB (1974) A technology for the fortification of tea with vitamin A : Proceedings of IV Int. Congress on Food Sci Technol V : 180-189

Garby L, Areekul S (1973) Iron supplementation in Thai Fish sauce. Annals Trop Med Parasit 24, 68(4): 467-476

Gillespie S, Mason J (1994) Controlling vitamin A deficiency. State-of-the-Art Series. Nutrition Policy Discussion Paper No. 14. ACC/SCN, Geneva

Hathcock JN (1985) Quantitative evaluation of vitamin safety. Pharma Times 51(5): 104-113

Indian Council of Medical Research (1994) Nutrient requirements and recommended dietary allowances for Indians. National Institute of Nutrition, Hyderabad

Iodine Deficiency Disorders Newsletter (1994) Universal Salt Iodization - The mid decade goal. 10(1) International Council for Control of Iodine Deficiency Disorders, Charlottesville, VA

International Nutritional Anaemia Consultative Group (1977) Guidelines for the eradication of iron deficiency anaemia. Washington DC

International Nutritional Anaemia Consultative Group (1990) Combating iron deficiency anaemia through food fortification technology. Washington DC

International Vitamin A Consultative Group (1988a) The safe use of vitamin A. Washington DC

International Vitamin A Consultative Group (1988b) Guidelines for the eradication of vitamin A deficiency and xeropthalmia. Washington DC

Jamison DT (1993) Disease control priorities in developing countries : An overview. In: Jamison DT, Mosley WH, Measham AR, Bobadilla JL (eds) Disease Control Priorities in Developing Counties. Oxford University Press for the World Bank, New York

Keith PW (1997) Vitamin A deficiency : health consequences and dietary solutions. Sight and Life Newsletter 3:11

King KW, Sebrell EL, Severingbaus, Storvick WO (1963) Lysine fortification of bread fed to Haitian school children. Am J Clin Nutr 12:36

Lamparelli RD, MacPhail AP, Bothwell TH, Ballot D, Danilewitz MD, MacFarlane BJ, Mayet F, Baynes RD (1987) Curry powder as a vehicle for iron fortification : effects on iron absorption. Am J Clin Nut 46: 335-340

Lan-Ing L, Parrish DB (1979) Biopotency of vitamin A in fortified flour and accelerated storage. J Agri Food Chem 27: 1134

Layrisse GMC, Martinez T, Reins M (1976) Sugar as a vehicle for iron fortification : further studies. Am J Clin Nutr 29: 274-279

Levin HMM (1986) A benefit-cost analysis of nutritional programs for anaemia reduction. Research Observer (World Bank) 1(2): 219-245

Mannar V (1988) Salt iodination. Part 2: Iodination Technique. IDD Newsletter 4(4): 11-16

Mannar V (1990) Fortification of salt with iron - a status report. In: Combating Iron Deficiency Anemia through Food Fortification technology. INACG, Washington DC

Marks J (1989) Vitamin safety : Status paper. Revised 4th edn, Hoffman La Roche & Co Basel, Switzerland

Mathew AE, Mohanti JP, Behera UC, Sivakumar B, Nair MK (1999) Why food fortification ? Nutrition 33(1): 12-22

McKigney JI (1983) Interventions for the prevention of vitamin A deficiency : A summary of experiences. In: Nutrition Intervention Strategies in National Development. Academic Press, New York

McKingney JI, (1992) Evaluation of interventions for the control of vitamin A deficiency in Guatemala. VITAL/ISTL, Washington DC

Mejia, LA, Arroyave G (1982) The effect of vitamin A fortification of sugar on iron metabolism in preschool children in Guatemala. Am J Clin Nut 36: 87-93

Ministry of Health and Family Welfare, Government of India/ UNICEF (1994) Report on Advocacy Policy Meeting on Iodine Deficiency Disorders. pp 5-6

Ministry of Industry, Salt Department/UNICEF (1994) Banning sale of edible non-iodized salt: An urgent measure. No 12

Molina MR (1991) Foods considered for nutrient additions: Sugar. In: Bauernfeind JC, Lachance PA (eds) Nutrient Additions to Foods. Food and Nutrition Press, Trumbell CT

Mora JO (1995) Issues in food fortification : Lesson from Latin America. Nutriview 4: 1-4

Muhilal D, Permeisih I, Azis, Saidin S, Jahari AB, Karyadi D (1988a) Vitamin A fortified monosodium glutamate and vitamin A status: a controlled field trial. Am J Clin Nut 48: 1265-1270

Muhilal D, Permeisih, Idjradinata YR, Muherdiyantinigsih, and Karyadi D (1988b) Vitamin A fortified monosodium glutamate and health, growth, and survival of children: a controlled field trial. Am J Clin Nut 48: 1271-1276

Murphy P, Hsu KW, Fratzke A, Hauck C (1987) Fortification of monosodium glutamate with "white" vitamin A in Indonesia. Dept. Food Technology. Iowa State University, USA

Murphy PB, Smith, Hauck C, O'Connor K (1992) Stabilization of vitamin A in synthetic rice premix. J Food Sci 57(2): 437-439

Musgrove P (1995) Economic aspects of food fortification. Nutriview 3: 1-5

Nadiger HA, Krishnamachari K, Naidu N, Rao BSN, Srikantia SG (1980) The use of common salt (sodium chloride) fortified with iron to control anemia: results of preliminary study. Brit J Nutr 43: 45-51

Nagy K (1982) Coloration and Vitaminization of Instant Beverage Powders. Haffman La Roche, Switzerland

Nilson A (1994) Staple food fortification. Nutriview 4: 1-6

National Institute of Nutrition Annual Report (1969) Fortification of foods. Indian Council of Medical Research, Hyderabad. pp 12-26

National Institute of Nutrition annual report (1970) Fortification of foods. Indian Council of Medical Research Hyderabad, pp 15-28

National Institute of Nutrition Annual Report (1977) A community trial with iron-fortified salt. Indian Council of Medical Research, Hyderabad

OMNI/Roche/USAID (1997) Fortification basics : Wheat flour. Nutriview Supplement 97/2

Orr E (1972) The use of protein-rich foods for relief of malnutrition in developing counties and analysis of experiences. Tropical Products Institute, London

Pandav CS, Carriere RC, Kochupillai N, Ma T, Nath LM (1987) The planning and management of IDD control programs. In: Hetzel BS, Dunn JT, Stanbury JB (eds) The Prevention Control of Iodine Deficiency disorders. Elsevier Press, Amsterdam

Pelletier O, Keith MO (1974) Bioavailability of synthetic and natural ascorbic acid. J Am Diet Assoc 64: 271

Peter U (1994) The state of world hunger. Nutr Rev 52(5): 151-161

Popkin BM, Bisgrove EZ (1988) Urbanization and nutrition in low-income countries. Food and Nut Bull 10(1): 3-23

Prabha S, Bagchi K (1978) Effect of lysine-fortified wheat on the nutritional status of children. In: Abrol YP, Pingle SV (ed) Wheat in the Indian diet: Proceedings of a Seminar. Roller Flour Miller's Federation of India, New Delhi. pp 25-27

Rao BSN, Vijayasarathy C (1975) Fortification of common salt with iron: effect of chemical additives on stability and bioavailability. Am J Clin Nutr 28: 1395-1401

Rao BSN (1978) Studies on iron deficiency anaemia. Ind J Med Res 68 : 58-69

Reiutlinger S (1993) Addressing hunger : An historical perspective of international initiatives. Washington DC : Background paper prepared for the Conference on overcoming Global Hunger, World Bank, Nov 29 - Dec 1

Richardson DP (1983) Food fortification. In: Ottaway PB (ed) The technology of vitamins in foods. Champan and Hall Press, NZ

Ristow KA, Gregory JF, Damron BL (1982) Thermal processing effects on folacin bioavailability in liquid model systems, liver and cabbage. J Agri Food Chem 30: 801

Rubin SH, Emodi A, Scialpi L (1977) Micronutrient additions to cereal grain products. Cereal Chem 54(4) 4895-904

Salt Commissioner's Report (1994) In: UNICEF. The Progress of Indian States : League Table

Scialpi LJ (1987) Process for preparing fat-soluble vitamin active beadlets. US patent 4 670 247

Solon FS, Latham MC, Florentino R, Williamson DF, Aguilar J (1985) Fortificaiton of MSG with vitamin A : The Philippines experience. Food Technol 39(11) : 71-77

Subrahmanyan V (1954) Investigation on rice substitute. CFTRI Bulletin 3(4): 180-183

Subrahmanyan V, Rao GR, Kuppuswamy S, Rao MN, Swaminathan M (1957) Indian multi-purpose food. Food Science 6(4): 76-80

UNICEF field reports (1997) In: Draft 1997. Progress in Controlling Vitamin A deficiency p 30

UNICEF (1995 a) The progress of Indian States : League Tables

UNICEF (1995 b) Universal Salt Iodization : Update - Vol 3

Walter T (1990) Combating iron deficiency : Chile : A case study. In: Combating Iron Deficiency Anemia through Food Fortification technology. INACG, Washington DC

WHO (1982) Control of vitamin A deficiency and xeropthalmia. Technical Report Series No 672, Geneva

WHO (1998) Life in the 21st century: A vision for all. World Health Organization, Geneva

WHO/UNICEF/ICCIDD (1994) Indicators for assessing iodine deficiency disorders and their control through salt iodization. WHO/NUT/94.6. WHO, Geneva

Wilson LA (1988) Proposed quality assurance guidelines for the fortification of MSG with white vitamin A in Indonesia project. Iowa State University, Department of Food Technology, Ames, Iowa

World Bank (1992) Investing in nutrition with World Bank assistance, World Bank, Washington DC

World Bank (1993) World development reports : Investing in health - world development indicators. World Bank, Oxford University Press

World Bank (1994) Enriching lives : Overcoming vitamin and mineral malnutrition in developing countries. Washington, DC

Zoller JM, Wolinsky I, Paden CA, Hoskin JC, Lewis KC, Lineback DR, McCarthy RD (1980) Fortification of non-staple food items with iron. Food Technol 34(1): 38-47

Received 13 August 1999; revised 30 August 1999; accepted 2 September 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 396-401

Determination of Thermal Process Schedules for Canned Gourds

L. SAIKIA* AND S. RANGANNA

International Food Technology Training Centre,
Central Food Technological Research Institute, Mysore - 570 013, India.

Thermal resistance studies carried out on *Clostridium sporogenes* (PA-3679, ATCC-7955) in canned brine gave two D and z values based on spore concentration of ~ 10^3 and ~10^4 tube^{-1}. Two methods of calculation of D-values were used. Five procedures for calculation of F-values and corresponding F_0 and BB by improved graphical and formula methods were done. When spore concentration was less (10^3 tube^{-1}), D values increased considerably. After checking with inoculated pack studies, Pt (actual processing time) recommended for commercial processing (canning) of bitter gourd, pointed gourd and ivy gourd were 27.0 min, 19.0 min and 16.0 min, respectively with corresponding F_0 values of 4.3 min, 4.5 min and 3.7 min, respectively at 115.6°C (240°F). F_0 values and the corresponding BB were found to be the highest values.

Keywords: Thermal processing, Thermal resistance, *Clostridium sporogenes*, Canning, Low-acid foods, Commercial sterilization, D-Value, F-value, Process time (BB), Actual Process time (Pt), Gourds.

Gourds are extensively grown all over the world (Warne and McCollum 1980. Pierce 1987). Gourds are generally consumed as a fresh vegetable. However, these are processed to candy and dehydrated forms to a limited extent (Siddique et al. 1990). Limited quantities of gourds are canned in India for export and also supplied to the Defence Forces. However, thermal process schedules followed for canning are empirical or based on experience gained in processing similar products, which often lead to over-processing, thereby causing severe losses of nutrients.

Low-acid foods having pH≥4.6 require processing at 115.6°C or above to inactivate *Clostridum botulinum*, which produces a potent exotoxin, the consumption of which may prove fatal (Ranganna 1986). Therefore, in fruits and some vegetables of tender nature, when the pH is ≥4.6, it is acidified to counter the risk of growing *C. botulinum* (Nath and Ranganna 1977, 1980). Random acidification is done in a number of canning industries in India to avoid this problem. Importers and consumers do not prefer acidified products, as these affect quality and palatability. Moreover, according to recent FDA regulations, the exporters of the canned products have to supply detailed process data with their consignments, which are meant for USA or European countries. The pH values of the fresh gourds vary from 4.6 to 5.8, but decrease slightly on canning (4.10 to 4.80). Hence, they are required to be processed as low-acid foods. *Clostridium sporogenes* (PA-367) is generally used as test organism in evolving thermal process as it is more heat resistant than *C. botulinum.* Process calculation and design of thermal processes for low-acid foods are usually done by the mathematical method of Ball (1923) rather than general method of Bigelow et al (1920) or improved graphical method of Schultz and Olson (1940). In the Ball (1923) method, single value of 10°C and jc of 1.41 was used, but the actual z and jc values were found to vary. Kao et al (1981) developed a mathematical process calculation method for different z and jc values using a hand model calculator. Recently, Saikia and Ranganna (1991, 1992, 1994) have reported that inactivation of bacterial spore is not logarithmic, and depending on spore concentration used, there were two D and z-values.

Not much work has been done on the development of a minimum thermal process schedule for gourds, to help indigenous and export-oriented canning industries, Hence, the present study was undertaken to develop safe thermal processes for bitter gourd (*Momordica charantia* Linn), Ivy gourd (*Coccinia cordifolia*) and pointed gourd (*Trichosanthes dioica*).

Materials and Methods

Preparation of vegetables : The tips of the gourds (ivy and pointed gourd) were trimmed. Bitter gourds were cut into pieces. The prepared material was blanched (3 min) and used for canning (A_1 Tall cans).

Determination of D-values : This was done following the method of Saikia and Ranganna (1992) using spore crop of *C. sporogenes* (PA-3679) and thermal death time (TDT) tube technique. The D values were calculated by the fraction negative

* *Corresponding Author; Present Address: Department of Horticulture, Assam Agricultural University, Jorhat-785013, India.*

method of Stumbo (1948) (termed D_1) and Stumbo et al (1950) (termed D_2).

Heat penetration studies were done according to the method of Saikia and Ranganna (1991). Heat penetration rates into the cans were measured using Ecklund non-projecting, plug-in, needle type thermocouple and manually operated Leeds and Northrup Potentiometer. Inoculated pack studies were done following the method of Saikia and Ranganna (1992) using 1 ml of inoculum containing $\sim 10^3$ or $\sim 10^4$ spores of *C. sporogenes* for each can after exhausting. Calculation of process time (BB), actual process time (Pt) by improved graphical and formula method of procedures A,B,C,D and E were done as described by Saikia and Ranganna (1991, 1994).

Results and Discussion

Table 1 shows the proximate composition of gourds and Table 2 the pH values. In canning, the most important biochemical characteristics of the food are their pH values, which influence the activity of heat resistance of enzymes and microogranisms (Schoeneman and Lopez 1973). The pH of the fresh gourds ranged from 4.6 to 5.8, which decreased with canning to 4.1 to 4.8. Decrease in pH due to canning was also reported by Pflug and Esselen (1956).

Thermal resistance : Heat resistance of *C. sporogenes* in vegetable and neutral phosphate buffer was expressed in terms of D_1 and D_2 values of Stumbo (1948) and Stumbo et al (1950), respectively. Correction for heating lag for the calculation of D_1 and D_2 values had significant effect on the magnitude of D values. The maximum difference between the graphical and formula methods of calculation of lethality contributed by the come-up time (Cut) in the determination of heat resistance was found to be 9.2 sec in bitter gourd brine and hence formula method was found to be better for application. Come-up time ranged from 1.75 to 4.10 min of which 33.02% had lethal effect. The lethality of come-up was almost the same as average value of 34% reported by Alstrand and Benzamin (1949).

Thermal resistance values in terms of D values (D_1 and D_2) for A ($\sim 10^4$ spores tube^{-1}) and B ($\sim 10^3$ spore tube^{-1}) concentrations of spores are shown in Table 3. The D_2 values ranged from 0.39 to 0.74 min and 0.69 to 1.03 min for A and B concentrations of spores, tube^{-1}, respectively. The average D_2 values for A and B concentrations were 0.54±0.10 and 0.85±0.12 min, respectively. Irrespective of method of calculation, D values found using spore concentration of B was 47.2% higher than D values for A concentration of spores for bitter gourd (Table 3 and Fig 1). Three-fold increase in the heat resistance was also reported by Berry and Bradshaw (1982), when bacterial population was reduced to 10^3 spores in mushrooms. The method of Stumbo

TABLE 1. PROXIMATE COMPOSITION OF GOURDS

Components	Bitter gourd	Ivy gourd	pointed gourd
Edible portion, g %	93.00	96.00	95.00
Moisture, g %	83.20	93.50	92.00
Protein, g %	2.10	1.20	2.00
Fat, g %	1.00	0.10	0.30
Minerals, g %	1.40	0.50	0.50
Fibre, g %	1.70	1.60	3.00
Carbohydrates, g %	10.60	3.10	2.20
Energy, Kcal	60.00	18.00	20.00
Calcium, mg %	23.00	40.00	30.00
Phosphorus, mg %	38.00	30.00	40.00
Iron, Mg %	2.00	1.40	1.70
Carotene, µg %	126.00	156.00	153.00
Thiamine, mg %	0.07	0.07	0.05
Riboflavin, mg %	0.06	0.08	0.06
Niacin, mg %	0.04	0.70	0.50
Vitamin C, mg %	96.00	15.00	29.00

TABLE 2. pH OF GOURD

Vegetable	Fresh	Canned: Laboratory	Canned: Factory
Bitter gourd	5.8	4.14	4.10
Ivy gourd	4.6	4.60	4.50
Pointed gourd	5.6	4.80	4.60

TABLE 3. CORRECTED D-VALUE AT 121.1°C AND Z VALUE OF *C. SPOROGENES* FOR GOURDS IN BRINE

Medium	Spores per tube	Calculation procedure	D, min	Z °F	r
Bitter gourd	A: 11200	D_1	0.70	17.85	0.9980
	A: 11200	D_2	0.74	17.95	0.9984
	B: 1380	D_1	1.03	18.62	0.9992
	B: 1380	D_2	1.09	18.64	0.9988
Ivy gourd	A: 11200	D_1	0.38	15.58	0.9978
	A: 11200	D_2	0.39	15.53	0.9977
	B: 1185	D_1	0.66	16.80	0.9975
	B: 1185	D_2	0.69	16.92	0.9974
Pointed gourd	A: 11200	D_1	0.48	15.62	0.9736
	A: 11200	D_2	0.50	15.79	0.9731
	B: 1280	D_1	0.76	16.52	0.9951
	B: 1280	D_2	0.78	16.43	0.9950

A : represents TDT tube containing $\sim 10^4$ spores, tube^{-1}

B : represents TDT tube containing $\sim 10^3$ spores, tube^{-1}

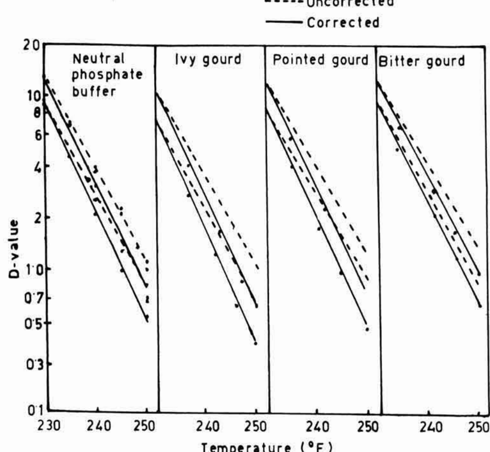


Fig. 1. Thermal resistance curve of *Clostridium sporogenes* in neutral phosphate buffer (NPB) and in gourds

et al (1950) (D_2) had given better safety margin for processing than the method of Stumbo (1948) (D_1).

z-values: Initially, process calculations by formula methods were done using single z value (assumed) of 18°F or 10°C (Ball 1923). In practical study, z-value was not found to be 18°F and it may be more than or less than 18°F (10°C), which greatly affects the process values, complicating practical applications by the canning industries. In the present study, the z-values obtained were less than or more than 18°F (Table 3). Higher z-values were found invariably for D_1 and D_2 values when spore concentrations used were ~10^3 $2ml^{-1}$ (i.e., 1380 spores $tube^{-1}$). It was highest (for D_1, 18.62°F; D_2 18.64°F) for bitter gourd. Similar was the case for ivy gourd and pointed gourd (Table 3). When spore concentration was ~10^4 $2ml^{-1}$ (11200 $tube^{-1}$), z values found for bitter gourd were lower (D_1, 17.85°F; D_2 17.95°F). Similar was the case for other gourds. It is evident from data (Table 3) that spore concentration has significant effect on z values as in the case of D values. Generally, z values found using ~10^3 spore $tube^{-1}$ were higher by 5.48% than those found using ~10^4 spores $tube^{-1}$. D_1 and D_2 methods of calculation made no significant difference in the z values.

Calculation of process time (BB): There are two methods of calculation of process schedule i.e., the general methods and the formula methods.

Improved graphical method : The lethality (L) in the improved graphical method (IGM) is calculated using the following expression.

$$L = \frac{1}{\log^{-1} \frac{250-T}{z}}$$

where L is the lethal rate time at 121°C (250°F), T is any lethal rate temperature in °C or °F and z, the number of °C or °F required for the slope of the TDT curve to traverse on log cycle was used for the present study for its most accuracy.

Using D_2 and experimental z values : Fig. 2 shows the heat penetration pattern into canned bitter gourd and the lethality curve corresponding to experimental z values of 17.95°F. Similar lethality curves were drawn for z value of 18.64°F and the classical value of 18°F. After drawing graphical interpolation curves, conversion of F_0 value corresponding to experimental z value to F_0 value. or vice versa was done as described by Saikia and Ranganna (1992).

Single segment approach : Pt required at 115.6°C (240°F) to achieve an F_0 value of 3.0 min was 23.5 (Procedure 1, Table 4) Five D of A corresponded to F_0 of 3.8 min which was equivalent to Pt of 26.3 min, (Procedure 2). 5D of B was equivalent to an F_0 of 5.2 min and Pt of 30.5 min

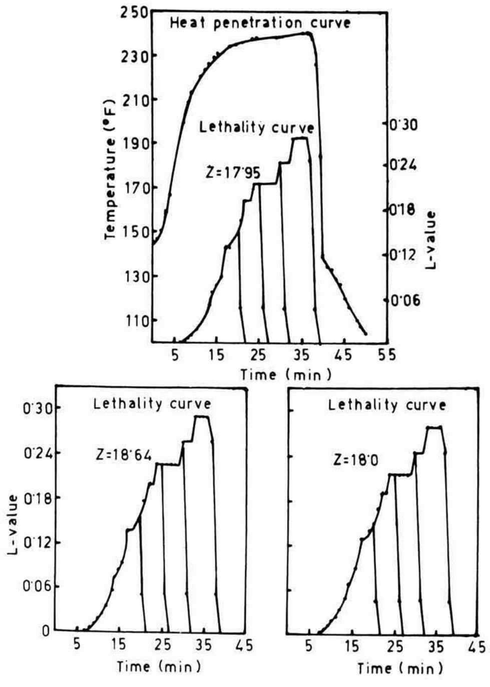


Fig. 2. Heat penetration pattern into canned bitter gourd and lethality curve corresponding to experimental z value of 17.95°F, 18.64°F and 18°F

TABLE 4. STERILIZATION VALUE (F_0) AND ACTUAL PROCESS TIME (Pt) AT 115.6°C FOR GOURDS IN BRINE (CANNED IN A_1 TALL CANS)

Particulars of calculation of F_0 value required	Improved graphical method: Using D_1 values, Using expt Z		Using D_2 values: Using exptl Z		Using D_2 values: Assuming Z as 10°C		Formula method: Using D_2 values and exptl. Z values, Eye fit:Process A		Linear fig:Proc. E	
	F_0, min	Pt, min	F_0, min	Pt, min	F_0, min	Pt, min	F_0, min	Pt, min	F_0, min	Pt, min
Bitter gourd										
F_0= 3.0			3.0	23.5	3.0	23.5	-	23.6	-	23.6
F = 5D of A	3.6	25.5	3.8	26.3	3.8	26.0	3.7	26.1	3.7	26.4
F = D of A (log a -3.0) + D of B (3-log bx)	3.7	25.7	3.7	25.7	3.7	26.0	3.6	25.5	3.6	25.8
F = D of A (log a-log ax) + D of B (log a -log 1.0)	4.5	28.5	4.7	29.1	4.9	29.8	4.7	29.7	4.7	30.1
F = 5D of B	4.9	29.7	5.2	30.5	5.1	30.5	5.2	31.4	5.2	31.6
Ivy gourd										
F_0= 3.0					3.0	13.5	-	14.5	-	14.5
F = 5D of A	2.5	11.5	2.5	11.5	1.9	9.5	2.5	12.5	2.5	12.4
F = D of A (log a -3.0) + D of B (3-log bx)	2.5	11.5	2.5	11.5	2.4	11.3	2.7	13.5	2.7	13.4
F = D of A (log a-log ax) + D of B (log a -log 1.0)	3.3	14.4	3.3	13.5	3.1	13.8	3.4	14.0	3.4	15.9
F = 5D of B	3.7	16.0	3.7	16.0	3.5	15.3	3.8	17.4	3.8	17.3
Pointed gourd										
F_0= 3.0					3.0	13.8	-	14.5	-	14.7
F = 5D of A	3.5	15.5	3.1	14.0	2.5	12.0	3.1	14.8	3.1	15.0
F = D of A (log a -3.0) + D of B (3-log bx)	3.7	16.0	3.8	16.5	3.2	14.5	3.6	17.3	3.8	17.5
F = D of A--- (log a-log ax) + D of B (log a -log 1.0)	4.0	17.0	4.0	17.0	3.5	15.0	4.1	18.3	4.1	18.5
F = 5D of B	4.4	18.5	4.5	19.0	3.9	17.0	4.5	19.8	4.5	20.0

* a refers to number of spores corresponding to A tube-1 (~10^4) multiplied by number of tubes, usually 6

b refers to number of survivors after heating; bx refers to number of survivors after heating tubes containing spores containing spores concentration corresponding to B (i.e., 10^3)

(Procedure 5). These represent the minimum and maximum requirements for processing.

Two segment approach Vs single segment approach : The two segment approach of Berry and Bradshaw (1982) gave F_0 value of 3.7 min and Pt of 25.7 min (Procedure 3). Procedure 4 which intended to reduce the spore concentration to less than one resulted in values closer to 5D of B, which was next to the maximum.

Considering z as 18°F (10°C) instead of experimental value : The z value found using spore concentration of A was 17.95°F and hence, use of 18°C in the calculation did not make any significant difference in the F_0 and Pt values. On the contrary, the z value found using spore concentration of B was 18.64°F. The F_0 corresponding to 5D of B and experimental z value of 18.64°F was 5.2 min but would be 5.1 min and Pt value of 30.5 min. Pt values of 23.5 min and 30.5 min were found to be minimum and maximum, respectively.

Using D_1 and experimental z values : F_0 values calculated using D_1 values and experimental z values as compared to D_2 values were either same or lower by 0.1 to 0.3 min. and Pt values were either the same or lower by 0.5 to 0.8.

Process calculation by formula method : Semilog plot of heat penetration data, except during initial lag yielded a straight line (Fig.3), Table 5 shows the heat penetration parameters. Slight difference in heat penetration parameters between statistical and eye fit curves made no significant difference in the Pt (Procedure A as compared to Procedure E). The variation in the value found by procedures B, C and D were insignificant. The F_0 and Pt values found using experimental z or classical z value of 18°F either by single or two segment procedures were similar to graphical method. Procedures requiring interconversion also did not make any marked difference.

F values for ivy gourd and pointed gourd : In ivy gourd, the F_0 value for 5 D of A and two segment approach using D_2 and experimental z

TABLE 5. HEAT PENETRATION PARAMETERS OF GOURDS

Vegetable	Curve fitting	RT °C	IT °C	Cut min	jh	fh min	jc	Cwt °C
Bitter gourd	Eye fit	115.6	62.2	6	0.83	13.10	1.23	30.0
	Linear fit	115.6	62.2	6	0.71	14.20	1.17	30.0
Ivy gourd	Eye fit	115.6	67.8	7	1.19	5.10	1.25	30.0
	Linear fit	115.6	67.8	7	1.03	5.25	1.14	30.0
Pointed gourd	Eye fit	115.6	60.0	7	0.52	6.50	1.00	29.4
	Linear fit	115.6	60.0	7	0.58	6.38	1.00	29.4

RT, Retort Temperature; IT, Initial temperature of the can; Cut, Come-up time of the Retort; jh and jc, heating and cooling parameters; Cwtf, cooling water temperature.

value were lower than the mandatory requirement of 3.0 min (Table 4), as the D and z values were low, which may be attributed to the pH in the region of 4.6 (Pflug and Odlaug 1978). 5D of B (Procedure 5), which resulted in F_0 value of 3.7 min with corresponding Pt value of 16.0 min by IGM method represents the ideal processing time. In formula method (Table 4), it corresponded to F_0 of 3.8 min, with Pt value of 17.4 min, which was close to IGM value.

In pointed gourd (Parval), the F_0 and Pt values calculated with D_2 and D_1 values corresponding to A and B concentration of spores by IGM or formula method and other procedures were lower than the bitter gourd but higher than the ivy gourd. An F_0 value of 4.5 min with corresponding Pt value of 19.0 min may be recommended for canning of pointed gourd.

Inoculated pack studies: The results obtained were confirmed by conducting inoculated pack studies according to procedure reported by Saikia and Ranganna (1994). Filled A_1 Tall cans were inoculated with 1000 of 10,000 spores of *C. sporogenes*, processed and stored for 90 days. After the experiment, Pt values of 27.0 min, 19.0 min and 16.0 min with corresponding F_0 values of 4.3, 4.5 and 3.7 min were found adequate for canning of bitter gourd, pointed gourd and ivy gourd.

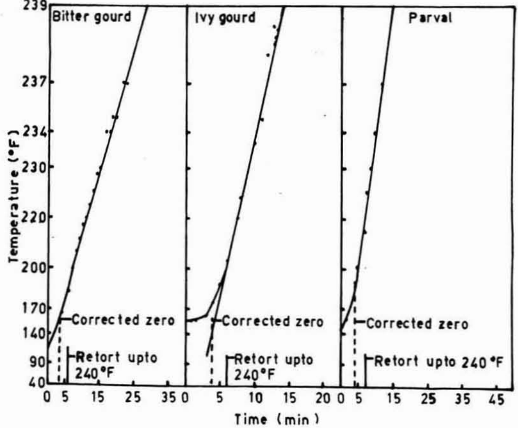


Fig. 3. Semilog plot of heat penetration data of gourds

Acknowledgements

The authors thank Dr. B.L. Amla, then Director, CFTRI, for keen interest in this investigation and M/s Vasundhara Canning Pvt. Ltd., Pardi, Gujarat, India for offering technical assistance. The senior research fellowship to the first author by the Indian Council of Agricultural Research, New Delhi is gratefully acknowledged.

References

Alstrand DV, Benzamin HA (1949) Thermal processing of canned foods in tin containers V. Effect of retorting procedures on sterilization values in canned foods. Food Res 14:253-260

Ball CO (1923) Thermal process time for canned foods. Natl Res Council Bull, Part I, 7(37):1-76, National Research Council, Washington DC

Berry Jr MR, Bradshaw Jr JG (1982) Heat penetration for sliced mushrooms in brine, processed in still and agitating retorts, with comparison to spore count reduction. J Food Sci 47:1698-1704

Bigelow WD, Bohart GS, Richardson AC, Ball CO (1920) In: Goldblith SA, Joslyn MA, Nickerson JTR (eds) An Introduction to Thermal Processing of Foods (1961), AVI Publishing Co, Westport. p 653

Kao J, Naveh D, Kopleman IJ, Pflug IJ (1981) Thermal process calculation for different Z and jc values, using a hand held calculator. J Food Sci 52:1084-1088

Nath N, Ranganna S (1977) Evaluation of thermal process for acidified canned muskmelon (*Cucumis melo*) J Food Sci 42:1306-1310

Nath N, Ranganna S (1980) Determination of thermal process schedule for acidified papaya. J Food Technol 15:251-256

Pflug IJ, Esselen WB (1954) Observation on the thermal resistance of putrefactive anaerobe 3679 in food substrates. Food Res 17:153-167

Ranganna S (1986) Hand Book of Analysis and Quality Control for Fruits and Vegetable Products, Tata McGraw-Hill Pub Co Ltd., New Delhi, p 716

Saikia L, Ranganna S (1991) Determination of thermal processes for curried brinjal (*Solanum melongena*). Indian Food Packer 45:37-48

Saikia L, Ranganna S (1992) Determination of thermal process schedules for canned drumstick, Okra, elephant yarn and potato. J Food Sci Technol 29:203-209

Saikia L, Ranganna S (1994) Determination of thermal process schedules for beans. Indian Food Packer 68(I):17-37

Schultz CT, Olson FCW (1940) Thermal processing of canned foods in tin containers. III. Recent improvement in the general method of process calculations on a special coordinate paper and methods of converting initial and retort temperatures. Food Res 5:399

Schoeneman DR, Lopez A (1973) Heat processing effects on physical and chemical characteristics of acidified canned tomatoes. J Food Sci 38:195-201

Siddique MI, Ahmed M, Awan JA, Rehman S, Ahmed A (1990) Production of wax gourd candy by using high sucrose syrup. J Food Sci Technol 27:205-208

Stumbo CR (1948) A technique for studying resistance of bacterial spores to temperatures in the higher range. Food Technol 2:228-240

Stumbo CR, Murphy JR, Cochran J (1950) Nature of thermal death time curves for PA-3679 and *Clostridum botulinum.* Food Technol 3:320-326

Warne GW, MeCollum JP (1980) In: Producing Vegetable Crops. The Interstate Printers and Publishers Inc, Illinois, p 313

Received 5 March 1998; revised 28 April 1999; accepted 9 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 402-407

Flow Properties and Physico-chemical Characteristics of 'Perlette' Grape Juice Concentrates

S. TEJINDER*, S. SUKHVINDER, K. BHUPINDER, K. HARINDER AND S.P.S. SAINI
Department of Food Science and Technology,
Punjab Agricultural University, Ludhiana - 141 004, India.

Flow properties and physico-chemical characteristics of concentrates of 'Perlette' grape juices extracted by cold-, hot-and pectolytic enzyme-break were investigated. Flow behaviour was studied at 10° to 50° C over the shear rates of 0.6-145.8 s^{-1}. The rheological data were fitted to power law, Herschel-Bulkley and Casson models. The concentrates were mildly non-Newtonian in nature. The values for flow behaviour index varied from 0.79-0.99. The consistency indices and yield stresses of the hot-break grape juice concentrates were considerably higher than those of the cold-break or enzyme-treated juice concentrates. The activation energy for the filtered juice concentrates were not significantly different than those of the unfiltered juice concentrates.

Keywords: Grape juice, Concentrate, Flow behaviour, Physico-chemical characteristics.

Grape (*Vitis vinifera L.*) cv. 'Perlette' is an important horticultural crop of economic consideration. Though, the major portion of the fruit is sold in the market for table purposes, it fails to fetch remunerative price for its low soluble solids, sour and astringent taste. Coincidence of glut harvesting of the crop with rainy season is another constraint that damages fruit quality and market potential. Utilization of 'Perlette' grape (especially watching the interest of the growers) for desirable food products has not been attempted so far. Rao et al (1984) studied the effect of concentration and low temperature on flow properties of concentrated grape juices. Kashara et al (1988), however, reported the preparation of 56° Brix concentrates from 'Thompson seedless' grapes for use in jams and jellies. Information regarding physico-chemical characteristics of 'Perlette' grape juice and rheological properties of their concentrates was not available. The present investigation was, therefore, carried out to prepare concentrates from 'Perlette' grapes to assess their physico-chemical properties, flow charateristics and to determine the suitability of rheological models.

Materials and Methods

Materials and sample preparation : Well matured and sound berries of grape cv. 'Perlette' were manually picked from the Research Orchard of the Punjab Agricultural University, Ludhiana. The grapes were destalked with hands, washed thoroughly with running tap water and drained. A weighed lot (180 kg) of the berries was crushed into a fine pulp using a high speed commercial grater (B Sen Berry, New Delhi). The grape crush was divided into three equal parts. One part of the crush was used for cold extraction of the juice. The second part was treated with pectolytic enzyme (Pectinex Ultra SP-L, Novo, Denmark, manufacturer's recommended dose of 100-200 g/tonne) at the rate of 0.5% (w/v), and the contents were stored at 4±0.1°C for 24h in a thermostatically controlled room. The third lot of the crush was heated to 63°C for 2 min. The juice was extracted by pressing the treated and untreated crush at a pressure of 100 psi using a hydraulic press.

Bulk lots of each juice (cold-, hot-, pectolytic enzyme-break) were divided into three equal parts. One part of each juice was filled directly into 750 ml glass bottles and sealed with crown corks. The second and third parts of the juices were pasteurized at 88°C for 2 min before bottling. To the third proportion of the juices, potassium metabisulphite (KMS) was added at the rate of 70 ppm. The bottled juices were sterilized in boiling water for 30 min, cooled slowly under running tap water and stored at ambient temperature (25°-30°C) for further use as desired.

Concentrates were prepared from the grape juice with and without filtration. Nearly 3 litres of each lot of the grape juice was filtered through a bed of Whatman No. 1 filter paper containing asbestos fibre. The filter paper pulp was placed in a Buckner funnel fitted on a heavy-walled flask and filteration was carried out using vacuum. The process was repeated 3-4 times to obtain sparkling clear juice. The juices were evaporated under vacuum at 35°C using rotary film evaporator (Heidolph Elektro GmbH & Co KG, Germany) to a concentration of 80% total solids (Singh 1989).

Rheological measurements : Flow characteristics of grape juice concentrates were determined using

* Corresponding Author

Rheotest 2 viscometer (VEB MLW Prufgerate-werk, Medingen, Germany) with coaxial cylinder attachment. The torque data were obtained at shear rates between 0.6 and 145.8 s^{-1} using H system (bob to cup ratio of 0.81). A measured amount of concentrates (17 ml) was introduced into the cup as slowly as possible. The samples were maintained at rest for 15 min to equilibrate to the experimental temperatures of 10°, 20°, 30°, 40° and 50°C, respectively, before measurements. Temperatures of the concentrates were controlled with a thermostatic-circulating water bath. Shear stress data were recorded first in the ascending order, followed by a rest of 5 min and then in the descending order.

The shear rate vs shear stress data were fitted to the power law (Harper and El Sahrigi 1965), Herschel-Bulkley (Herschel and Bulkley 1926) and Casson models (Charm 1978). The power law flow behaviour, 'n' (dimensionless) and consistency index, K ($Pa.s^n$) were obtained from the regression of log τ vs log γ data. The Casson yield stress τ_{oC}(Pa.s) was derived from the regression line between square root of shear stress ($\sqrt{\tau}$) and square root of shear rate ($\sqrt{\gamma}$). The Herchel-Bulkley flow behaviour index (n_H), yield stress (t_{oH}) and consistency index (K_H) were calculated as described by Rao et al (1981). Influence of temperature on consistency index of the grape juice concentrates was examined using the Arrhenius model. The activation energy (ΔE) was derived from the regression of inverse of absolute temperature vs log consistency index.

Physico-chemical characteristics : Processed juice as well as 10% aqueous solution (w/v) of grape juice concentrates were evaluated for colour, total soluble solids (°Brix) and pH. Titratable acidity (% tartaric acid) and reducing sugars (% glucose) were estimated according to the procedures of AOAC (1990). Tannins were determined by the Folin-Dennis reagent method. Ascorbic acid contents were determined using 0.4% solution of 2,6-dichlorophenol indophenol dye (AOAC 1990). Pectin was determined by the modified procedure of Carre and Haynes (1922). Diluted samples of juice or concentrate were boiled for 1 h, cooled and filtered. One hundred ml of the filtrates were mixed with 10 ml of 1 N sodium hydroxide and allowed to stand at ambient temperature for overnight. The alkali was neutralized with 1N acetic acid. The contents were filtered, mixed with 25 ml of 1N calcium chloride solution and boiled for 60 sec. The precipitates of calcium pectate were filtered, dried and weighed. The results were expressed as % calcium pectate.

The results of analyses and flow characteristics were examined statistically by the analysis of variance according to the statistical graphics system of Statgraphics (1991). The least significant difference (LSD) was used as the test for significance and the values are given in the tables.

Results and Discussion

Physico-chemical characteristics of grape juice and concentrate : Juice yield by cold-break of grapes was nearly 70.2%. Grape crushes heated to 63°C for 2 min or treated with pectolytic enzyme yielded higher juice contents by about 3% and 10%, respectively, than those of the cold-break.

The total soluble solids (TSS) and titratable acidity of hot-break and pectolytic enzyme-break grape juices were higher than those of the cold-break ones (Table 1). This was in accordance with the observations of Sistrunk and Morris (1982). Differences in the values for pH of the various grape juices were negligible. The reducing sugar contents

TABLE 1. PROCESSING CONDITIONS AND PHYSICO-CHEMICAL CHARATERISTICS OF GRAPE JUICE

Processing conditions Break	Treatment	TSS[a] °Brix	pH	Acidity[b], %	Reducing sugars glucose, %	Pectin[c], %	Tannins[d], mg/100 ml	Ascorbic acid, mg/100 ml
Cold	UPA[e]	14.0	3.54	0.69	13.4	0.54	149	8.4
	PA[f]	14.5	3.54	0.71	13.5	0.60	155	7.8
	PA+KMS[g]	14.5	3.58	0.71	13.7	0.56	148	8.1
Hot	UPA	15.0	3.52	0.79	14.0	0.68	179	4.5
	PA	16.0	3.51	0.81	14.2	0.70	183	2.7
	PA+KMS	16.0	3.53	0.82	14.3	0.69	180	2.6
PE[h]	UPA	14.0	3.52	0.78	13.2	0.50	161	5.4
	PA	15.0	3.53	0.79	13.2	0.52	164	4.5
	PA+KMS	15.0	3.53	0.80	13.9	0.49	162	4.5
LSD (0.05)		0.01	0.01	0.08	0.68	0.08	6	0.2

[a] total soluble solids, [b] As tartaric acid, [c] As calcium pectate, [d] As tannic acid, [e] Unpasteurized, [f] Pasteurized, [g] Potassium metabisulphite, [h] Pectolytic enzyme

of the hot-break grape juices were marginally higher than those of cold - or enzyme-break juices (Table 1). However, their pectin contents were higher, possibly, due to inactivation of natural enzymes present in the grapes. Considerably higher tannin contents of hot-and enzyme-break juices than those of the cold-break corroborated the findings of Flora (1977). It is possible that both the heat and pectolytic enzyme treatments enhanced extraction of tannins from the grapes. However, the values for ascorbic acid contents of these juices were discernibly low.

Compared with the unpasteurized juices, the influence of pasteurization as well as addition of KMS on the various physico-chemical parameters was negligible, except for those of TSS, ascorbic acid and colour intensities (Table 1). The values for TSS of the pasteurized and chemically treated juices were significantly ($P<0.05$) higher than those of the unpasteurized juices. Pasteurization caused destruction of vitamin C, possibly, due to oxidative degradation of ascorbic acid.

Characteristics of 10% aqueous solutions of various grape juice concentrates are given in Table 2. Filtration of juices prior to concentration had negligible influence on pH, titratable acidity and reducing sugars of the concentrates. However, they showed lower pectin contents than those of the unfiltered juices, possibly, due to removal of microfibres from the juices. Concomitantly higher pH and considerably lower values for titratable acidity, reducing sugars and pectin contents of all the concentrates as compared to those of the corresponding juices (Table 1) might be an artifact of concentration of grape juices.

TABLE 2. PHYSICO-CHEMICAL CHARACTERISTICS OF GRAPE JUICE CONCENTRATES

Processing conditions: Break	Treatment	pH	Acidity[a], %	Reducing sugars glucose, %	Pectin[b], %
Unfiltered					
Cold	UPA[c]	3.69	0.41	8.8	0.32
	PA[d]	3.71	0.43	8.9	0.33
	PA+KMS[e]	3.72	0.43	9.0	0.31
Hot	UPA	3.73	0.46	9.1	0.36
	PA	3.75	0.48	9.2	0.35
	PA+KMS	3.78	0.50	9.3	0.34
PE[f]	UPA	3.64	0.55	8.9	0.28
	PA	3.69	0.56	9.0	0.28
	PA+KMS	3.71	0.59	9.1	0.26
Filtered					
Cold	UPA	3.63	0.39	8.8	0.14
	PA	3.65	0.42	8.9	0.17
	PA+KMS	3.67	0.43	8.9	0.13
Hot	UPA	3.59	0.48	9.0	0.16
	PA	3.63	0.51	9.2	0.15
	PA+KMS	3.67	0.54	9.2	0.15
PE	UPA	3.58	0.57	8.8	-
	PA	3.63	0.59	8.8	-
	PA+KMS	3.64	0.61	8.8	-
LSD (0.05)		0.05	0.02	0.11	0.01

[a] As tartaric acid, [b] As calcium pectate, [c] Unpasteurized, [d] Pasteurized, [e] Potassium metabisulphite, [f] Pectolytic enzyme

Flow characteristics : Starting with the lowest speed, the shear rate vs shear stress data obtained for ascending and descending curves were close together, indicating time-independant flow of the grape juice concentrates. When fitting the power law and Herschel-Bulkley model to the experimental data, high correlation coefficients were obtained, which ranged from 0.990 to 0.999 for the various concentrates. Suitability of power law model was also substantiated by the straight line relationship between the log shear stress vs log shear rate data (Fig. 1).

The mean values for flow behaviour indices for the various concentrates ranged from 0.79 to 0.99 (Table 3), indicating their mildly non-Newtonian to nearly Newtonian nature (Muller 1973). The concentrates prepared from juices extracted by cold-and pectolytic enzyme-break showed nearly identical Newtonian behaviour, whereas those of hot-break were mildly non-Newtonian. It is possible that more pectin in hot-break juices (Table 1)

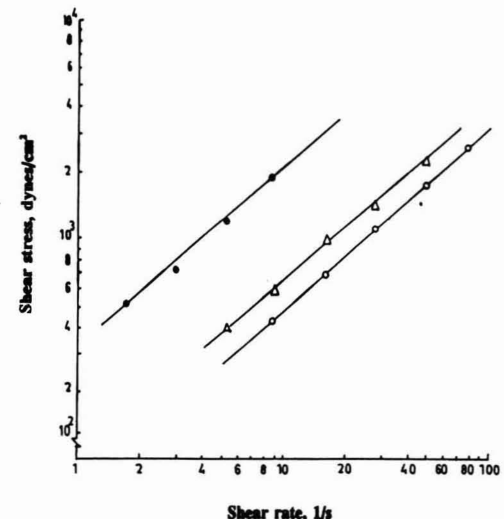


Fig. 1. Shear stress vs shear rate plots for cold break unfiltered grape juice concentrates at 30°C. O–O Unpasteurized Δ–Δ Pasteurized ●–● Pasteurized + potassium metabisulphite

TABLE 3. FLOW BEHAVIOUR INDEX AS CALCULATED BY HERSCHEL-BULKLEY (n_H) MODELS FOR GRAPE JUICE CONCENTRATES AT VARIOUS TEMPERATURES

Processing conditions		Temperature °C				
Break	Treatment	10	20	30	40	50
		n_H	n_H	n_H	n_H	n_H
Unfiltered						
Cold	UPA[a]	0.92	0.90	0.91	0.89	0.86
	PA[b]	0.91	0.90	0.89	0.87	0.85
	PA+KMS[c]	0.91	0.90	0.87	0.85	0.84
Hot	UPA	0.86	0.85	0.82	0.80	0.80
	PA	0.84	0.84	0.83	0.79	0.79
	PA+KMS	0.86	0.85	0.84	0.82	0.80
PE[d]	UPA	0.93	0.93	0.92	0.91	0.90
	PA	0.92	0.92	0.92	0.91	0.89
	PA+KMS	0.93	0.92	0.91	0.90	0.89
Filtered						
Cold	UPA	0.96	0.94	0.93	0.92	0.92
	PA	0.92	0.90	0.89	0.88	0.88
	PA+KMS	0.94	0.92	0.90	0.89	0.88
Hot	UPA	0.90	0.89	0.88	0.87	0.86
	PA	0.87	0.85	0.85	0.81	0.81
	PA+KMS	0.90	0.88	0.88	0.84	0.83
PE	UPA	098	0.95	0.94	0.93	0.92
	PA	0.97	0.96	0.96	0.94	0.93
	PA+KMS	0.96	0.94	0.93	0.92	0.91
LSD (0.05)		0.01	0.01	0.01	0.01	0.01

[a]Unpasteurized, [b]Pasteurized, [c]Potassium metabisulphite, [d]Pectolytic enzyme

resulted in more bound water in the cell wall materials, which caused high flow resistance. Pasteurization, addition of KMS, filtration of juices prior to concentration and increasing the experimental temperature of the concentrates from 10°C had negligible influences on the values for flow behaviour indices. Rao et al (1981), Speers and Tung (1986) and Rani and Bains (1987), also reported practically no significant effect of temperature on the flow behaviour of the various food products.

Yield stress : Results on the effects of processing conditions and temperature on yield values for the various grape juice concentrates are presented in Table 4. The mean values for yield stress of hot-break grape juice concentrates were significantly ($P<0.05$) higher than those of the cold-break, whereas those of the pectolytic enzyme-break were the lowest (Table 4). This might be attributed to their pectin contents (Table 2). Spectacularly, lower values of yield stress for pectolytic enzyme-treated juice concentrates might be due to breakdown of their pectic substances by the hydrolytic enzyme. These differences in the yield stress values were in accordance with the observations of Rani and Bains (1987) for tomato juice concentrates. The concentrates prepared from the filtered juices

TABLE 4. YIELD STRESS AS CALCULATED BY CASSON (τ_{OC}) AND HERSCHEL-BULKLEY (τ_{OH}) MODELS FOR GRAPE JUICE CONCENTRATES AT VARIOUS TEMPERATURES

Processing conditions		Temperature °C									
		10		20		30		40		50	
Break	Treatment	τ_{OC} Pa.S	τ_{OH} Pa.S	τ_{OC} Pa.S	τ_{OH} Pa.S	τ_{OC} Pa.S	τ_{OH} Pa.S	τ_{OC} Pa.S	τ_{OH} Pa.S	τ_{OC} Pa.S	τ_{OH} Pa.S
Unfiltered											
Cold	UPA[a]	16.1	25.1	5.5	13.7	2.7	6.2	2.6	5.7	1.2	1.8
	PA[b]	15.6	26.1	8.7	20.0	4.8	13.0	1.8	6.5	1.6	5.1
	PA+KMS[c]	12.8	25.2	7.0	23.9	3.6	11.3	2.4	5.6	1.6	2.7
Hot	UPA	13.8	21.5	4.9	7.0	3.9	5.3	3.1	2.7	2.7	1.4
	PA	18.6	27.1	12.5	18.5	9.0	9.0	7.0	5.4	6.9	8.2
	PA+KMS	23.1	35.2	11.0	30.4	7.6	25.4	1.5	14.4	1.3	8.0
PE[d]	UPA	10.2	26.1	8.6	10.1	3.8	7.5	0.4	2.5	0.9	1.2
	PA	8.7	22.2	4.2	16.1	1.0	13.0	1.3	8.2	0.4	2.3
	PA+KMS	11.6	25.2	9.3	18.1	2.9	11.7	3.7	11.3	0.9	2.9
Filtered											
Cold	UPA	15.1	25.3	7.3	15.1	2.3	13.2	0.5	6.4	0.5	2.9
	PA	13.9	36.1	10.2	25.3	3.1	12.6	0.9	6.4	0.9	3.1
	PA+KMS	14.1	27.3	5.9	18.9	3.1	9.4	1.3	4.7	1.3	1.9
Hot	UPA	16.6	26.4	8.8	13.2	4.0	6.6	3.2	2.8	2.8	1.9
	PA	19.3	37.1	13.1	27.2	3.6	18.6	2.9	9.4	1.2	4.7
	PA+KMS	14.9	36.7	8.9	27.5	6.1	13.7	3.2	6.8	3.2	3.5
PE	UPA	4.0	27.6	1.5	14.0	0.6	6.9	0.6	3.6	0.3	0.3
	PA	3.2	24.0	0.8	15.0	0.5	12.0	0.5	5.9	0.3	2.9
	PA+KMS	7.7	14.0	4.0	7.4	1.8	3.7	1.1	3.6	0.6	2.7
LSD (0.05)		2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2

[a]Unpasteurized, [b]Pasteurized, [c]Potassium metabisulphite, [d]Pectolytic enzyme

exhibited lower values for yield stresses than those from the unfiltered juices (Table 4). This might be the artifact of removing microfibrillar system i.e. the insoluble carbohydrate polymers, especially, pectin, cellulose, and fibre from the juices upon filtration. The microfibrillar system serves as a nuclei for aggregation of soluble solids forming an amorphous network, which contributes to viscosity and resistance to flow (Xu et al. 1986).

Increases in the testing temperatures of the concentrates from 10° to 50°C consistently decreased the yield stress values from 14.0-37.1 Pa.s to 0.3-2.8 Pa.s (Table 4). At 40°C, the yield stress values were nearly 60% to 94% lower than those at 10°C. Further decreases in the values were less marked with increases in the temperature from 40°C to 50°C.

Consistency index : The consistency index values for various concentrates were considerably influenced by the processing conditions of the grape juices (Table 5). Like yield stress, the values for consistency indices were perceptibly high for hot-break juice concentrates, intermediate for cold-break juice concentrates and low for pectolytic enzyme-break juice concentrates. High temperature for extraction of juice, possibly, inactivated the natural pectolytic enzymes in the juice (Luh and Daoud 1971) and leached more soluble pectic substances from the cell walls, which formed an amorphous network in conjunction with the debris of collapsed cell walls, resulting in high consistency indices. Similarly, pasteurization of juices and addition of KMS (70 ppm) also inactivated the enzymes, resulting in higher magnitudes of consistency indices over those of the corresponding unpasteurized juice concentrates. Differences in the consistency index values due to filtration were similar to those obtained for yield stress values (Tables 4 and 5).

Effect of temperature on consistency index : The consistency index data successfully followed the Arrhenius model. The coefficient of correlation (r^2) between inverse absolute temperature (1/T) and in consistency index values varied from 0.984 to 0.998. The values of activation energy of flow (ΔE) for various concentrates are given in Table 6. The consistency index values for all concentrates decreased gradually with increases in temperatures (Table 5). Decreases in the values for hot-break and pectolytic enzyme-break grape juice concentrates were similar to those of the cold-break juice concentrates. This also conformed with their similar activation energy values (Table 6). Though the consistency indices of the filtered grape juice concentrates were considerably lower than those of the unfiltered juices, decreases in the values, with increasing temperatures were similar (Table 5). This was also evident from their activation energy values which were similar to those of the unfiltered juice concentrates (Table 6). These results suggested that

TABLE 5. CONSISTENCY INDEX AS CALCULATED BY HERSCHEL-BULKLEY (K_H) MODELS FOR GRAPE JUICE CONCENTRATES AT VARIOUS TEMPERATURES

Processing conditions		Temperature °C				
		10	20	30	40	50
		K_H	K_H	K_H	K_H	K_H
Break	Treatment	$Pa.S^n{}_H$	$Pa.S^n{}_H$	$Pa.S^n{}_H$	$Pa.S^n{}_H$	$Pa.S^n{}_H$
Unfiltered						
Cold	UPA[a]	247	110	42	14.9	4.7
	PA[b]	395	412	68	20.7	7.0
	PA+KMS[c]	820	298	79	24.8	10.4
Hot	UPA	301	109	30.7	17.1	5.5
	PA	393	138	50	17.1	4.6
	PA+KMS	1569	443	158	44	11.5
PE[d]	UPA	177	86	32.2	15.4	5.1
	PA	259	110	39	17.6	6.9
	PA+KMS	342	128	53	17.4	6.0
Filtered						
Cold	UPA	122	56	24.0	9.0	5.0
	PA	158	62	30.2	10.9	4.9
	PA+KMS	251	98	36.1	16.2	5.3
Hot	UPA	168	62	30.5	9.9	3.1
	PA	276	98	39.7	14.4	5.1
	PA+KMS	761	164	76	26.1	7.3
PE	UPA	90	38	17.5	8.1	3.5
	PA	123	55	19.9	8.5	3.9
	PA+KMS	238	70	30.1	10.2	5.0
LSD (0.05)		7.7	7.7	7.7	7.7	7.7

[a]Unpasteurized, [b]Pasteurized, [c]Potassium metabisulphite, [d]Pectolytic enzyme

TABLE 6. CONSISTENCY INDEX (K_H) AS A FUNCTION OF TEMPERATURE FOR GRAPE JUICE CONCENTRATES

Processing conditions		Unfiltered ΔE	Filtered ΔE
Break	Treatment	K cal/g mol	K cal/g mol
Cold	UPA[a]	17.3	20.8
	PA[b]	18.3	18.7
	PA+KMS[c]	21.9	19.8
Hot	UPA	21.1	17.1
	PA	17.9	15.4
	PA+KMS	17.8	16.3
PE[d]	UPA	17.6	18.9
	PA	16.7	16.7
	PA+KMS	18.9	15.3
LSD (0.05)		0.9	0.9

[a]Unpasteurized, [b]Pasteurized, [c]Potassium metabisulphite, [d]Pectolytic enzyme

the energy inputs required to align the particles in both type of concentrates were possibly the same.

Conclusion

Nearly 3-10% more juice could be obtained from grapes using hot-break and pectolytic enzyme treatment than those of the cold-break. The grape juice concentrates were mildly non-Newtonian in nature. The consistency index and yield stress values calculated using Herschel-Bulkley model for the hot-break grape juice concentrates were higher than those of the cold-break or enzyme-treated juice concentrates. These values decreased with increasing temperatures from 10° to 50°C.

References

AOAC (1990) Official Methods of Analysis, 15th edn. Association of Analytical Chemists, Washington, DC

Carre MH, Haynes D (1922) The estimation of pectin as calcium pectate and application of this method to determination of soluble pectin in apples. Biochem J 16:60-64

Charm SE (1978) The Fundamentals of Food Engineering, 3rd edn. Van Nostrand Reinhold/AVI, New York

Flora LF (1977) Processing and quality characteristics of Muscadine grapes. J Food Sci 42:935-938

Harper JC, El-Sahrigi AF (1965) Viscometric behavior of tomato concentrates. J Food Sci 30:470-476

Herschel WH, Bulkley R (1926) Konsistenzmessungen von gummibenzollosunge. Kolloid-Z 39:291-300

Kashara GI, Garrido BF, Vargas FB (1988) Formulation of jellies and jams from concentrated juices and pulp from table grapes. Alimentos 13:7-10

Luh BS, Daoud HN (1971) Effect of break temperature and holding time on pectin and pectic enzymes in tomato pulp. J Food Sci 36:1039-1043

Muller HG (1973) An Introduction to Food Rheology. William Heinemann Ltd., Heinemann, London

Rani U, Bains GS (1987) Flow behaviour of tomato ketchups. J Texture Stud 18:125-135

Rao MA, Bourne NC, Cooley HJ (1981) Flow properties of tomato concentrates. J Texture Stud 12:521-538

Rao MA, Cooley HJ, Vitali AA (1984) Flow properties of concentrated juices at low temperatures. Food Technol 38:113-117

Singh T (1989) Flow behavior and functional properties of malt extract concentrates. J Texture Stud 20:479-497

Sistrunk WA, Morris JR (1982) Influence of cultivar, extraction and storage temperature and time on quality of Muscadine grape juice. J Am Soc Horti Sci 107:1110-1113

Speers RA, Tung MA (1986) Concentration and temperature dependence of flow behavior of xanthan gum dispersions. J Food Sci 51:96-98

Statgraphics (1991) Statistical Graphics System. Version 5.0 Reference Manual. Statistical Graphics Corp, Princeton, NJ

Xu S-Y, Shoemaker CF, Luh BS (1986) Effect of break temperature on rheological properties and microstructure of tomato juices and pastes. J Food Sci 51:399-402

Received 8 May 1998; revised 8 June 1999; accepted 9 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 408-412

Crushing Characteristics of Mustard with Commercial Oil Expeller

R.T. PATIL* AND NAWAB ALI
Central Institute of Agricultural Engineering,
Nabi bagh, Berasia Road, Bhopal - 462 038, India.

The rapeseed mustard contains about 35-40% oil, which is generally expelled by screw type mechanical oil expellers or motorised rotaries. A commercial 9 bolt oil expeller was tested for mustard oil expulsion at 4.6% moisture content of raw seed. The oil recovery was found to be 19.18%, 35.78%, 24.04% and 6.01% in the first, second, third and fourth passes, respectively. The temperature of the barrel and the thickness of the cake were measured during oil expulsion process. The average throughput was 135 kg/h of seed and the efficiency of oil expression in three passes was 79% at seed oil content of 35%. The average speed of the worm was 46 rpm. The pattern of barrel temperature indicated higher temperature at bolt numbers 5,6, and 7. The average temperature of the barrel was 66.1°C.

Keywords: Crushing characteristics, Oil expeller, Mustard oil, Costing.

Edible oil is the main source of fat consumed by Indian population. The oil is expelled from the oilseeds by mechanical as well as chemical methods. In mechanical methods, the seeds are compressed or squeezed and the oil is recovered. The types of oil expelling devices are hydraulic press, the bullock drawn ghanies, mechanical ghanies or rotaries, the baby oil expeller and high capacity high pressure expellers. In the initial stage of compression, decrease in volume of voids in total mass of oil bearing material takes place due to rearrangement of oilseeds.

A comprehensive analysis of processing high oil content seeds in continuous screw press was reported by Ward (1976), who stated that each stage in expelling as shown in Fig. 1, is equally important as that of pressing to achieve best results. The oil in the seed is contained in sacs or fibrous capillaries. The application of pressure causes capillaries to be reduced in volume and the oil to be expelled, but by the same effect, capillaries are narrowed and sealed eventually by the application of increasing pressure. This puts a practical limit, even with all four operations ideally performed, (cleaning, seed preparation, cooking and pressing) on the lowest residual oil content obtained by high pressure screw press.

Ajibola (1989) found that the yield of mechanically expressed oil is dependent on pre-pressing factors such as seed moisture content, particle size, pre-heat temperature, hold period at this temperature, cake thickness and applied pressure. Adeeko and Ajibola (1990) reported that maximum oil yield was obtained when moisture content was reduced to 5% level. Becker et al (1983) evaluated a screw expeller for use on the farm to determine its performance in expressing sunflower oil for use in diesel engines. They reported that the oil extraction efficiency ranged from about 56% to 84% at low and high relative barrel pressures, respectively. The greatest oil extraction rate was 12.3 kg/h at a feed rate of 40kg/h with an extraction efficiency of about 80%. Galal (1990) studied the effect of cottonseed oil extraction, by either the screw or hydraulic pressing systems. Results proved that oil yield was lower in the screw pressing system than in case of hydraulic pressing. Crude cottonseed oil quality (free fatty acids and colour), besides cottonseed cake quality (available lysine and *in vitro* protein digestibility) were greatly affected by screw than by hydraulic pressing.

There are two steps involved in the expulsion of oil through the expeller 1) disintegration and 2) the pressing. The expellers work on the principle of pressure differential applied to incoming oilseeds versus that applied to the discharged material and it is termed as compression ratio. The radial pressure is generated due to volumetric compression along the screw barrel. Under the ideal conditions, the flow of material along the worm shaft should be purely axial and the compression ratio should be quite low.

The preparatory operation helps improve the oil expression. The process coagulates the proteins in the seeds causing coalescence of oil droplets and makes the seed permeable to the flow of oil. The pre-treatments also decrease the affinity of oil for the solid surfaces of the seed so that best yield of oil is obtained on subsequent pressing.

Though the technology has been developed from the ghanies to expeller, it is mainly through

* *Corresponding Author*

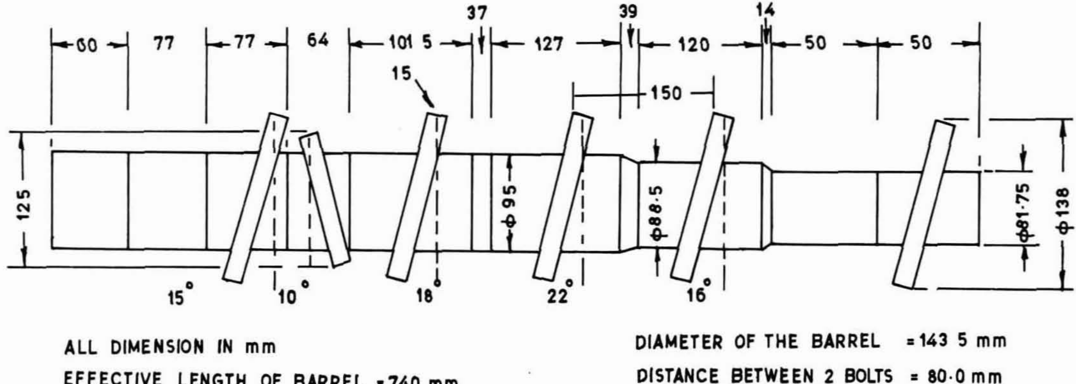


Fig. 1. Configuration of the screw of commercial oil expeller

the experience and very little scientific investigations on the working of these devices for different oilseeds have been documented. This paper reports the use of a commercial screw press for extraction of mustard oil. The extraction efficiency in each pass was evaluated. The temperature developed during processing was recorded. The economics of the screw press was also worked out to determine the cost of processing.

Materials and Methods

Oil expeller : A commercial 9 bolt heavy duty oil expeller was used in the study. The unit consisted of 2 parts, (i) feeding section and (ii) expeller section. In feeding section round feeding box was provided with a stirrer arrangement. The stirrer got its motion through bevel set provided at the bottom. The bevel set was rotated by a horizontal shaft having a flat pulley connected to expeller pulleys.

The expeller section consisted of expeller body, expelling chamber, gear box and drive arrangement. The expeller body was assembled in 4 pieces with an oil tray and casted pipe connections. The expeller section stood on two casted legs. The bodies were held together with tie rods. In the expeller section, worm set was keyed on the main shaft. The worm assembly was housed in the cage bar housing secured by chamber bolts (Fig. 1).

The drive to the expeller was the most important component of the expeller assembly. The 3 phase, 20HP, 1000 rpm motor was used as prime mover. The gear box was housed in two bodies, main gear with 60 teeth and other gear with 52 teeth. The 60 teeth gear got its drive from 12 teeth gear and 52 teeth gear got from 20 teeth gear, respectively, which were keyed to the shaft. The 60 teeth gear drove the worm assembly and 52 teeth gear was for feed assembly.

Running of the expeller : The pressure worm shaft rotated in anti clockwise direction from driving to the expeller end. In the beginning, the pressure cone was withdrawn from the barrel. Oilseeds from the feeding box were fed to the expeller through the discharge chute and compressed in the expeller worm. While passing through the length of the expeller, the material was compressed, screwed, crushed and the oil was freed from the seed. The pressure was applied through diminishing pitches of the worm and final pressure was applied by the pressure cone. In the beginning of the operation, the pressure cone was withdrawn completely and meal was allowed to pass freely from the cake discharge.

When the meal came out in regular round annular shape, the cone was advanced into the machine gradually i.e., in stages. While constant working also, the pressure cone was moved back and forth so that it did not get fixed at one position. The heaviest flow of oil occurred from feed section, if the expeller was running properly. The proper feeding of the expeller was very important for efficient operation and feeding was kept such that feed spout was always full.

Mustard seeds : The mustard seeds grown on the Nabi Bagh farm in season of 1995-96 was obtained for the experiment. The seeds were cleaned by motorised air screen cleaner cum grader. The moisture content of the seeds was determined by OHAUS moisture balance at 130°C for 90 min. The

moisture was added to the seeds before expressing to get optimum moisture content corresponding to better oil expression. Water was sprinkled on the mustard seeds batch of 50 kg and mixed thoroughly in a container and kept covered for equilibration for 2 h.

Results and Discussion

The moisture content of the mustard seeds was 4.6% wet basis. Two kilograms of water was added per batch of 50 kg. The resultant superficial moisture content of the seeds was found to be 8.25%. The pressure applied by the expeller system was low in the first pass, as squeezing oil was relatively easy. Hence, the cake thickness was also bigger in the first pass. The cake thickness in each pass is shown in Fig. 2. The reductions in thicknesses were 35%, 59% and 77%, respectively in second, third and fourth passes over the first pass.

The temperature of the oil and cake during expression in each pass are shown in Fig. 3. Since the oil and solids mixture formed a fluid mass, the temperature generated due to friction and compression was equally shared by the liquid as well as solid phases in the fluid. Hence, there was no significant difference in the oil as well as cake temperature. However, the temperature was higher in subsequent passes. This was due to additional pressure exerted, as passes increased due to movement of the pressure cone inside the barrel. The temperature was 45°C in the first pass whereas, it was raised to 60°C in the fourth pass. The average temperature of cake and oil during expressing was 46°C.

The temperature of the oil and cake was result of the barrel temperature. When barrel temperature was higher, it made the fluid hot and even gave the cake charred appearance and discolored oil was obtained. Hence, the temperature of barrel at each bolt was measured (Fig. 4). As the material size reduction starts in feed zone, the back pressure starts developing due to partial plugging action of the pressure cone. The temperature in the feed section also starts rising. The temperature at the first bolt was 65°C and kept on rising upto third bolt. The highest temperature was 80°C at third bolt. As the oil was separated from the crushed material, it started oozing out through the slots, going from higher pressure to nil pressure and cooled down. As effective volume squeezed was reduced, as the cake devoid of oil passed to the discharge end and the pressure in the barrel at these points also got reduced. This reduction in pressure also resulted in lowering the temperature, thus giving the overall cooling effect in the subsequent bolt positions. The temperature profile of the barrel was very similar to the theoretical pressure curve as shown in Fig. 5. The radial pressure increased upto bolt 3 position and thereafter, it started decreasing due to expression of oil through the slots.

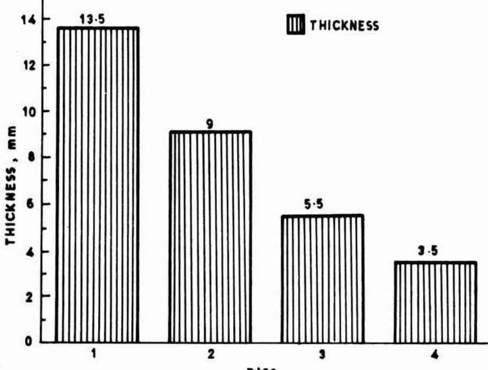


Fig. 2. Thickness of the cake in each pass

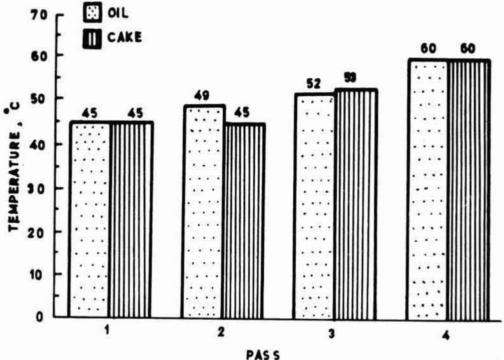


Fig. 3. Temperature of oil and cake in each pass

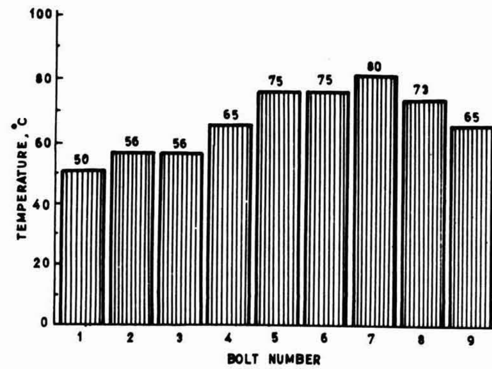


Fig. 4. Temperature variations in barrel

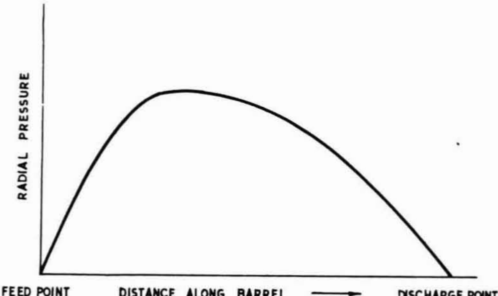


Fig. 5. Radial pressure in screw press barrel (Ward 1976)

The maximum radial pressure was generated at the feed end of the compressed plug, as upto this stage, the flow of the material was axial. Thereafter, it fell as the flow of material was in both axials as well as radial direction, thereby reducing the compression ratio and hence, the radial pressure started falling. The axial pressure in the barrel also followed the similar pattern.

The most important point about which the user of such mills is concerned about is the throughput and yield of cake and oil. The throughput was also a function of position of the pressure cone. In the first pass, the throughput was 135 kg/h, much higher as against the third (108 kg/h) and fourth pass (98 kg/h). However, the oil recovery was higher in the second pass. This indicated that in the first pass, the seeds got ruptured physically but not all crushed. This also could be seen by physical observation of the cake, which showed the flattened seeds still retaining their identity. In the second pass, the seeds were crushed, and oil was expressed. The results of the throughput, oil and cake yield in each pass are shown in Fig. 6. The

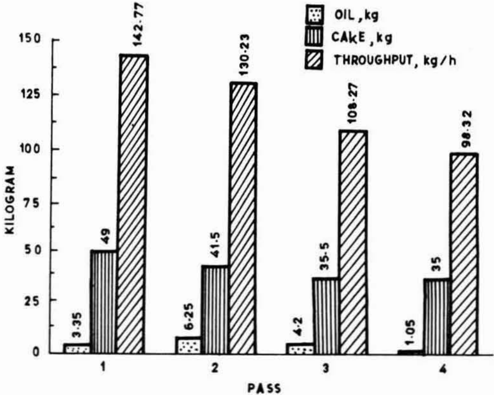


Fig. 6. Recovery and throughput of expeller in each pass

93% of total expressible oil was recovered in three passes. The oil content in the seed was found to be 35%. That means about 79% oil was expressed in three passes from the mustard seeds by this expeller.

Economics of expeller operation : The cost of the this expeller was Rs. 85,000.00 and the prime mover about Rs. 35,000.00. The total costs came to Rs. 120,000.00. The assumptions for economic analysis were as follows:

Rent/housing = 2000.00/month

Unskilled labour= 1

Skilled labour = 1

Operating hours = 8 h/day

Yearly operation = 240 days

Useful life of the machine = 10 years

Salvage value = 10% of initial cost

Rate of interest = 18%

Wages of skilled operator = Rs. 60/day

Wages of unskilled operator = Rs. 40/day

Plant runs for 2 shifts

Fixed costs

Machinery and equipment		
Commercial 9 bolt expeller	=	85,000.00
Electric motor, 20 hp, 3 phase, 1000 rpm	=	35,000.00
Filter press	-	20,000.00
Accessories	=	25,000.00
Total		**165,000.00**
Electrical fittings, plumbing etc. 10% of machine costs.	-	16,500.00
Total	=	**181,500.00**
Variable costs		
Salaries and wages		
Skilled labour @ Rs. 60/days x 2	-	3600.00 per month
Semi-skilled labour @ Rs. 40/day x 2	=	2400.00 per month
Total	-	**6000.00 per month**
Raw material: mustard @ Rs. 12.5/kg	=	225.000.00 per month
Utilities: Electricity, 15 units/h @ Rs. 2.50/unit	=	18,300.00 per month
Water		200.00 per month
Total	=	**18,500.00 per month**
Other expenses		
Building rent	=	2000.00 per month
Maintenance @ 6%/year on Rs. 165,000	=	825.00 per month
Insurance @ Rs. 4/1000	=	55.00 per month
Office expenses	-	500.00 per month
Total	-	**3380.00 per month**

Operating cost

Salary and wages	=	6,000.00 per month
Raw material	=	225,000.00 per month
Utilities	-	18,500.00 per month
Other expenses	=	3380.00 per month
Total	=	**252,880.00 per month**
Operating cost for 2 months	=	**505,760.00**
Fixed cost per year		
Machinery and equipment	=	181,500.00
Operating cost for 2 months		505,760.00
Total	=	**687,260.00**
Depreciation @ 10 per year on 165,000.00	=	16,500.00
Interest @ 18% year	=	32,670.00
Total	=	**49,170.00**
Variable cost per year		
Salary and wages	-	72,000.00
Raw material	-	2.700,000.00
Utilities	=	222,000.00
Other expenses	=	40,560.00
Total	=	**3.034,560.00**
Production cost per year	=	**3,083,730.00**
Total production		
Oil @ 14.5 kg/h	-	5800 kg/month
Cake @ 30.5 kg/h	=	12200 kg/month
Return from production		
Return from oil/year @ Rs. 35/kg	=	2,436,000.00
Return from cake/year @ Rs. 5/kg		32,000.00
Cost of production/kg oil		
Total cost of production-Cost of cake/oil produced per year	=	Rs. 33.80
Per kg variable cost	=	Rs. 33.10
Per kg fixed cost	=	Rs. 0.70

Net profit per year

Return-cost = 3,168,000.00, 3,083,730 - 84,270.00

Break even point

Fixed cost/ (Sale price-Variable cost) = 49,170.00/(35.00-33.80)

= 49170/1.2 = 40975 kg of oil

= 40975/ (232 kg/day) = 177 days

Return on investment

Net Profit* 100/Fixed cost = 84,270*100/687260 = 12.3%

Pay back period

Capital Investment/Net profit=181,500,/84,270
= 2.15 years = 516 Days

The cost analysis showed that oil expelling unit of the size evaluated in this study was a feasible enterprise. About 177 days of operation were required to break even. The return on investment was 12.3%, which would be just marginal. This showed the need for suitable pre-treatment to the oilseed to increase the expelling efficiency and to get good quality cake to fetch better price. This system provided chemical free oil expression requiring minimal processing. The major advantage was that the plant could be installed in the village, where sufficient quantity of good quality raw material was available.

From the studies on expelling characteristics of the mustard seeds in commercial oil expeller, it could be concluded that the temperature profile of the barrel was very similar to the theoretical pressure curve in the barrel. The temperature of the barrel showed increasing trend upto bolt 3 position and thereafter, decreasing trend due to expression of oil through the slots. The 93% of total expressible oil was recovered in three passes, which was about 79% of the oil present in the mustard seeds. The cost of crushing was estimated to be Rs. 33.80/kg of oil and the break even point was achieved after 177 days of operation and return on investment was 12.3%. This showed that there was a need to develop the process to make it more efficient and more profitable.

References

Adeeko KA, Ajibola OO (1990) Factors affecting the expression of oil from groundnut. J Agric Engng Res 45(2):45-53

Ajibola OO (1989) A study of some factors affecting the hydraulic pressing of palm kernel. J Food Sci Technol 20(4):213-217

Becker LF, Jacobsen L, Olson JC (1983) Farm scale recovery and filtration characteristics of sunflower oil for use in diesel engines. J Am Oil Chem Soc 60(8):1558-1560

Galal SM (1990) Effect of cottonseed pressing methods on the quality characteristics of oil and cake. Bulletin Faculty Agriculture, Cairo University Egypt, 40(1):225-240

Ward JA (1976) Processing high oil content seeds in continuous screw press. J Am Oil Chem Soc 53(6):261-264

Received 4 May 1998; revised 3 May 1999; accepted 9 August 1999

Effect of Hot Deboning and Polyphosphate on Refrigerated Storage Stability of Quail Sticks

R.P. SINGH*, S.K. ANAND AND S.S. VERMA

Central Avian Research Institute, Izatnagar - 243 122, India.

Physico-chemical, bacterial and sensory quality of sticks prepared from hot deboned (un-chilled; ≤ 60 min post-mortem) or cold deboned (1h slush ice + 4 h aging at 4±1°C) spent quail hen meat treated with or without 0.5% sodium tripolyphosphate (STP) was evaluated under refrigerated (4±1°C; 80-85% RH) storage. Although the product showed significant (P< 0.01) increase in percent weight loss with storage time, the differences between deboning and STP treatments were non-significant. Moisture, crude proteins and ether extractives of sticks, which averaged 68.5, 19.4 and 9.6%, respectively, also did not differ significantly between processing treatments or storage periods. However, addition of STP raised emulsion pH by about 0.30 unit, improved emulsion stability, inhibited lipid oxidation and restricted bacterial multiplication, resulting in the extension of organoleptically acceptable shelflife of the product upto 9 days, as against 6 days for samples without STP, regardless of deboning methods.

Keywords: Hot/cold deboning, Polyphosphate, Spent quail sticks, Keeping quality.

Hot deboning of poultry carcasses over conventional chilled and aged deboning has economic advantages like energy conservation and reduction in processing time (Hamm 1981). Some workers have, however, reported increased toughness of products prepared from hot deboned poultry meat due to interference with muscle tenderization during post-mortem aging process (Wardlaw et al. 1973; Lyon et al. 1983). Incorporation of sodium chloride and/or polyphosphate to tenderize hot cut chicken meat by soaking, injection or tumbling has shown desirable effects on alleviating the toughness problem (Peterson 1977; Ang and Hamm 1986), but these processes require additional handling, labour, time and equipment, which could offset the economic advantages of hot deboning of carcasses. Alternatively, blending of hot or cold deboned meat with these additives either singly or in combination was suggested for the enhancement of the functionality of meat and quality of finished products (Kijowski et al. 1982; Young and Lyon 1997). Although considerable work has been done on the development of comminuted products from spent hen meat including chicken sticks (Baker et al. 1966), such a study in case of Japanese quail has not been reported. The purpose of this study was, therefore, to evaluate the keeping quality of meat sticks formulated with hot or cold deboned spent laying quail meat, edible by-products and polyphosphate under refrigerated storage condition.

Materials and Methods

One hundred forty spent Japanese quail layers of 58 weeks, reared in cages under identical feeding and managemental conditions, were procured from the experimental farm of this institute and slaughtered conventionally in two equal groups. One group of warm (un-chilled) eviscerated carcasses were manually deboned within 1 h post-mortem, while the other group was deboned after chilling the carcasses in slush-ice (1:2 w/v) for 1 h to an internal temperature of 3°C followed by aging for 4 h at 4±1°C. The time interval between bleeding and deboning was so adjusted that both hot and cold deboned meats could be simultaneously fabricated into sticks. The lean meat was ground once through 6 mm plate of Hobard food mincer whereas skin, gizzard and heart (SGH) were minced twice in natural proportion (67.3:24.8:7.8).

The minced meat and SGH of each deboning treatments were mixed separately in the ratio of 85:15 and one half of the lot of each type of meat-SGH mix was blended with 0.5% sodium tripolyphosphate (STP), while the remainder served as respective control groups. The emulsion was prepared from each treatment, which contained 10% refined vegetable oil, 5% refined wheat flour, 2.5% each garlic and ginger, 2% each spice mix and common salt and 7% chilled water. The emulsion was formed into blocks in stainless steel moulds and frozen (−18°C) for 16h. The frozen blocks were tempered, cut into stick pieces of 1 cm thickness, wrapped individually in parchment paper and six pieces were packaged in each low density polyethylene (250 G) pouch and held under refrigeration (4±1°C; 80-85% RH) for keeping quality evaluation at periodic intervals.

The percent loss in weight of the product was calculated from the difference between initial and stored weights. Ten gram samples were homogenized

* *Corresponding Author*

with 100 ml distilled water and the pH of slurry was determined using a Beckman pH meter. Emulsion stability (Baliga and Madaiah 1971), TBA values (Tarladgis et al. 1960), moisture, crude protein and ether extract contents (AOAC 1980) of the product were determined. Standard methods (Speck 1976) were followed for bacterial counts. All these determinations were made in triplicates. The product cooked under pressure (1.1 kg/cm^2 for 10 min) was evaluated organoleptically for appearance, texture, juiciness, flavour and overall acceptability on a 7-point Hedonic scale (7= highly desirable; 1= highly undesirable) by at least seven experienced sensory panel members of this institute. Data were subjected to analysis of variance and means were compared for significant differences by Duncan's multiple range test (Snedecor and Cochran 1968).

Results and Discussion

Physico-chemical characteristics reported in Table 1 indicated that a significant ($P<0.01$) increase in weight loss occurred with storage time. Sticks prepared from either hot deboned (HB) or cold deboned (CB) meat, containing polyphosphate registered relatively lower weight loss than the respective control groups, which might be attributed to increased water holding capacity of the phosphate-treated meat (Pearson and Tauber 1984). As expected, incorporation of STP in meat emulsions brought about a significant ($P<0.01$) increase in their pH values, which fluctuated within a narrow range during storage. However, almost identical increase observed in pH of STP-treated hot and cold deboned groups is contrary to the finding of Young and Lyon (1994), who reported greater pH increase for STP-treated hot deboned turkey meat. This might be because of variation in the rigor state of muscles during addition of polyphosphate.

The proximate constituents were neither influenced significantly by processing treatments nor by storage periods. However, both HB and CB sticks devoid of STP had relatively lower contents of moisture than those containing added phosphate. Protein hydration due to increased solubility of myofibrillar proteins (Hamm 1960) might have accounted for comparatively more moisture in phosphate treated sticks. Deboning methods appeared to have no significant influence on the emulsion stability (ES), but addition of STP resulted in a significant ($P<0.01$) improvement in the ES of both hot and cold deboned groups. This might be due to the increased pH and specific effect of phosphate in increasing the myofibrillar proteins solubility (Prusa and Bowers 1984), which upon heating, promoted greater entrapment of water and fat globules within the interwoven protein matrix. Several workers (Baker et al. 1972; Choi et al. 1987; Kondaiah et al. 1992) also reported superior ES of comminuted meat products containing polyphosphates. Storage period had no significant influence on the emulsion stability regardless of treatments. On the other hand, a progressive increase in thiobarbituric acid (TBA) values occurred with the advancement of storage period. However, consistently lower TBA values found in phosphate treated than in control groups might be due to the inhibitory effect of STP on lipid oxidation by sequestration of catalytic heme and non-heme iron of meat (Tims and Watts 1958; Ang and Hamm 1986). The reason for comparatively higher TBA values of HB than of CB sticks in the absence of added phosphate is not obvious. It is likely that higher temperature of hot deboned meat and/or its rigor state might have enhanced the catalytic effect of heme pigments on lipid oxidation.

The results of bacterial counts (Table 2) indicated that the initial total aerobic plate counts (APC) of control and phosphate-treated samples were almost similar. The APC observed in this study was higher than the initial counts of log 4.0/g in restructured beef steaks (Van-Laack and Smulders 1991), but lower than that (log 5.0/g) of freshly ground poultry meat (Maxcy et al. 1973). Eventhough, a gradual increase in both APC and psychrophilic count occurred with storage time, polyphosphate treatment extended the lag phase of aerobic mesophilic bacteria as evident from little increase in APC in treated samples upto the 6th day but thereafter, it showed faster multiplication. Almost identical rate of bacterial multiplication observed in HB and CB sticks was in accordance with the finding of Van-Laack and Smulders (1991). Polyphosphate treatment appeared more effective in inhibiting the growth of mesophiles than that of psychrophiles. Although the exact mechanism of anti-microbial action of polyphosphate like STP is not yet known, postulated modes of its inhibitory action include chelation of metal ions, interaction with cell membrane to hinder transport functions and enzyme inhibition (Sofos 1986).

For the sake of brevity, only data pertaining to overall acceptability scores are presented in Table 2. Although the mean acceptability scores tended to decline with storage time regardless of treatments, the product prepared from hot or cold deboned meat containing polyphosphate was

TABLE 1. EFFECT OF DEBONING METHOD AND POLYPHOSPHATE ON PHYSICO-CHEMICAL QUALITY OF QUAIL STICKS DURING REFRIGERATED (4±1°C) STORAGE

Days of storage	Treatments HB	HB+STP	CB	CB+STP	Mean
			Weight loss, %		
3	0.31 ± 0.05	0.26 ± 0.02	0.25 ± 0.03	0.24 ± 0.04	0.26^{A} ± 0.02
6	0.46 ± 0.04	0.38 ± 0.04	0.43 ± 0.03	0.36 ± 0.04	0.41^{B} ± 0.02
9	0.62 ± 0.05	0.59 ± 0.07	0.68 ± 0.06	0.56 ± 0.05	0.61^{C} ± 0.03
Mean	0.46 ± 0.05	0.41 ± 0.05	0.45 ± 0.07	0.38 ± 0.05	
			pH		
0	6.32^{a} ± 0.04	6.63^{b} ± 0.03	6.24^{a} ± 0.03	6.58^{b} ± 0.05	6.44 ± 0.05
3	6.30^{a} ± 0.04	6.60^{b} ± 0.03	6.36^{a} ± 0.06	6.62^{b} ± 0.07	6.47 ± 0.05
6	6.38^{a} ± 0.08	6.67^{b} ± 0.04	6.31^{a} ± 0.02	6.69^{b} ± 0.06	6.51 ± 0.06
9	6.43^{a} ± 0.03	6.69^{b} ± 0.05	6.46^{a} ± 0.03	6.64^{b} ± 0.05	6.55 ± 0.04
Mean	6.35^{a} ± 0.03	6.65^{b} ± 0.02	6.34^{a} ± 0.03	6.63^{b} ± 0.03	
			Moisture, %		
0	68.63 ± 0.66	69.28 ± 0.87	69.29 ± 0.78	69.38 ± 1.36	69.15 ± 0.41
3	67.88 ± 0.98	69.45 ± 1.28	67.89 ± 0.60	70.03 ± 0.15	68.82 ± 0.48
6	68.06 ± 0.44	67.36 ± 0.91	68.97 ± 1.12	69.03 ± 1.48	68.35 ± 0.50
9	66.64 ± 0.69	68.38 ± 0.89	66.43 ± 0.77	68.96 ± 0.47	67.60 ± 0.45
Mean	67.81 ± 0.38	68.62 ± 0.49	68.15 ± 0.49	69.35 ± 0.47	
			Crude protein, %		
0	19.52 ± 0.51	18.48 ± 0.48	18.78 ± 0.37	19.56 ± 0.64	19.08 ± 0.26
3	20.16 ± 0.58	19.06 ± 1.16	19.96 ± 0.33	18.90 ± 0.51	19.52 ± 0.39
6	18.93 ± 0.77	20.19 ± 0.53	18.75 ± 0.67	19.06 ± 0.90	19.22 ± 0.35
9	19.88 ± 0.71	19.76 ± 0.60	20.16 ± 1.04	19.77 ± 0.30	19.89 ± 0.31
Mean	19.62 ± 0.35	19.37 ± 0.37	19.41 ± 0.34	19.31 ± 0.28	
			Ether extractives, %		
0	9.35 ± 0.69	8.82 ± 0.56	10.38 ± 1.04	9.68 ± 0.63	9.56 ± 0.36
3	8.94 ± 0.26	9.06 ± 0.84	9.96 ± 0.82	10.31 ± 0.94	9.57 ± 0.37
6	10.26 ± 0.38	8.94 ± 0.22	10.05 ± 0.63	9.88 ± 0.35	9.78 ± 0.83
9	9.71 ± 0.80	10.07 ± 0.16	9.56 ± 0.46	8.56 ± 0.65	9.47 ± 0.29
Mean	9.56 ± 0.28	9.22 ± 0.25	9.98 ± 0.34	9.61 ± 0.35	
			Emulsion stability, %		
0	11.19^{a} ± 0.48	8.15^{b} ± 0.36	12.08^{a} ± 0.85	8.01^{b} ± 0.46	9.86 ± 0.59
3	12.09^{a} ± 0.93	7.14^{b} ± 0.65	11.93^{a} ± 1.18	10.25^{c} ± 1.14	10.35 ± 0.73
6	9.86^{a} ± 0.43	8.73^{a} ± 0.48	12.26^{b} ± 0.62	9.32^{a} ± 0.26	10.04 ± 0.45
9	13.44^{a} ± 0.69	10.05^{b} ± 0.58	12.54^{a} ± 1.25	8.69^{b} ± 0.76	11.18 ± 0.68
Mean	11.64^{a} ± 0.48	8.52^{b} ± 0.38	12.20^{a} ± 0.44	9.06^{b} ± 0.40	
			TBA value mg malonaldehyde/kg		
0	0.23 ± 0.03	0.12 ± 0.02	0.19 ± 0.03	0.16 ± 0.02	0.17^{A} ± 0.02
3	0.49^{a} ± 0.07	0.26^{b} ± 0.03	0.31^{ab} ± 0.05	0.23^{b} ± 0.04	0.32^{A} ± 0.04
6	0.73^{a} ± 0.04	0.32^{b} ± 0.07	0.64^{a} ± 0.06	0.37^{b} ± 0.04	0.51^{B} ± 0.05
9	0.82^{a} ± 0.06	0.38^{b} ± 0.04	0.66^{a} ± 0.09	0.45^{b} ± 0.05	0.58^{B} ± 0.06
Mean	0.57^{a} ± 0.07	0.27^{b} ± 0.03	0.45^{ab} ± 0.06	0.30^{b} ± 0.04	

Means bearing similar superscript or no superscript did not differ significantly ($P<0.01$)

HB: Hot deboned, CB : Cold deboned, STP: Sodium tripolyphosphate

consistently preferred over untreated control group throughout the storage. This reflected the beneficial role of STP in producing a significantly more stable stick mix with improved juiciness and in inhibiting lipid peroxidation (Table 1), resulting in less alteration in flavour of the product during storage. The desirable effect of polyphosphates on the moderation of cooked flavour of pork patties was also demonstrated by Keeton (1983). Both HB and CB sticks devoid of STP developed off-flavour after 6 days of storage and became organoleptically unacceptable, whereas the phosphate-treated samples remained fairly acceptable for upto 9 days, regardless of deboning methods.

TABLE 2. EFFECT OF DEBONING METHOD AND POLYPHOSPHATE ON BACTERIAL AND SENSORY QUALITY OF REFRIGERATED (4±1°C) QUAIL STICKS

Days of storage	Treatments HB	HB+STP	CB	CB+STP	Mean
			Aerobic plate count, log/g		
0	4.54 ± 0.04	4.52 ± 0.03	4.55 ± 0.03	4.51 ± 0.03	4.53^{A} ± 0.01
3	4.72 ± 0.01	4.55 ± 0.03	4.74 ± 0.02	4.65 ± 0.02	4.67^{A} ± 0.02
6	5.17^{a*} ± 0.03	4.59^{b} ± 0.03	5.18^{a} ± 0.06	4.62^{b} ±0.04	4.89^{A} ± 0.09
9	6.50^{a*} ± 0.02	5.02^{b} ± 0.02	6.48^{a} ± 0.01	5.00^{b} ± 0.01	5.95^{B} ± 0.22
Mean	5.23^{a*} ± 0.22	4.67^{b} ± 0.06	5.24^{a} ± 0.23	4.70^{b} ± 0.05	
			Psychrophilic count, log/g		
0	2.34 ± 0.03	2.33 ± 0.02	2.53 ± 0.01	2.51 ± 0.01	2.43^{A} ± 0.03
3	2.58 ± 0.02	2.37 ± 0.02	2.52 ± 0.01	2.38 ± 0.02	2.46^{A} ± 0.03
6	3.57 ± 0.01	3.26 ± 0.07	3.56 ± 0.01	3.15 ± 0.05	3.38^{B} ± 0.06
9	4.59 ± 0.02	4.30 ± 0.03	4.58 ± 0.01	4.25 ± 0.03	4.43^{C} ± 0.05
Mean	3.27 ± 0.26	3.06 ± 0.24	3.30 ± 0.25	3.07 ± 0.22	
			Sensory (overall acceptability) score +		
0	6.43 ± 0.37	6.71 ± 0.18	6.57 ± 0.28	6.71 ± 0.28	6.61^{A} ± 0.14
3	5.57 ± 0.43	6.14 ± 0.26	5.71 ± 0.36	6.00 ± 0.31	5.86^{B} ± 0.17
6	4.71^{ab*} ± 0.42	5.43^{a} ± 0.37	4.57^{b} ± 0.43	5.43^{a} ± 0.48	5.03^{C} + 0.21
9	2.28^{a*} ± 0.36	4.57^{b} ± 0.37	2.43^{a} ± 0.48	4.28^{b} ± 0.42	3.39^{D} ± 0.21
Mean	4.75^{a*} ± 0.35	5.71^{b} ± 0.21	4.82^{a} ± 0.35	5.61^{b} ± 0.25	

Means bearing similar superscript or no superscript did not differ significantly ($P<0.01$; *$P<0.05$)
HB : Hot deboned, CB: Cold deboned, STP: Sodium tripolyphosphate
+ 7: Extremely desirable, 1: Extremely undesirable

These results indicated that the quality and storage stability of sticks prepared from HB meat were in no way inferior to those from CB meat with added advantages of energy saving and shortened processing time. In addition, incorporation of STP at permitted level (0.5%) appeared advantageous in improving the product quality and extending its refrigerated (4±1°C) shelf-life by 3 days over control group.

References

Ang CYW, Hamm D (1986) Effect of salt and sodium tripolyphosphate on shear, thiobarbituric, sodium and phosphorus values of hot-stripped broiler breast meat. Poult Sci 65:1532-1538

AOAC (1980) Official Methods of Analysis. 13th edn. Association of Official Analytical Chemists, Washington, DC

Baker RC, Darfler JM, Vadehra DV (1972) Effect of selective additive on the acceptability of chicken frankfurters. Poult Sci 51:1616-1619

Baliga BR, Madaiah N (1971) Preparation of mutton sausage. J Food Sci 36:607-610

Choi YI, Kastner CL, Kropf DH (1987) Effect of hot boning and various levels of salt and phosphate on protein solubility, functionality and sausage characteristics of preblended pork used in frankfurters. J Food Protec 50:1025-1036

Hamm D (1981) Unconventional meat harvesting. Poult Sci 60:1666

Hamm R (1960) Biochemistry of meat hydration. Adv Food Res 10:355-463

Keeton JT (1983) Effect of fat and NaCl/phosphate levels on the chemical and sensory properties of pork patties. J Food Sci 48:878-881,885

Kijowski J, Niewiarowicz A, Kujawska-Biernat B (1982) Biochemical and technological characteristics of hot chicken meat. J Food Technol 17:553-560

Kondaiah N, Anjaneyulu ASR, Salahuddin M. Singh RP. Panda B (1992) Effect of phosphate and spent hen yolk on the quality of chicken patties and *kababs* from spent hens. J Food Sci Technol 29:363-365

Lyon CE, Hamm D, Thomson JE, Hudspeth JP, Ayres JL, Marion JE (1983) Effect of hot or cold deboning on functional properties of dark meat and quality of sausage. Poult Sci 62:965-970

Maxcy RB, Froning GW, Hartung TE (1973) Microbial quality of ground poultry meat. Poult Sci 52:486-491

Pearson AM, Tauber FW (1984) Processed Meats. 2nd edn. AVI Publ Co, Connecticut, USA

Peterson DW (1977) Effect of polyphosphate on tenderness of hot cut chicken breast meat. J Food Sci 42:100-101

Prusa KJ, Bowers JA (1984) Protein extraction from frozen, thawed turkey muscle with sodium nitrite, sodium chloride and selected sodium phosphate salts. J Food Sci 49:709-713

Snedecor GW, Cochran WG (1968) Statistical Methods. 6th edn. Oxford and IBH Publ Co, Calcutta

Sofos JN (1986) Use of phosphates in low sodium meat products. Food Technol 40:52-68

Speck ML (1976) Compendium of Methods for Microbiological Examination of Foods. APHA Inc., Washington, DC

Tarladgis BG, Watts BM, Younathan T (1960) A distillation method for the quantitative determination of malonaldehyde in rancid foods. J Am Oil Chem Soc 39:44-48

Tims MJ, Watts BM (1958) Protection of cooked meats with phosphates. Food Technol 12:240-249

Van-Laack RJM, Smulders FJM (1991) Microbiological condition of restructured steaks prepared from pre-and post-rigor beef. J Food Protec 54:357-359

Wardlaw FB, McCaskill LH, Acton JC (1973) Effect of postmortem muscle changes on poultry meat loaf properties. J Food Sci 38:421-423

Young LL, Lyon CE (1994) Effect of rigor state and addition of polyphosphate on the colour of cooked turkey meat. Poult Sci 73:1149-1152

Young LL, Lyon CE (1977) Effect of post-chill aging and sodium tripolyphosphate on moisture binding properties, colour and Warner-Bratzler shear values of chicken breast meat. Poult Sci 76:1587-1590

Received 30 April 1998; revised 1 June 1999; accepted 9 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 418-423

Nutritional Evaluation of Liver and Body Flesh Lipids of Ray Fish, *Dasyatis bleekeri* (Blyth)

DEBASISH PAL, TARUN K. PATRA, DIPANKAR BANERJEE, JOYDEEP DE AND AMITABHA GHOSH*[1]

Department of Chemistry, Bose Institute,
93/1, Acharya Prafulla Chandra Road, Calcutta - 700 009, India.

Fatty acid compositions and lipid levels in body flesh and liver of sting ray, *Dasyatis bleekeri* (Blyth) were determined. Nutritionally important values were calculated and compared with different sub-classes of lipids. Total lipids from liver and body flesh obtained were 63.4% and 1.3%, respectively. The level of triacylglycerol (92.7%) in liver was higher, whereas phospholipid (59.9%) was major lipid fraction in body flesh. In both the samples, phosphatidylcholine (>50%) was the major component. High levels of ω3 PUFA were found in triacylglycerol (21.7%) in liver and phosphatidylethanolamine (37.6%) and sphingomyelin (33.7%) in body flesh. ω3 PUFA docosahexaenoic acid, eicosapentaenoic acid and ω 6 PUFA arachidonic acid were present in considerable amounts in both the samples.

Keywords: *Dasyatis bleekeri*, Docosahexaenoic acid, Eicosapentaenoic acid, Lipids, Phospholipids.

Lipids and particularly the polyunsaturated fatty acids (PUFA) have long been known to be essential for the maintenance of good health of any individual. Interest in effects of fish oil on human health was accelerated with the studies carried out by Dyerberg et al (1975). They reported on the rarity of heart disease among Greenland Eskimos and its relationship with consumption of lipids high in ω3 PUFA. Lipids are necessary as a source of energy, the transport of fat soluble vitamins and the source of certain essential fatty acids. However, studies have been carried out on the effects of certain fatty acids, especially PUFA (Pigott and Tucker 1987). The fraction of particular interest in fish oils involves the omega-3 fatty acids, especially eicosapentaenoic acid (20:5 ω3, EPA) and docosahexaenoic acid (22:6 ω3, DHA). Studies have shown that dietary EPA prevents medical disorders in heart and circulatory diseases (Simopoulos 1991; Connor and Connor 1997), reverses impairment of endothelium-dependent relaxation (Chin and Dart 1994), inhibits platelet activation (Hay et al. 1982), reduces monocyte attachment to arterial endothelium and suppresses release of toxic, mitogenic and prothrombotic agents (Kim et al. 1990), DHA is also effective in skin disorders, aids brain development and forms a good part of the retina of the eye (Lee et al. 1985).

The sting ray, *Dasyatis bleekeri* (Blyth) belongs to the Family Dasyatidae of the class Chondrichthyes, occupying the lowest group of all living vertebrates with movable jaws and paired appendages. *D. bleekeri* is a dominating cartilagenous fish of the lower zone of the Hooghly-Matlah estuarine complex of West Bengal, India. The present experiment was undertaken to determine the levels of lipids and fatty acids of the body flesh and the liver of *D. bleekeri* as well as explore the possibilities of commercial exploitation of the abundantly available species as a source of marine oil and polyunsaturated fatty acids of -ω3 series.

Materials and Methods

The sting ray, *Dasyatis bleekeri* was collected from the waters of Sunderbans, West Bengal, situated between the latitude 21°31' and 22°30' N and longitudes 88°02' and 88° 16' E. The fish flesh and liver were dissected to separate from the other parts of the body and immediately frozen and stored at -20°C until analyzed.

Extraction of lipid : The total lipids were extracted from the flesh and liver by the method of Bligh and Dyer (1959). BHT (butylated hydroxy toluene) was added at a level of 100 mg/litre to the lipids as antioxidant. The extracted lipids were stored under nitrogen in redistilled hexane and kept at -20°C for future use.

Column chromatography : A portion of the total lipids was resolved into neutral, glyco-and phospholipids by column chromatography on silicic acid (Mallinckrodt, 300 mesh) (Rouser et al. 1967). Each class of lipids was estimated by weighing in a micro balance and stored in redistilled hexane at -20°C.

Thin layer chromatography (TLC) : Thin layer chromatography was performed on 20 x 20 cm^2 chromatoplates covered with silica gel G (0.25-0.50 mm thickness). The neutral lipids were fractionated

* *Corresponding Author;* [1]*Present Address : Drug Research and Development Centre, 1078, Jessore Road, Calcutta 700 028, West Bengal, India.*

by preparative TLC, using light petroleum ether (40°C-60°C)-diethyl ether-acetic acid (80:20:1, v/v/v) (Mangold 1969). While sterylester, 1-*O*-alkyldiacylglycerol, triacylglycerol and sterols were separated, the overlapping hydrocarbon and wax ester bands could only be resolved by rechromatography using light petroleum ether (40°C-60°C)-diethyl ether (98:2, v/v) as solvent system (Mishra and Ghosh 1991). Phospholipids were separated into different classes viz., phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI) and sphingomyelin (SPH), using a solvent system of chloroform-methanol-28% ammonia (65:25:5, v/v/v) (Rouser et al. 1976). Phospholipids were estimated according to Bartlett (1959). Spots of separted lipid components were detected by iodine vapour and by specific spray reagents. All the lipids were identified by comparing the R_f with those of authentic standards. Neutral lipid components were estimated by direct weighing.

Saponification of various lipids : Aliquots of neutral lipids (NL), phospholipids (PL) and separated components of neutral lipids viz., wax esters (WE), steryl esters (SE), 1-*O*-alkyldiacylglycerol (ADAG), triacylglycerol (TG) and phospholipid components, viz., PE, PC, PI and SPH were saponified separately (Christie 1982).

Methylation of fatty acids : Fatty acids of each fraction obtained by the above process were methylated using an etherial solution of diazomethane (Schlenk and Gallerman 1960).

Analysis of fatty acid methylesters (FAME) by gas liquid chromatography (GLC) : Gas liquid chromatography of FAME was done on a Hewlett Packard instrument, model 5890 series II (Hewlett Packard Company, Avondale, PA, USA), equipped with glass column (1.8 m x 2 mm i.d.) and flame ionisation detector (FID). Quantitation was done by an integrator (Hewlett Packard model 3394A) attached to GLC machine. Column used was packed column of 10% diethylene glycol succinate polyester (DEGS) supported on chromosorb-W (HP) 80-100 mesh. Oven temperature was kept constant at 196°C. Detector and injection port temperatures were uniform at 250°C. Nitrogen flow rate was 30 ml/min. Identification of fatty acids was done by (i) comparison of retention times of authentic standards, as well as with the chromatographically purified fatty acids (99.0%) of cod liver oil, as secondary standard as suggested by Ackman and Burgher (1965); (ii) semi-logarithmic plots (Ackman el al. 1963) of relative retention times (RRT) against carbon chain lengths; (iii) comparison of equivalent chain length (ECL) values of the component acids with those reported in the literature (Mishra et al. 1983; Ackman 1969) and (iv) comparison of the chromatogram of catalytically reduced fatty acid methylesters with that of the original sample (Ghosh and Dutta 1972).

Results and Discussion

The total lipid and other various classes and sub-classes of lipids of body flesh and liver of *Dasyatis bleekeri* are presented in Table 1. The total lipids of liver were considerably high (63.4%) whereas, body flesh was low (1.3%). These results are in accordance with the many previous studies on lipids of marine organisms (Ackman 1989; Banerjee et al. 1997)

Among the various classes of lipids, composition of neutral lipids of liver was highest (91.8%) followed by glycolipids and phospholipids. High levels of neutral lipids, particularly, rich in triacylglycerol is very characteristic of liver oils of marine organisms (Pigott 1996). In contrary, the body flesh lipids of *D. bleekeri* indicated high levels (59.9%) of phospholipids and relatively low level (17.2%) of neutral lipids. Phospholipids and glycolipids of body flesh oils together were as high as 82.8% in *D. bleekeri* and comparable to the Atlantic cod, *Gadus morhua* (Addison et al. 1968).

TABLE 1. COMPOSITION OF VARIOUS CLASSES OF LIPIDS OBTAINED FROM THE BODY FLESH AND THE LIVER OF *DASYATIS BLEEKERI*

Lipids	Percent, w/w	
	Body flesh	Liver
Total lipids (TL)[a]	1.3	63.4
Neutral lipids (NL)[b]	17.2	91.8
Glycolipids (GL)[b]	22.9	4.9
Phospholipids (PL)[b]	59.9	3.3
Neutral lipids		
Hydrocarbons (HC)[c]	10.8	3.3
Wax ester (WE)[c]	3.0	0.8
Steryl ester (SE)[c]	7.5	1.7
1-*O*-Alkyl-2-3-diacylglycerol (ADAG)[c]	1.2	1.3
Triacylglycerol (TG)[c]	36.7	92.7
Sterol (ST)[c]	40.8	0.2
Phospholipids		
Phosphatidylethanolamine (PE)[d]	12.4	29.5
Phosphatidylcholine (PC)[d]	53.1	52.5
Phosphatidylinositol (PI)[d]	20.5	12.1
Sphingomyelin (SPH)[d]	14.0	5.9

[a] Expressed as % w/w of west tissue
[b] Expressed as % w/w of total lipids
[c] Expressed as % w/w of neutral lipids
[d] Expressed as % w/w of total phospholipids

High levels of phospholipids in body flesh are reported to be similar to other animals of Sunderbans ecosystem (Dutta et al. 1986; Chattopadhyay 1993). Among the phospholipid fractions, phosphatidyl-choline (PC) is the major fraction in the liver (52.5%) as well as the body flesh (53.1%) and other components are PE (29.5%), PI (12.1%) and SPH (5.9%). In the body flesh of *D. bleekeri*, PE was as low as 12.4% whereas, PI (20.5%) and SPH (14%) were higher than those in the liver lipids. There were altogether seven components present in the neutral lipid fraction of which triacylglycerol was the major component (92.7%) in the liver, whereas, sterol in the body flesh was abnormally high (40.8%).

Fatty acid composition : The fatty acids of neutral lipids and their distribution among different lipid classes are summarized in Table 2. Table 3 shows the total -ω3 fatty acids, total -ω6 fatty acids, -ω3/-ω6 ratios, total polyunsaturated fatty acids (PUFA) and saturated to unsaturated ratio for each component.

The major fatty acids (>4% of neutral lipids) in body flesh of *D. bleekeri* were 16:0, 18:0, 18:1ω9, 18:2ω6, 20:4ω6 and 22:6ω3 acids. Minor components were 16:1ω9, 20:5ω3 and 22:5ω6, whereas, other fatty acids were present in small amounts only. In liver neutral lipids, major fatty acids were 16:0, 16:1ω9, 18:0, 18:3ω3, 20:5ω3 and 22:6ω3 and minor fatty acids were 14:0 and 22:5ω3. Unsaturated fatty acids of liver neutral lipids predominated distinctly over NL of body flesh. Comparison of the results with body flesh revealed that, in liver, NL-ω3 series of fatty acids predominated distinctly over the -ω6 series, as evident from Table 3.

Each neutral lipid class was characterised by a distinct pattern of fatty acids. The ratio of saturates/unsaturates of liver lipids indicated that unsaturated fatty acids predominated over the saturates in TG, whereas, WE, SE and ADAG were rich in saturates. In body flesh, WE and TG contained a little higher levels of total unsaturates than the saturates, while, in SE and ADAG, the levels of total saturates were more than the total unsaturates. The ω3 fatty acids of liver were higher in TG and ADAG, but WE and SE were rich in -ω6 fatty acids, of which proportions of 24:5ω6 in

TABLE 2. FATTY ACID COMPOSITION OF NEUTRAL LIPIDS AND ITS FOUR DIFFERENT SUB-CLASSES OF BODY FLESH AND LIVER OF *D. BLEEKERI*

	NL[c]		WE[c]		SE[c]		ADAG[c]		TG[c]	
Fatty acids[b]	BF	L	BF	L	BF	L	BF	L	BF	L
14:0	0.2	4.0	1.1	6.6	0.6	6.5	1.6	1.2	1.1	1.3
16:0	30.8	23.2	28.2	31.5	36.8	33.5	37.9	32.4	34.3	22.0
16:1ω9	3.8	24.1	12.2	4.7	3.0	3.1	8.6	12.0	15.7	21.0
18:2ω6	0.5	1.2	0.8	0.6	0.4	0.9	-	0.6	0.7	2.9
18:0	15.9	7.1	16.1	14.8	26.7	18.4	16.8	11.5	11.6	9.5
18:1ω9	15.2	11.8	12.6	18.2	10.2	13.0	8.2	18.3	16.3	13.6
18:2ω6	5.2	1.0	2.2	4.5	2.2	2.3	1.3	1.4	6.6	1.2
20:0	-	-	0.5	0.9	1.1	1.1	0.4	0.2	0.4	1.1
18:3ω3	1.9	4.7	2.5	1.3	2.6	1.1	4.7	7.6	2.6	5.0
18:4ω6	0.2	-	0.3	-	-	-	-	0.2	-	-
18:4ω3	0.2	0.5	-	0.2	0.2	0.3	0.3	0.1	0.2	0.6
20:3ω9	-	0.4	-	0.1	-	0.1	-	0.5	-	0.6
22:0	0.4	0.4	1.3	2.0	1.6	2.4	1.2	1.0	0.4	0.4
20:4ω6	6.9	1.9	5.1	3.0	4.8	2.5	4.8	2.1	3.1	1.9
22:2ω6	0.3	0.4	0.6	0.6	0.2	1.0	1.1	2.0	0.2	0.4
20:5ω3	3.7	9.8	6.9	0.1	2.7	0.1	1.1	1.2	3.4	9.8
24:0	-	-	-	-	-	-	-	1.5	-	-
22:1ω9	0.6	-	0.3	-	0.7	-	2.4	-	0.2	-
22:4ω6	1.8	1.2	1.0	1.3	0.8	1.7	1.4	0.5	0.5	1.1
22:5ω6	2.8	1.2	0.9	1.7	1.3	1.1	2.8	2.2	0.5	1.2
22:5ω3	1.5	2.1	2.7	0.5	0.5	0.7	0.8	0.4	0.3	2.1
22:6ω3	7.8	4.5	5.3	1.3	2.4	1.9	3.0	2.2	1.7	4.2
24:5ω6	0.7	0.5	-	6.1	1.2	8.3	1.6	1.1	-	0.6

[a] Abbreviation : NL: Neutral lipid; WE: wax ester; SE: sterylester; ADAG: Alkyl-diacylglycerol; TG: Triacylglycerol; BF: Body flesh; L: Liver; '-' denotes data not detected

[b] First and second figures represent carbon chain length: number of double bonds. The -ω values represent the number of carbon atoms by which terminal methyl is far from the closest olefinic carbon atom

[c] Expressed as % w/w of total fatty acids present in each component

TABLE 3. FATTY ACID LEVELS OF THE BODY FLESH AND LIVER OF *D. BLEEKERI*

	Total-ω3[b]		Total-ω6[c]		-ω3/-ω6		Total PUFA		Saturate/ unsaturate	
Components[a]	BF	L	BF	L	BF	L	BF	L	BF	L
NL	15.1	21.6	18.4	7.4	0.8	2.9	33.5	29.4	0.9	0.5
WE	16.8	3.4	10.9	17.8	1.5	0.22	28.3	21.3	0.9	1.3
SE	8.4	4.1	10.9	17.8	0.8	0.2	19.3	22.0	2.0	1.6
ADAG	9.9	11.5	13.0	9.9	0.8	1.2	22.9	21.9	1.4	1.1
TG	8.2	21.7	11.8	9.3	0.7	2.3	20.0	31.6	0.9	0.5
PL	28.5	24.2	25.8	18.1	1.1	1.3	54.3	42.5	0.3	0.7
PE	37.6	16.0	20.3	16.5	1.8	1.0	57.9	32.9	0.2	1.4
PC	26.1	11.8	24.0	14.0	1.1	0.8	50.1	26.3	0.3	1.0
PI	14.2	13.3	21.8	15.7	0.6	0.8	36.1	29.2	0.9	0.9
SPH	33.7	17.8	26.5	9.9	1.3	1.8	60.2	28.1	0.4	0.9

[a]Expressed as % w/w of total fatty acids present in each component
[b]Includes 18:3ω3
[c]Includes 18:2ω6

both the latter two samples is noteworthy. Comparison of the fatty acid composition of body flesh indicates that TG and ADAG are rich in -ω6 fatty acids, whereas, WE is rich in -ω3 fatty acids, in contrast to the results of the liver lipids. The ratios of saturated-to-unsaturated of body flesh were very similar to those of the air breathing fish, *B. boddaerti* (Banerjee et al. 1997) of Sunderbans ecosystem.

Table 4 shows fatty acid profiles of phospholipids and their individual classes of body flesh and liver lipids, which indicated that unsaturated fatty acids were predominant over the saturated in PL and in four classes of body flesh.

TABLE 4. FATTY ACID COMPOSITION OF DIFFERENT CLASSES OF PHOSPHOLIPIDS OF BODY FLESH AND LIVER OF *D. BLEEKERI*

	PL[c]		PE[c]		PC[c]		PT[c]		SPH[c]	
Fatty acids[b]	BF	L	BF	L	BF	L	BF	L	BF	L
14:0	0.3	0.6	0.3	0.6	0.8	1.3	0.4	1.8	0.2	2.3
15:0	0.1	-	-	-	0.1	-	-	-	-	-
16:0	10.2	25.4	5.3	26.7	16.0	36.2	16.9	20.1	12.0	37.0
16:1ω9	5.7	7.0	4.2	2.2	4.7	10.5	1.2	10.0	1.9	7.0
16:2ω6	1.4	0.4	0.9	0.7	0.4	0.4	0.5	0.5	0.3	0.6
18:0	14.7	16.2	12.6	28.8	7.2	10.8	30.9	25.1	15.5	7.5
18:1ω9	14.2	7.5	19.5	6.5	20.8	13.0	13.8	11.9	9.3	16.8
18:2ω6	0.8	0.6	1.5	1.0	0.9	1.2	1.3	1.1	1.0	1.5
20:0	0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.2	0.2	0.2
18:3ω3	1.6	1.5	1.7	2.5	1.4	4.4	1.3	5.6	1.2	9.2
18:4ω6	0.3	0.1	0.7	-	-	-	0.2	-	-	-
20:3ω9	-	0.3	-	0.4	-	0.5	-	0.2	-	0.4
22:0	0.2	0.5	-	1.0	0.1	0.6	0.4	0.7	0.5	0.5
20:4ω6	16.7	10.5	11.0	5.9	15.4	8.2	16.8	13.3	19.9	4.7
22:2ω6	0.1	0.3	0.1	0.7	0.1	0.5	0.1	-	0.1	0.5
20:5ω3	5.2	4.6	3.0	1.6	5.0	2.5	1.4	4.0	4.7	1.3
24:0	-	0.2	-	2.1	-	1.2	-	0.6	-	0.6
24:1ω9	0.1	-	0.1	-	0.1	-	0.1	-	0.4	-
22:4ω6	2.8	1.4	2.4	1.5	3.1	1.2	1.1	0.4	2.5	1.4
22:5ω6	2.6	4.1	3.2	4.4	3.7	2.1	1.2	0.2	1.5	1.2
22:5ω3	2.7	2.6	2.8	1.2	2.6	0.8	2.1	1.7	2.8	1.0
22:6ω3	18.7	15.4	29.4	10.7	17.1	4.1	9.3	2.0	25.0	6.3
24:5ω6	1.4	0.7	1.2	2.3	0.4	0.4	0.8	0.2	1.2	-

[a] Abbreviation : PL: Phospholipid; PE: Phosphatidylethanolamine; PC: Phosphatidylcholine; PI: Phosphatidylinositol; SPH: Sphingomyelin; BF: Body flesh; L: Liver; '-' denotes data not detected
[b]Shorthand notations have been clarified under Table 2
[c]Expressed as % w/w of total fatty acids present in total phospholipid and in each individual class of phospholipid

In liver phospholipids, proportions of saturated fatty acids were considerably higher in PE, in comparison to unsaturates. In PC, PI and SPH, total saturates to unsaturates were very close to 1.0. Highest ratio of -ω3/-ω6 was in PE of body flesh and also in SPH of liver. Lowest ratio of -ω3/–ω6 was in PC of liver and PI of body flesh. Predominance of 20:4ω6 and 22:6ω3 among the two series of fatty acids were observed in both liver and flesh.

All the lipid classes of body flesh contained relatively high levels of PUFA compared to liver except SE and TG. Higher proportion of PUFA was present in TG of liver, comparable to that in SPH of flesh. High levels of PUFA especially EPA and DHA in liver and body flesh lipids are supported by many previous studies (Ackman 1989; Pastoriza and Sampedro 1992). PUFA levels of fish have been reported to affect vascular function (Chin and Dart 1995) which contributes to a decrease in the overall risk of cardiovascular disease. Incorporation of these PUFA into cardiovascular tissue membranes occurs readily, mainly at the expense of PUFA of the -ω6 type (18:2 and 20:4). The benefits of fish oil-ω3 fatty acids against thrombogenic cardiovascular risk factors are achieved via a favourable eicosanoid profile. Compared to the prostaglandins and thromboxanes of the 2-series (PGI_2 and TXA_2) derived from membrane arachidonic acid (20:4ω6), -ω3 PUFA result in the production of PGI_3 and TXA_3 (Weber 1998). The -ω3 series of eicosanoids derived from -ω3 PUFA have different thrombogenic potencies in comparison to those derived from -ω6 PUFA. Thus, PGI_3 is equipotent to PGI_2 in antithrombotic actions, while TXA_3 possesses only weak biological activity. Thus the risk for ischemic heart disease is reduced (Fitzgerald et al. 1989).

This study has shown that liver and flesh of ray fish, *Dasyatis bleekeri* is a good source of marine oils as well as -ω3 PUFA and they would be suitable for inclusion in the formulation of highly unsaturated diets.

Acknowledgements

Authors thank Department of Biotechnology (DBT), Government of India for financial assistance. Thanks are also due to Professor P.K. Ray, Director, Bose Institute and Professor P.C. Sen, Chairman, Department of Chemistry, Bose Institute for encouragement and providing laboratory facilities.

References

Ackman RG, Burgher RD (1965) Cod liver oil fatty acids as secondary reference standars in the GLC of polyunsaturated fatty acids of animal origin: Analysis of a dermal oil of the Atlantic leather-back turtle. J Am Oil Chem Soc 42:38-42

Ackman RG, Burgher RD, Jangaard PM (1963) Systematic identification of fatty acids in gas-liquid chromatography of fatty acid methyl esters: A preliminary survey of seal oil. Can J Biochem Physiol 41:1627-1641

Ackman RG (1969) Gas-liquid chromatography of fatty acids and esters. In: Lowenstein JM (ed) Methods in Enzymology. Academic Press, New York, Vol 14, pp 329-381

Ackman RG (1989) Fatty acids. In : Ackman RG (ed) Marine Biogenic Lipids, Fats and Oils. CRC Press Inc, Boca Raton. Vol 2, pp145-178

Addison RF, Ackman RG, Hingley J (1968) Distribution of fatty acids in cod flesh lipids. J Fish Res Board Can 25:2083-2090

Banerjee D, Pal D, Patra TK; Misra S, Ghosh A (1997) Lipids and fatty acids of air breathing fish. *Boleophthalmus boddaerti.* Food Chem 60 (3):303-309

Bartlett GR (1959) Phosphorous assay in column chromatography. J Biol Chem 234: 466-468

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911-917

Chattopadhyay G (1993) Investigation on the lipid profiles of the estuarine bivalve *Macoma brimanica* (Philippi) of Sunderbans Mangrove Complex, India. Ph.D. Thesis, University of Calcutta, Calcutta, India

Chin JPF, Dart AM (1994) Therapeutic restoration of endothelial function in hyper-cholesterolemic subjects: Effect of fish oils. Clin Experi Pharmacol 21:749-755

Chin JPF, Dart AM (1995) How do fish oils affect vascular function? Clin Expert Pharmacol 22:71-81

Christie WW (1982) Lipid Analysis. 2nd edn. Pergamon Press, Oxford, England

Connor SL, Connor WE (1997) Are fish oils beneficial in the prevention and treatment of coronary artery disease? Am J Clin Nutr 66 (Suppl 4):1020S-1031S

Dutta AK, Pal PK, Ghosh A, Misra S, Nandi S, Choudhury A (1986) Lipids and fatty acids of the gastropod mollusc *Cerethidea cingulata.* J Am Oil Chem Soc 63:223-225

Dyerberg J, Bang HO, Hjorne N (1975) Fatty acid composition of the plasma lipids in Greenland Eskimos. Am J Clin Nutr 28:958-966

Fitzgerald GA, Braden G, Fitzgerald DJ, Knapp HR (1989) Fish oils in cardiovascular disease. J Intern Med 225 (Suppl 1): 25-29

Ghosh A, Dutta J (1972) A multi-thydrogenator for micro scale hydrogenation of fatty acids. Trans Bose Res Inst 35:13-15

Hay CRM, Durber AP, Saynor R (1982) Effect of fish oils on platelet kinetics in patients with ischaemic heart disease. Lancet 1:1269-1272

Kim DN, Schmee J, Thomas WA (1990) Dietary fish oil added to hyperlipidemic diet for swine results in reduction in the excessive number of monocytes attached to the arterial endothelium. Atherosclerosis 81:209-216

Lee TH, Hoover RL, Williams JD, Sperling RI, Ravalse J, Spur BW, Robinson DR, Corey EJ, Lewis RA, Austin KF (1985) Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on *in vitro* neutrophil and monocyte leukotriene generation and neutrophil function. New Engl J Med 312 (19):1217-1224

Mangold HK (1969) Aliphatic lipids. In: Stahl E (ed) Thin layer Chromatography. Springer, New York, pp 363-415

Misra S, Dutta AK, Dhar T, Ghosh A, Choudhury A, Dutta J (1983) Fatty acids of the mud skipper *Boleophthalmus boddaerti*, J Sci Food Agric 34:1413-1418

Misra S, Ghosh A (1991) Analysis of epicuticular waxes. In: Linskens HF, Jackson JF (eds) Modern Method of Pant analysis. Springer Verlag, Berlin, Vol 12, pp205-229

Pastoriza L, Sampedro G (1992) Chemical composition of the muscle tissue of ray (*Raja radiata*) from *Terranova*, Food Chem 44(2):127-129

Pigott MG (1996) Marine oils. In: Hui YH (ed) Bailey's Industrial Oil and Fat Products, 5th edn. John Wiley & Sons, Inc, New York, Vol 3, pp 225-254

Pigott GM, Tucker BW (1987) Science opens new horizons for marine lipids in human nutrition. Food Rev Int 3 (1&2): 105-138

Rouser G, Kritchevsky G, Yamamoto A (1967) Column chromatographic and associated procedures for separation and determination of phosphatides and glycolipids. In: Marinetti GV (ed) Lipid Chromatographic Analysis. Marcel Dekker Inc, New York, Vol 1, pp 99-103

Rouser G, Kritchevsky G, Yamamoto A (1976) In: Marinetti GV (ed) Lipid Chromatographic Analysis. Marcel Dekker Inc, New York, Vol 3, pp713-727

Schlenk H, Gallerman JL (1960) Esterification of fatty acids with diazomethane on a small scale. Anal Chem 32:1412-1414

Simopoulos AP (1991) Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 54:438-463

Weber PC (1989) Clinical studies on the effects of n-3 fatty acids on cell and eicosanoids in the cardiovascular system. J Intern Med 225 (Suppl 1):61-68

Received 14 March 1998; revised 26 April 1999; accepted 9 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 424-427

Diffusion of Sodium Chloride and Citric Acid in Raw and Fried *Paneer* at Different Temperatures

K. JAYARAJ RAO*[1] AND G.R. PATIL[2]
Division of Dairy Technology,
National Dairy Research Institute, Karnal - 132 001, India.

Diffusion rates of sodium chloride and citric acid were determined in raw and fried *paneer* at 30°, 50° and 70°C. The temperature had a significant effect on the diffusion rate showing higher diffusion rate at higher temperature. Frying of *paneer* slightly retarded the diffusion of sodium chloride and citric acid. At 70°C, the diffusion tended to show "Non-fickian" behaviour. The diffusion coefficient values of NaCl were more than those of citric acid. The activation energy values of citric acid for raw *paneer* were less than those of fried *paneer*, whereas reverse was true with sodium chloride.

Keywords: *Paneer*, Sodium chloride, Citric acid, Diffusion, Water activity, Humectant.

Combined process technique (Hurdle Technology) is currently being advocated for food preservation (Leistner 1994). In this technique, the combined effect of water activity (a_w), pH, heat, redox potential etc., is used to preserve a food so that the sensory and nutritional qualities are not altered much. This necessitates methods to be evolved to adjust the a_w and pH of foods to desired levels, especially in cases, where the integrity of solid food pieces is to be kept unaffected.

One of the techniques to lower the a_w of foods is the moist infusion process - also called osmotic dehydration - in which solid pieces of fresh food are soaked and /or cooked in an appropriate solution to result in a final product having the desired a_w (Kaplow 1970). Differences in a_w between food and the solution cause migration of water out of the food into surrounding solution. However, there is a simultaneous diffusion of the surrounding solutes into the food (Favetto et al. 1981). Humectants and acids are allowed to diffuse into the food particles in order to bring down the a_w and pH, respectively. The rate at which diffusion takes place depends on the type of food as well as humectant and the temperature.

Paneer, an acid coagulated dairy product having soft, sliceable body is very amenable for preservation by hurdle technology (Rao et al. 1991). Humectants and acids can be infused into *paneer* cubes to lower the a_w and pH. In the absence of studies on any diffusion aspects in *paneer* and in order to understand the diffusion phenomenon, one of the common osmotic agents used to lower the a_w viz., sodium chloride and an acidulant (citric acid) were used in this study. Use of citric acid also facilitated to study the diffusion of two compounds of significantly different molecular weights.

Materials and Methods

Raw material : Fresh *paneer* having 55.2% moisture, 23% fat, 16.4% protein, 0.2% acidity (lactic) and pH 5.7 was procured from the Experimental Dairy of the Institute. *Paneer* cubes were fried in refined vegetable oil at 175°-180°C for about 10 min. Frying process resulted in slight reduction in dimensions of *paneer* cubes. Hence, after frying, the cubes of 2 cm size were selected for the experiment, whereas raw *paneer* was directly cut into 2 cm cubes. The fried *paneer* had a composition of 29.7% moisture, 38.1% fat, 25.6% protein, 0.22% acidity (lactic) and pH 5.9.

Diffusion process : Diffusion of sodium chloride and citric acid into *paneer* cubes-raw and fried-was studied according to the method adapted from Favetto et al (1981). Apparatus used for the studies is shown in Fig. 1. Approximately 3 l of humectant solution (8%) or acidulant solution (5%) was taken in 5 l-beaker, which was kept half-immersed in a circular water bath maintained at desirable temperature. A mechanical agitator was provided to agitate the solution continuously (at 50 rpm). Twenty *paneer* cubes were taken in an aluminium basket made of Al-wire, specially devised to hold cubes inside the solution. It was hung just above the agitator. The basket had a lid also made of Al-wire, which contained *paneer* cubes within the basket. The beaker was closed with stainless steel ring plates to minimise evaporation losses. Diffusion was carried out at three temperatures viz., 30°, 50° and 70°C for about 15, 10 and 4 h, respectively to allow complete equilibration. One *paneer* cube was taken out at regular intervals and

* *Corresponding Author: Present Address:[1] D.T. Section, N.D.R.I. (SRS), Adugodi, Bangalore-560 030.*
[2] *Dept of AH & Dairying, Agricultural College, Parbhani, India.*

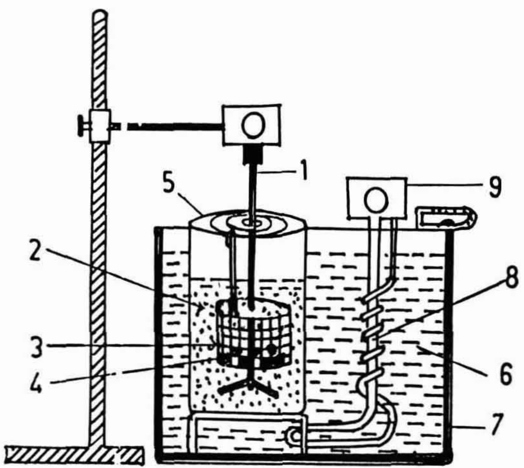


Fig. 1. Apparatus used for diffusion studies
1. Agitator, 2. Humectant/acidulant solution, 3. Aluminium wire basket, 4. *Paneer* cubes, 5. Stainless steel ring plates, 6. Water, 7. Insulated jacket of water bath, 8. Heater coil, 9. Thermostat

its citric acid or sodium chloride contents were determined.

Analytical techniques : Moisture content in *paneer* samples was determined by the procedure described in BIS (1981). The *paneer* cube taken out from the solution was kept on a metal sieve and allowed to drain for 30 sec. The free solution on its surface was removed by gently touching its surface with tissue paper and the cube's weight recorded. It was ground thoroughly in a mortar with a pestle and sodium chloride and citric acid contents were analysed.

Sodium chloride content was determined by International Standard Method (1961), involving the titration of acid-digested sample with standard silver nitrate solution. Citric acid content was estimated by titrating the *paneer* sample against standard sodium hydroxide as per the procedure followed for acidity (AOAC 1984). Initial acidity in terms of citric acid was subtracted from the total acidity to get the final acid content in *paneer*.

Diffusion coefficient (D) and activation energy (E_a) : The sodium chloride or citric acid contents at any given time were expressed as percentage in the moisture present in the *paneer* sample at that time (Mt). At the end of diffusion process, a few *paneer* cubes were left soaked for infinite time (till constant NaCl/citric acid content was obtained), and sodium chloride or citric acid contents (%) present in moisture of *paneer* samples were determined (M_∞). The diffusion coefficient was then calculated by the equation given by Crank (1975) as follows :

$$\frac{Mt}{M_\infty} = 2\left[\frac{D.t}{\pi.1^2}\right]^{1/2}$$

where, 1= half thickness of the *paneer* cube (i.e. 1 cm)

T= time at which sample was taken (min)

D-value at a given temperature was calculated by subjecting the linear portion of Mt/M_∞ Vs time 1/2 plot to simple linear regression. The log D values were plotted against the inverse of the corresponding absolute temperatures and activation energy for diffusion was determined using the following equation:

$$D = A_0 e^{(-E_a/RT)}$$

where, D = Diffusion coefficient

A_0 = Arrhenius constant

E_a = Activation energy

R = Universal gas constant (8.314 J/mol°K)

T = Absolute temperature

Results and Discussion

Sodium chloride content in *paneer* gradually increased with time, indicating the progress of diffusion of sodium chloride, which was linear up to a certain extent (linearity limits : 0.73 and 0.68 Mt/M_∞ at 30°C, 0.65 and 0.73 at 50°C, 0.60 and 0.83 at 70°C in raw and fried *paneer*, respectively) and then the rate of diffusion declined. Linear regression equations for the linear portion of the diffusion plot are given in Table 1. It was also observed that the rate of diffusion increased with temperature, as evident from the diffusion coefficients shown in Table 2. These values also showed that diffusion of sodium chloride was faster in raw *paneer*. The diffusion coefficient-temperature plot (Fig. 2) showed two ranges, 30°-50°C and 50°-70°C. Activation energy values were also more for raw *paneer* (Table 2).

In literature, no reports on diffusion of sodium chloride in *paneer* have been found. However, the D-values obtained in this work can be compared with those of beef cubes reported by Favetto et al (1981). The D-values of sodium chloride in raw and fried *paneer* at 30°C were higher than those of NaCl in beef cubes (0.94 X 10^{-5} cm^2 s^{-1}). This difference is because of basic nature of product. Meat has a compact, closely knit texture, whereas *paneer* has a porous, yet a cohesive texture. Frying slightly retarded the diffusion rate, as this process is

TABLE 1. REGRESSION EQUATIONS FOR LINEAR PORTION OF DIFFUSION

Product	Humectant/ acidulant	Temperature of diffusion	Regression equation	Correlation coefficient
		30°C	Y = 0.257 + 0.00392 X	0.97
Raw *paneer*	NaCl	50°C	Y = 0.215 + 0.00750 X	0.97
		70°C	Y = 0.198 + 0.02026 X	0.96
		30°C	Y = 0.220 + 0.00361 X	0.99
Fried *paneer*	NaCl	50°C	Y = 0.270 + 0.00400 X	0.98
		70°C	Y = 0.226 + 0.00880 X	0.98
		30°C	Y = 0.163 + 0.00266 X	0.98
Raw *paneer*	Citric acid	50°C	Y = 0.193 + 0.00331 X	0.98
		70°C	Y = 0.136 + 0.00929 X	0.96
		30°C	Y = 0.220 + 0.00171 X	0.97
Fried *paneer*	Citric acid	50°C	Y = 0.156 + 0.00371 X	0.97
		70°C	Y = 0.133 + 0.00959 X	0.96

$Y=Mt/M_\infty$, X= time of diffusion (min)

TABLE 2. DIFFUSION COEFFICIENTS AND ACTIVATION ENERGIES OF DIFFUSION OF SODIUM CHLORIDE AND CITRIC ACID IN *PANEER* AT DIFFERENT TEMPERATURES

	Diffusion coefficient, D (cm^2 s^{-1})			Activation energy, Ea (kJ/mole)		
Product	Temperature, °C	D, NaCl	D, Citric acid	Temperature, °C	E_a, NaCl	E_a, Citric acid
Raw *paneer*	30	3.64 X 10^{-3}	2.10 X 10^{-3}	30-50	19.3708	13.8272
	50	5.86 X 10^{-3}	2.95 X 10^{-3}	50-70	39.9411	60.6125
	70	13.96 X 10^{-3}	11.00 X 10^{-3}			
Raw *paneer*	30	2.86 X 10^{-3}	1.10 X 10^{-3}	30-50	11.4544	28.2000
	50	3.79 X 10^{-3}	2.20 X 10^{-3}	50-70	39.4457	68.3304
	70	8.93 X 10^{-3}	9.70 X 10^{-3}			

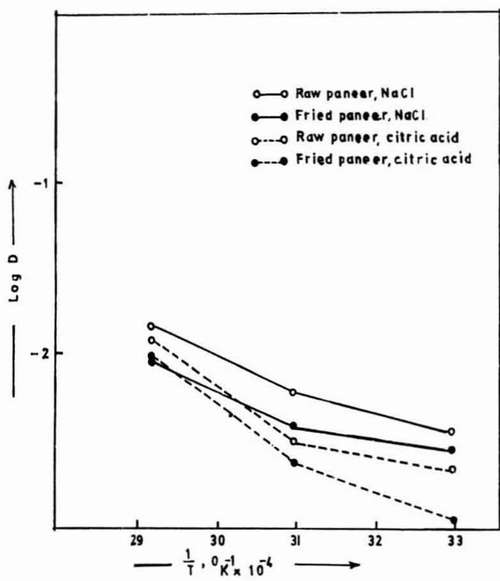


Fig. 2. Diffusion constant (D)-temperature (T) plot for sodium chloride and citric acid in raw and fried *paneer* cubes

known to result in the compaction, which completely vanishes granularity of raw *paneer* (Kalab et al. 1988). The activation energy value of 19.37 kJ/ mole (equivalent to 4.63 kcal/mole) obtained in this study for raw *paneer* was comparable to that reported for beef (4.4 kcal/mole) by Favetto et al (1981).

In case of citric acid, the diffusion progressed up to a certain stage (linearity limits: 0.90, 0.97 and 0.90 Mt/M_∞ at 30°, 50° and 70°C, respectively for raw *paneer*, 0.88, 0.90 and 0.92 Mt/M_∞ at above temperatures for fried *paneer*). Regression equations for linear portion of diffusion plot are given in Table 1. At 50° and 70°C, "Non-fickian" behaviour was observed, which could be ascribed to the transient sample temperature during initial stages of immersion (Favetto et al. 1981). This behaviour was neglected, while determining the D-values and activation energies. The D-values of citric acid at all temperatures in fried *paneer* were less than in raw *paneer*. This trend was the same as for sodium chloride (Table 2). The diffusion coefficient-temperature plot (Fig. 2) showed two ranges viz., 30°-50°C and 50°-70°C. The activation energy values of fried *paneer* were higher than those for

raw *paneer* reversing the trend obtained with sodium chloride. It was also observed that the D-values of sodium chloride were higher than those of citric acid at all temperatures except at 70°C, which was ascribable to prominent "Non-fickian" behaviour of diffusion in fried *paneer*. The higher D-values of sodium chloride could be attributed to its lower molecular weight (60.5) than that of citric acid (162).

The results of this study have indicated that the diffusion is an important phenomenon, when a_w of solid food pieces has to be adjusted without affecting their integrity. The diffusion rate mainly depends on the temperature, molecular weight of humectant and the texture of a product. For example, in fried *paneer*, the diffusion was slightly retarded. These results may be helpful in the product development studies for determining equilibration periods.

References

AOAC (1984) Official Methods of Analysis. Association of Official Analytical Chemists, Inc., Virginia, USA

BIS (1981) Handbook of Food Analysis: Dairy Products. SP: 18 Part IX. Bureau of Indian Standards, New Delhi, p165

Crank J (1975) The Mathematics of Diffusion. Oxford University Press, London, UK

Favetto G, Chirife J, Bartholomai GB (1981) A study of water activity lowering in meat during immersion-cooking in sodium chloride-glycerol solutions. I. Equilibrium considerations and diffusional analysis of solute uptake. J Food Technol 16:609-619

International Standard Method (1961) Determination of the Salt Content of Cheese FIL-IDF-17: p 2

Kalab M, Gupta SK, Desai HK, Patil GR (1988) Development of microstructure in raw, fried and cooked *paneer* made from buffalo, cow and mixed milks. Food Microstructure 7:83-91

Kaplow M (1970) Commercial development of intermediate moisture foods. Food Technol 24:53-57

Leistner L (1994) Food design by hurdle technology and HACCP. Publishers, Adalbert-Raps Foundation, Adalbert-Raps Str 1, Kulmbach, Germany

Rao JK, Dresel J, Leistner L (1991) Anwendung der Hurden technologie in entwicklungs landern zum Beispiel fur paneer. Annual report of Federal Center for Meat Research, Kulmbach, Germany

Received 27, March 1997; revised 13 July 1999; accepted 9 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 428-433

Physico-Chemical Changes During Storage of Papaya Fruit (*Carica papaya* L.) Bar (*Thandra*)

K. ARUNA*[1], V. VIMALA[1], K. DHANALAKSHMI[2] AND VINODINI REDDY[3]

[1]Department of Foods and Nutrition, Post-Graduate and Research Centre,
[2]Department of Microbiology, College of Veterinary Science,
Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad - 500 030
[3]National Institute of Nutrition, Hyderabad - 500 007, India.

Papaya fruit bar (*Thandra*) was stored at room temperature (25-45°C) for nine months and the physico-chemical and microbiological changes were studied during the storage period. It was also stored at different temperatures and organoleptic changes were evaluated. Sensory evaluation of fruit bar revealed higher deterioration in colour, appearance and texture on 6 and 9 months storage at higher temperature. The losses of total carotenes, β-carotene and vitamin C were 54, 46 and 43%, at the end of the storage period. The products stored for 6 months were found to be superior from the textural and odour points of view and with minimum physico-chemical changes. Microbiological count was observed on 6 months storage and increased with increase in storage temperature. Equilibrium relative humidity studies revealed that 3 mil high density polyethylene could be used to store the product for 10 months.

Keywords: Papaya *thandra*, Papaya fruit bar, Physico-chemical changes, Sensory qualities, Microbiological quality, Equilibrium relative humidity.

India is the fourth largest producer of papaya (FAO 1991) with an output of about 9.05 lakh tonnes (Chadha 1995). Though papaya is a nutritious fruit, it is not widely used in products due to its odd flavour, which is not acceptable to many people (Aruna 1995). *Thandra* is a term acquired from Telugu script to represent a dry fruit (bar) product, (e.g., mango *thandra* otherwise called as mango fruit bar). *Thandra* is an age old traditional method of preserving this fruit and it is widely accepted by all age groups (Nanjundaswamy et al. 1976; Rao and Roy 1980a). Due to processing, the odour of papaya decreases as the volatiles evoporate and thus becomes acceptable to many consumers (Morales and Duque 1987). The present study was undertaken to develop *thandra* from papaya and assess its keeping qualities.

Materials and Methods

Preparation of the pulp : Pulp was prepared from evenly ripened fruit procured in bulk from the local wholesale market. The fruit was washed thoroughly in water and then in one per cent solution of potassium permanganate to prevent it from being infected. The cleaned, peeled and cut papaya pieces were put into the pulper with 50 mesh sieve. The pulp was analyzed for total soluble solids (TSS), pH and total acidity. The acidity of the pulp was increased to 0.7% by adding citric acid, heated to 100°C, filled into sterilized bottles and crown corked. The bottled pulp was stored at room temperature (9 to 24°C) and used within 5 days for the preparation of papaya fruit bar.

Preparation of papaya fruit bar or thandra : Fruit bar was prepared by dehydration method. Acidity and °Brix of the pulp were adjusted to 0.7% and 30 °Brix, mixed, spread evenly on trays (250g/sq.ft) and each layer was dried at 60°C for 8 h. The proportion of ingredients, was calculated for 10 kg. of the pulp (CFTRI 1990). The method of preparation of bar is given in the flow diagram (Fig. 1).

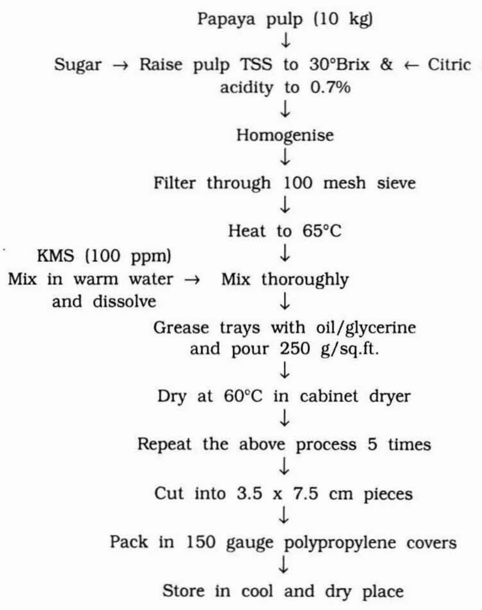


Fig. 1. Flow sheet for the preparation of papaya bar

* Corresponding Author

Storage studies : The prepared papaya fruit bar was wrapped in 150 gauge polyethylene covers and stored in a stainless steel tin at room temperature (25-45°C) with relative humidity (RH) of 35 to 75%, for 9 months. Physico-chemical characteristics were evaluated intermittently at intervals of 0, 3, 6 and 9 months during storage at 25 to 45°C. Papaya fruit bar was stored at different temperatures and microbiological and organoleptic changes were assessed.

Physico-chemical characteristics : The chemical constituents present in papaya fruit influence the nutritional and storage qualities of the product. Physico-chemical characteristics such as bulk density (Griswold 1962), acid insoluble ash (AOAC 1965), non-enzymatic browning (Baloch et al. 1973), total acids, sulphur dioxide and pectin (Ranganna 1986), moisture (AOAC 1984), total ash (AOAC 1984), total sugars and reducing sugars (Lane and Eynon 1923), vitamin C (AVC 1966) and total carotenes and β-carotene (Zakaria et al. 1979; Nelis and DeLeenher 1983) were analyzed at the end of 0, 3, 6 and 9 months of storage at 25 to 45°C.

Microbiological count : Microbiological count was carried out in papaya fruit bar at 0, 3, 6 and 9 months storage period. The products were stored at 5 to 8°C (refrigerator temperature), 9 to 24°C (winter temperature), 25 to 34°C (summer temperature) and 35 to 45°C (peak summer temperature). The viable counts of bacteria, yeast and mould were determined by Pour-Plate method (Cruickshank et al. 1975).

Organoleptic evaluation : Sensory evaluation was done using 20 trained panelists as per the standard procedure (CFTRI 1986). The products were stored at 5 to 8°C (refrigerator temperature), 9 to 24°C (winter temperature), 25 to 34°C (summer temperature) and 35 to 45°C (peak summer temperature). In order to evaluate the products, a score card was developed. Five point scale was used for ranking i.e., 5=excellent, 4=very good, 3=good, 2=fair, 1=poor and 0=very poor.

Equilibrium relative humidity (ERH) studies : The ERH of papaya fruit bar was studied by using Wink (1946) weight equilibrium method.

Statistical analysis : Various physico-chemical characteristics of papaya fruit bar were subjected to one way analysis of variance. The critical differences were also calculated (Snedecor and Cochran 1967).

Results and Discussion

Physico-chemical changes : During dehydration process of papaya fruit bar, the weight and volume of the product decreased, resulting in a significant deterioration in bulk density (Table 1). The prepared

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF PAPAYA FRUIT BAR (PER 100G PRODUCT)

Physico-chemical characteristics	Storage period, months				
	0 (a)	3 (b)	6 (c)	9 (d)	C.D. values
Weight, g#	44.60	47.36^{a}	43.69^{b}	43.34^{b}	2.3013*
Volume, ml	41.59	44.78^{a}	41.60	41.34	1.9883**
Bulk density, g/ml	1.07	1.06	1.05^{a}	1.05^{a}	0.0115**
Total acids, g (as citric acid)	1.20	1.25^{a}	1.30^{ab}	1.39^{abc}	0.0460***
pH#	4.05	3.94	3.85^{a}	3.69^{abc}	0.1549***
Acid insoluble ash, mg	0.05	0.04	0.05	0.04	NS
Non-enzymatic browning, OD#	0.10	0.12	0.16^{ab}	0.19^{ab}	0.0237***
Sulphur dioxide, ppm	900.00	500.00^{a}	225.00^{ab}	160.00^{abc}	76.3674***
Pectin, g (as calcium pectate)	1.66	1.59	1.62	1.59	NS
TSS, °Brix#	82.93	82.63	81.83^{ab}	81.50^{ab}	0.3501*
Moisture,	19.62	19.68	18.63	17.40^{ab}	1.2916***
Total ash, g	1.60	1.60	1.59	1.59	NS
Total sugars, g	68.28	68.72	67.43^{ab}	66.90^{ab}	0.6286***
Reducing sugars, g	58.82	59.86^{a}	59.09^{b}	59.21^{b}	0.5568***
Non-reducing sugars, g	9.46	8.86^{a}	8.34^{a}	7.69^{abc}	0.5908***
Vitamin C, mg	53.90	43.12^{a}	35.36^{ab}	24.75^{abc}	2.7762***
Total carotene, mg	3.35	2.95^{a}	2.42^{ab}	1.81^{abc}	0.0797***
β-carotene, mg	0.99	0.86^{a}	0.73^{ab}	0.56^{abc}	0.0545***

NS: Not Significant; *Significant at 5% level; ** Significant at 1% level; *** Significant at 0.1% level; C.D. Critical difference-at 0.001 level; # Indicates product values

Superscripts of a, b and c indicate significant differences with those columns of the same row

papaya fruit bar had 82.93° Brix initially, which decreased to 81.53° Brix, on 9 months storage. The significant increase in total acids can be attributed to loss of moisture, resulting in the concentration of the product during storage (Nanjundaswamy et al. 1976; Rao and Roy 1980 b; Mir and Nath 1993). As a result of increase in acidity, a significant decrease in the pH of papaya fruit bar, was noticed. The acid insoluble ash and total ash were not altered during storage.

A drastic increase was noticed in the non-enzymatic browning of the product and the increase was significant on 9 months storage. This increase in non-enzymatic browning might be due to decrease in sulphur dioxide (Swaminathan 1987; Mir and Nath 1993), higher temperature (Meyer 1966), as expected from Arrhenius equation (Mir and Nath 1993) and concomitant with heat and loss of sulphur dioxide content of the product (Rao and Roy 1980 b). This might be due to reaction of sulphur dioxide with food constituents (sugars, pectins, proteins and lipids), oxidation or due to volatilisation (Bolin and Boyle 1972; Echkoff and Okos 1986).

Sulphur dioxide showed a significant and drastic decline from 900 ppm to 160 ppm, during the study. Since sulphur dioxide is an antioxidant (Meyer 1966), it provided protection initially from autooxidation and as storage temperature and period of storage increased, sulphur dioxide decreased. Mir and Nath (1993) and Rao and Roy (1980 b) in mango bar studies observed similar changes.

The pectin content of papaya fruit bar decreased in a non-significant manner as the period of storage increased. This probably was due to the conversion of protopectin (insoluble) into pectinic acid and pectic acid further to D-galacturonic acid (Meyer 1966; Singh et al. 1983). A similar finding was reported by Singh et al (1983) on storage of guava cheese for 120 days.

The moisture content of papaya bar decreased significantly on storage and it may be attributed to evaporation of water from bar due to high storage temperature and loss of sulphur dioxide (Rao and Roy 1980 b). Papaya fruit bar like mango fruit bar was hygro-emissive (Nanjudaswamy et al. 1976) and thus kept well in the range of 50 to 60% RH.

Significant decreases in total and non-reducing sugars in papaya fruit bar might be due to significant increases in reducing sugars (Mir and Nath 1993) by acid hydrolysis of total and non-reducing sugars and thereby inversion of total (Rao and Roy 1980 a) and non-reducing sugars to reducing sugars (Meyer 1966; Roy and Singh 1979).

Vitamin C (53.9 mg per 100g), total carotenes (3.35 mg per 100g) and β-carotene (0.99 mg per 100g) decreased drastically on storage for 9 months. The changes in physico-chemical characteristics were greater on storage for 9 months and were minimum when stored for 3 months. Vitamin C content of fruit bar decreased (Table 1) significantly and progressively during storage due to its thermolabile nature. Retention of vitamin C was fairly good upto 3 months storage, due to adequate amounts of sulphur dioxide in the product, but after 3 months storage, higher loss of vitamin C with concomitant loss of sulphur dioxide was observed. Thus, sulphur dioxide might be protective to vitamin C. During storage, the loss of vitamin C was high (54%), which might be due to exposure to light, air, product area exposed and length of storage period.

The losses of total carotenes (46%) and β-carotene (43%) in papaya fruit bar were significantly high during storage period. The losses could be due to non-oxidative changes (*cis-trans* isomerization, epoxide formation or thermal degradation) or oxidative changes. Such changes altered the colour of the product and lowered the flavour and nutritive value of the product (Land 1962; Eskin 1979)). Changes in carotenoids have been reported in mango products (Ranganna and Siddappa 1961; Goday and Rodriguez-Amaya 1987; Mir and Nath 1993). Greater retention values of β-carotene and total carotenoids were observed upto 3 months in papaya fruit bar and during this period, the sulphur dioxide content was also more. The higher retention of β-carotene in papaya fruit bar during 3 months storage was due to the protective action of sulphur dioxide (Foda et al. 1972; Bolin and Stafford 1974; Rao and Roy 1980 b).

During storage, significant changes were not observed for acid insoluble ash, total ash and pectin, whereas significant changes were noticed in weight, volume, bulk density and TSS. Significant changes were noticed in moisture, total acids, pH, vitamin C, total carotenes, β-carotene, pectin and sulphur dioxide on 3, 6 and 9 months storage.

Microbiological count : Microbiological count (Table 2) was not observed upto 3 months storage. But on 6 months storage, yeast and mould counts were noticed and these increased further on 9 months storage. The increase in microbiological count was proportionate to storage temperature.

TABLE 2. MICROBIAL LOAD OF PAPAYA FRUIT BAR DURING 9 MONTHS STORAGE

Storage temperature	Microbiological counts	Storage period, months 0	3	6	9
5 to 8°C	Yeast and mould counts	Nil	Nil	Trace	Trace
	Bacterial counts	Nil	Nil	Trace	Trace
9 to 24°C	Yeast and mould counts	Nil	Nil	2.0 x 10^1	3.0 x 10^1
	Bacterial counts	Nil	Nil	1.0 x 10^1	2.0 x 10^1
25 to 34°C	Yeast and mould counts	Nil	Nil	3.0 x 10^1	1.5 x 10^2
	Bacterial counts	Nil	Nil	2.0 x 10^1	8.0 x 10^1
25 to 34°C	Yeast and mould counts	Nil	Trace	1.5 x 10^2	2.0 x 10^2
	Bacterial counts	Nil	Trace	4.0 x 10^1	9.0 x 10^1

Note: Yeast and mould and bacterial counts were not observed on 0 and 3 months storage

Organoleptic evaluation : Scores for papaya fruit bars initially were 26.01 (for 30) and on storage decreased to 24.93, 24.39, 22.13 and 19.80 (Table 3), when stored at 5 to 8°C (refrigerator-temperature), 9 to 24°C (winter-temperature), 25 to 34°C (summer temperature), 35 to 45°C (peak summer-temperature) respectively. Significant differences were observed in colour and appearance of papaya fruit bar, when stored at 25°C and above (Chan and Cavaletto 1978) due to increase in the non-enzymatic browning, which doubled (0.19 OD) on 9 months storage and thus resulted in decreases in the mean scores of colour and appearance. Significant deteriorative changes were noticed in papaya fruit bar texture due to stickiness developed during storage at 5 to 8°C and 25°C and above. The increase in stickness might be due to increase in acidity and absorption of moisture on storage. The panel members did not detect papaya aroma in products stored at different temperatures and this could be mainly due to the evaporation of the volatiles responsible for the aroma during the preparation of the product. An odd odour different from the natural odour of papaya was detected by some of the panel members, when papaya fruit bar was stored at 25°C and above. The product was considered by some panel members as slightly hard in texture with a sticky mouthfeel, irrespective of storage temperature. The stickiness might have been due to increases in acidity and reducing sugars. The organoleptic acceptability decreased with increase in storage temperature.

Equilibrium relative humidity (ERH) : Initial moisture content of the product was 7.72%, on dry weight basis. At 60% relative humidity (RH), the product retained its characteristics like good shape,

TABLE 3. ORGANOLEPTIC QUALITIES OF PAPAYA BAR STORED AT DIFFERENT TEMPERATURES

Storage temperature	Sensory parameters	Storage period, months 0	3	6	9	Significance
5 to 8°C	Colour & appearance	4.75	4.75	4.71	4.68	NS
	Texture	4.50	4.38	4.00	3.75	*
	Mouthfeel	4.25	4.25	4.20	4.20	NS
	Aroma/odour	3.75	3.75	3.75	3.65	NS
	Taste	4.38	4.40	4.35	4.32	NS
	Overall quality	4.38	4.40	4.40	4.33	NS
	Total	**26.01**	**25.93**	**25.41**	**24.93**	
9 to 24°C	Colour & appearance	4.75	4.59	4.25	4.04	NS
	Texture	4.50	4.38	4.35	4.05	NS
	Mouthfeel	4.25	4.25	4.20	4.18	NS
	Aroma/odour	3.75	3.75	3.65	3.62	NS
	Taste	4.38	4.35	4.33	4.30	NS
	Overall quality	4.38	4.29	4.25	4.20	NS
	Total	**26.01**	**25.61**	**25.03**	**24.39**	
25 to 34°C	Colour & appearance	4.75	4.38	3.88	3.38	**
	Texture	4.50	4.38	4.00	3.75	*
	Mouthfeel	4.25	4.13	4.00	4.00	NS
	Aroma/odour	3.75	3.75	3.63	3.25	NS
	Taste	4.38	4.13	4.00	4.00	NS
	Overall quality	4.38	4.25	4.00	3.75	NS
	Total	**26.01**	**25.02**	**23.51**	**22.13**	
35 to 45°C	Colour & appearance	4.75	4.13	3.38	3.00	**
	Texture	4.50	4.28	3.90	3.35	**
	Mouthfeel	4.25	4.01	3.84	3.20	*
	Aroma/odour	3.75	3.65	3.35	3.15	NS
	Taste	4.38	4.13	3.85	3.60	*
	Overall quality	4.38	4.20	3.90	3.50	*
	Total	**26.01**	**24.40**	**22.22**	**19.80**	

Maximum scores for each parameter is 5; NS Not significant; * Significant at 5% level; ** Significant at 1% level

texture and colour. The critical point was 10.17% equilibrium moisture content (EMC) at 60% RH and danger point was at 8.33% EMC at 55% RH and initial point was at 7.72% EMC at 53% RH (Table 4). To store 45 g product with surface area of 177.4 square cm for 6 months, the maximum permissible water vapour transmission rate (WVTR) was 45 x 10^{-8} cc/cm^2/sec/cm/ of Hg at 25°C and 65% RH. Based on WVT (30 mil), 3 mil high density polyethylene (0.954) was recommended to store for 10 months.

Cost of the product : The cost of papaya fruit bar was calculated as per the existing prices, at the time of the study. The cost of papaya bar was Rs. 450/-per 10 kg. The cost of papaya bar was Rs. 4.50 per 100 g. whereas the cost of mango bar was Rs. 10 to 15/-per 100 g. Thus, the cost

TABLE 4. EQUILIBRIUM RELATIVE HUMIDITY DATA OF PAPAYA FRUIT BAR AT ROOM TEMPERATURE

EMC %	RH %	No. of days required to reach equilibrium	Remarks
0.21	0	140	Shrunken, dry, brittle and dark colour
1.33	10	140	Shrunken, dry, brittle and dark colour
2.00	20	140	Shrunken, dry, brittle and dark colour
3.33	30	140	Shrunken, dry, brittle and slightly dark colour
4.64	40	140	Shrunken, dry, slightly brittle and slightly dark colour
6.59	50	100	Good shape, slightly dry and good colour
10.36	60	81	Good shape, good texture and good colour
16.08	70	53	Moist, light colour and mould growth
22.50	80	18	Moist, light colour and mould growth
29.16	90	12	Watery, light colour and mould growth
40.15	100	8	Watery, light colour and mould growth

EMC: Equilibrium moisture content RH: Relative humidity

of similar product was two-fold more than the cost of the papaya fruit bar.

Conclusion

Physico-chemical, organoleptic and microbial changes were studied in papaya fruit bar during storage at room temperature. Significant changes were noticed in bulk density, total acids, pH, non-enzymatic browing, sulphur dioxide, pectin, TSS, moisture, total sugars, non-reducing sugars, vitamin C, total carotenes and β-carotene. On storage, the retentions of vitamin C, total carotenes and β-carotene were 46 to 57%. To minimise changes, storage at low temperature was found to be suitable. Since the product deteriorated on 9 months storage and deterioration was minimal on 6 months storage, the papaya fruit bar should not be stored beyond 6 months.

Acknowledgements

The first author is grateful to Acharya N.G.Ranga Agricultural University, Hyderabad, for granting fellowship to undertake the study. The authors thank Central Food Technological Research Institute Regional Centre, Hyderabad and National Institute of Nutrition, Hyderabad for extending laboratory facilities.

References

AOAC (1965) Official Methods of Analysis, 10th edn. Association of Official Analytical Chemists, Washington, DC

AOAC (1984) Official Methods of analysis, 14th edn. Association of Official Analytical Chemists, Arlington

Aruna K (1995) Propagation, processing and storage studies of papaya (*Carica papaya* L.) and its products, Ph.D. Thesis, Andhra Pradesh Agricultural University, Hyderabad

AVC (1966) Official Methods of Analysis, 3rd edn. Association of Vitamin Chemists, New York

Baloch AK, Buckle KA, Edwards RA (1973) Measurement of non-enzymatic browning of dehydrated carrot. J Sci Food Agric 24:389-398

Bolin HR, Boyle FP (1972) Effect of storage and processing on sulphur dioxide in preserved fruit. Food Product Development 7:82-86

Bolin HR, Stafford AE (1974) Effect of processing and storage on pro-vitamin A activity and vitamin C in apricots. J Food Sci 39:1034-1035

CFTRI (1986) Sensory analysis and consumer acceptance-Workshop on Sensory Analysis of Foods held at CFTRI, pp 1-5

CFTRI (1990) Papaya in India: Production, Preservation and Processing. Industrial Monograph Series, CFTRI, Mysore, India

Chadha KL, (1995) Changing Scenario of Horticulture in India. Dr. P.G. Krishna Memorial Lecture delivered at Birla Science Museum Naubit Pahar, p 8

Chan Jr HT, Cavaletto CG (1978) Dehydration and storage stability of papaya leather. J Food Sci 43:23-25

Cruickshank R, Duguid JP, Marmion BP, Swain RHa (1975) Medical Microbiology - The Practice of Medical Microbiology. Churchill Livingstone, Edinburgh, UK

Echkoff SR, Okos MR (1986) Kinetics of sulfite reactions in corn grain. J Agri Food Chem 34:239-245

Eskin NAM (1979) Plant pigments, flavours and textures. The Chemistry and Biochemistry of Selected Compounds. Academic Press, London

Foda YH, Hegazi SM, Selem SA (1972) Changes occurring in certain chemical components of sun-dried apricot sheets with relation to discolouration during storage. Sudan J Food Sci 4:57-60

FAO (1991) FAO Production Year Book 45:164-170

Goday HT, Rodriguez-Amaya DB (1987) Changes in individual carotenoids on processing and storage of mango slices and puree. Inter J Food Sci Technol 22:451-460

Grisworld RM (1962) Experimental Study of Foods. Houghton Mifflin Co, Boston, New York, USA

Land DG (1962) Stability of plant pigments. Advanced Food Research 2:50-56

Lane JH, Eynon LJ (1923) In: Handbook of analysis and quality control for fruit and vegetable products by Ranganna S, Tata McGraw-Hill Publishing Co Ltd., New Delhi, pp14-16

Meyer LH (1966) Food Chemistry, Reinhold Publishing Corporation, New York

Mir MA, Nath N (1993) Storage changes in fortified mango bars. J Food Sci Technol 30:279-282

Morales AL, Duque C (1987) Aroma constituents of the fruit of the mountain papaya (*Carica pubescens*) from Colombia. J Agric Food Chem 35:538-540

Nanjundaswamy AM, Radhakrishnaiah Setty G, Saroja S (1976) Studies on the development of newer products from mango. Indian Food Packer 30:95-103

Nelis HJCF, DeLeenheer AP (1983) Isocratic non-aqueous reversed-phase liquid chromatography of carotenoids. Analyt Chem 55:270-275

Ranganna S (1986) Handbook of Analysis and Quality Control for Fruit Vegetable Products. Tata McGraw-Hill Publishing Co Ltd, New Delhi

Ranganna S, Siddappa GS (1961) Effect of processing on the absorption spectra of carotenoid pigments of Badami mango. Food Technol 16:204-206, 308-309

Rao VS, Roy SK (1980 a) Studies on dehydration of mango pulp I. Standardization for making mango sheet/leather. Indian Food Packer 34:64-71

Rao VS, Roy SK (1980 b) Studies on dehydration of mango pulp. II. Storage studies of the mango sheet/leather. Indian Food Packer 34:72-79

Roy SK, Singh RN (1979) Studies on utilization of bael fruit (*Aegle marmelos correa*) for processing: III. Preparation and prservation of bael fruit products. Indian Food Packer 33:9-14

Snedecor GW, Cochran WG (1967) Statistical Methods. Iowa State University Press Ames, Iowa, USA

Singh R, Kapoor AC, Gupta OP (1983) The effect of cultivars, seasons and storage on the nutritive value and keeping quality of guava cheese, Indian Food Packer 37:71-77

Swaminathan M (1987) Food Science, Chemistry and Experimental Foods. The Bangalore Printing and Publishing Co Ltd., Bangalore

Wink WA (1946) Determining the moisture equilibrium curves of hygroscopic materials. Industrial Engng Chem Analytical Edition 18:251-252

Zakaria M, Simpson K, Brown PR, Krustulovic A (1979) Use of reversed phase HPLC analysis for the determination of pro-vitamin A carotenes in tomatoes, J Chromatogr 176:10

Received 1 June 1999; revised 17 August 1999; accepted 19 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 434-435

Effect of Parboiling, Hand-pounding and Machine-milling on Chemical Composition of Rice

SADHNA SINGH*, MANORANJAN KALIA AND S.R. MALHOTRA
Department of Food Science and Nutrition,
HP Krishi Vishvavidyalaya, Palampur-176 062, India.

The effect of parboiling, hand-pounding, machine-milling and genotype on chemical composition of rice was studied. Parboiled rice was found to have higher protein and ash contents and low free amino acids and free fatty acids as compared to non-parboiled rice. In contrast to machine-milled rice, hand-pounded rice contained not only higher protein, fat and ash, but also high free fatty acids, indicating poor storage quality. Irrespective of various treatments, different genotypes under experiment showed significant variations for proteins, free amino acids, fat, free fatty acids and total ash contents.

Keywords : Parboiling, Paddy, Hand-pounding, Machine-milling, Genotype, Chemical composition.

Parboiling of rice is the common practice in India and in some south Asian countries. As a result, about half the rice produced in the region is consumed as parboiled rice (Bhattacharya 1985). Parboiled rice is produced by soaking paddy in water till saturation, steaming it to gelatinize the starch, followed by drying and milling either by hand-pounding using such devices as wooden pestle and mortar or in small power-driven huller or large mechanized rice mills. In ancient times, dehusking was done, using stone pestle and mortar or chakki (stone mills), but in the present day, it is being done in rice mills. The present communication reports the results of a study on the effects of parboiling, hand-pounding, and machine-milling on chemical composition of rice.

Paddy samples of nine genotypes were procured from the Rice Research Station, Malan (Kangra) and Research sub-station, Katrain (Kullu) of Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India.

Parboiling : Paddy samples were cleaned and parboiling was done by soaking them in warm water (70°±2°C for 3.5 h, draining and autoclaving at1.05 kh/cm^2 for 15-25 min, depending upon the genotype, till no white core was left. The paddy was then shade-dried till 12±2% moisture level was attained.

Machine-milling : Paddy samples were dehusked in a laboratory type sheller (Satake Rice Sheller) and polished in McGill Miller No. 2 to obtain six per cent degree of polish.

Hand-pounding : Hand-pounding of paddy samples was done using stone pestle and wooden mortar and partially milled rice samples were separated from the husk.

Chemical analysis : Crude protein, fat and total ash were determined according to AOAC (1990) procedures. Free amino acids were determined by the method of Lie (1973) with a slight modification in the extraction by extracting one gram of finely ground samples with 100 ml of 70% ethanol for 1h. Ethanol was evaporated at 40°C and volume was made up to 50 ml. A 2 ml aliquot was reacted with one ml of colouring reagent (containing Na_2HPO_4-100g, potassium dihydrogen phosphate - 60 g, ninhydrin - 5 g and fructose - 3 g/litre). Glycine was used as a standard. The free fatty acids were determined according to AACC (1976) procedure.

Statistical analysis : Data on various parameters were replicated thrice and analysed using factorial design (Steel and Torrie 1960). All the values have been reported on dry weight basis.

Results of the study revealed that parboiled samples contained higher protein and ash contents as compared to non-parboiled rice, while fat, free fatty acids and free amino acids were significantly lower (Table 1). Damir (1985) also reported similar results. However, Raghavendra Rao and Juliano (1970) found slightly lower range for protein in parboiled rice as compared to raw brown rice.

Statistically significant differences were also observed among hand-pounded and machine-milled rice for crude proteins, fat, free amino acids and free fatty acids (Table 1). Machine-milling decreased the protein fat and ash contents. Saikia and Bains (1990) also found a lower range of fat in milled rice as compared to brown rice. The free fatty acid contents were found to be lower in machine-milled rice as compared to hand-pounded rice, indicating better storage quality of machine-milled rice.

The different genotypes studied also varied significantly with respect to proteins, fat, ash, free

* Corresponding Author

TABLE 1. EFFECT OF PARBOILING HAND-POUNDING AND MACHINE-MILLING ON CHEMICAL COMPOSITION OF RICE

Nutrient	Non-parboiled	Parboiled	Hand-pounded	Machine-milled	C.D. (0.05)
Crude proteins, %	8.40	8.64	8.77	8.26	0.11
Crude fat, %	1.45	0.79	1.20	1.03	0.08
Total ash, %	0.92	1.00	1.17	0.74	0.06
Free fatty acids, % as oleic acid	0.28	0.06	0.19	0.11	0.01
Free amino acids, % as glycine	0.21	0.10	0.21	0.09	0.004

* Values are mean of nine genotypes

TABLE 2. EFFECT OF GENOTYPES ON CHEMICAL COMPOSITION OF RICE

Genotype	Category	Crude protein, %	Fat, %	Total ash, %	FFA, % as oleic acid	FAA, % as glycine
'HPU 2216'	*Indica*	7.98	1.27	0.83	0.19	0.15
'RP 2421'	*Indica*	9.52	1.04	1.02	0.17	0.14
'HPU 845'	*Indica*	8.89	1.04	1.05	0.18	0.15
'Kasturi'	*Indica*	10.47	1.15	0.79	0.20	0.10
'China purple'	*Indica*	8.19	0.89	0.76	0.20	0.15
'Naggar dhan'	*Japonica*	7.06	1.23	0.84	0.16	0.38
'Norin 18'	*Japonica*	7.84	1.20	0.74	0.13	0.13
'Matali'	*Japonica*	8.46	1.25	1.36	0.17	0.16
'Jatoo'	*Japonica*	8.25	1.04	1.25	0.15	0.10
CD (0.05)		0.22	0.16	0.13	0.01	0.01

fatty acids and free amino acids (Table 2). The maximum crude protein was recorded in long grained 'kasturi'. In general, protein content was higher in long grained *Indica* genotypes as compared to medium and short-grained *Japonicas*. The maximum fat content was recorded in 'HPU 2216'. The fat contents of genotypes under study ranged from 0.89 to 1.27%, while a range of 0.36 to 0.69% fat in 18 *Indica* and *Japonica* rice varieties has been reported by Charkraborthy et al (1972). Free fatty acid contents were found to be the highest in 'China Purple' and 'Kasturi', while the lowest values were found in 'Norin 18'. The ash contents of different genotypes ranged from 0.74 to 1.36%.

It may be concluded that parboiling of paddy should be encouraged and milling as desired to increase the storage quality of rice should be done, but over-milling should be avoided.

References

AACC (1976) Approved Methods of Analysis. American Association of Cereal Chemists. St. Paul Minn, USA

AOAC (1990) Approved Methods of Analysis. Association of Official Analytical Chemists, Washington, DC, USA

Bhattacharya KR (1985) Parboiling of Rice In: Rice Chemistry and Technology. American Association of Cereal Chemists. St. Paul Minn, USA. pp 289–348

Chakraborthy TK, Dwarakanath KR, Bhatt PB (1972) Studies on physico-chemical properties of some varieties of rice. J Food Sci Technol 9(3):140–143

Damir AA (1985) Comparative studies on the physico-chemical properties and microstructure of raw and parboiled rice. Food Chem 16(1):1–14

Lie S (1973) The E.B.C. Ninhydrin method for determination of free amino nitrogen. J Inst Brew 79:37–41

Raghavendra Rao SN, Juliano BO (1970) Effect of parboiling on some physico-chemical properties of rice. J Agric Food Chem 18:289

Saikia, L, Bains GS (1990) Studies on some Assam rice varieties for processing and nutritional quality. J Food Sci Technol 27(5):345–348

Steel RGD, Torrie JH (1960) Principles and Procedures of Statistics. McGraw-Hill Book Co., New York

Received 13 October 1997; revised 30 July 1999; accepted 10 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 436-437

Non-destructive Measurement of Vacuum in Canned Water

S.G. PATWARDHAN

Central Food Technological Research Institute, Regional Centre,
Bhavan's College Campus, Andheri (West), Mumbai-400 058, India.

Boiling point of canned drinking water was determined. Vapour pressure related to boiling point was deducted from atmospheric pressure to arrive at vacuum in mm. Vacuum was also measured by vacuum gauge can tester. No significant disagreement was found in the two measurements of vacuum.

Keywords : Vacuum, External measurement of vacuum, Vapour pressure.

In case of heat-processed or vacuum-packed canned products, estimation of vacuum is of paramount importance. Presently, vacuum is measured by vacuum gauge can tester. During measurement, the can gets punctured and the product cannot be stored any more. If the vacuum could be estimated without puncturing the can, it would be most welcome by the canning industry. For this, vacuum exposure of cans is a suggested method (Howard 1949). In a consignment to be purchased, the buyer can measure vacuum in a number of cans to get a feel of the shelf life of the product without much objection, as the can remains intact. However, the method requires vacuum pump, etc. and therefore, is of no use for field test.

When one starts heating water, at a certain point, continuation of supply of heat produces vapour bubbles. This is called boiling point or simmering point of water. This is the point when vapour pressure produced due to heating equals to that of the environment, i.e., water boils or simmers when the vapour pressure of the heated water equalises with the atmospheric pressure above the surface of water i.e., 760 cm. at sea level.

The boiling or simmering point of canned water can be determined to relate to vapour pressure inside the can. The difference between the external atmospheric pressure and the vapour pressure in the closed container is called vacuum (Hans-Joachim Longo 1983). In this experiment, 10 old canned drinking water samples were analysed to study the boiling point vacuum relation.

In case of canned water, boiling water, naturally free of dissolved gases, was filled in the cans, cooled and heat processed for commercial sterilisation. In this process, vacuum was created inside the can, to enable the water inside the can would boil at a lower temperature than 100°C, which is the boiling point of water at sea level.

* *Corresponding Author*

A canned water sample was heated on a gas stove over a wire gauge. "RIEGE" German make thermometer (minus 20° to 360°C) was held by hand vertically touching the top surface of the can. When the water inside the can started boiling/ simmering, the vapour bubbles produced inside the can gave a simmering sensation to the hand. holding the thermometer. This was confirmed by 5 persons. This was recorded as simmering point, which is considered equivalent to boiling point.

For a system to undergo a change of phase, heat must be added to it, or taken from it, quite apart from the heat necessary to bring its temperature to the required value. Here, it can be stated (Robert and David 1995) that a process that takes place in such a way that no heat flows into or out of the system is called an adiabatic process. The words "boiling point" or "simmering point" wherein change of phase takes place are used freely without any demarcation.

It may be necessary to clarify whether by touching the thermometer on the can, one can assess the boiling point. For calibrating temperature against pressure in an autoclave, Western Regional Instrumentation Centre, Vidyanagari, Mumbai, India adopted the following satisfactory method. Water (2 litres) was filled in an Al American autoclave (capacity 25 quarts) with metal to metal joint with lid. (dia. 13" height 12"). The autoclave was heated on a gas stove. The probe of a sensitive temperature recorder was touched on to the top of the autoclave. As the pressure went on rising, the temperatures were recorded. It was found that the autoclave was working quite satisfactorily and the temperature and pressure correlated well.

After recording the simmering/boiling point of the closed canned water sample measured using hand held thermometer, the can was cooled in flowing water and the vacuum was measured, using a vacuum gauge can tester at room

temperature. It has to be noted that if the heating is continued the simmering point/boiling point will go on rising as the vapour pressure inside the can goes on rising similar as in autoclave and unlike water boiling in open atmosphere.

An embossed can code/manufacturing date, simmering/boiling point, vapour pressure, vacuum arrived by deducting vapour pressure from atmosphere pressure and vacuum measured by vacuum gauge are shown in Table 1. It can be seen that the new method almost tallies with the old vacuum gauge method. Errors originating from thinning of headspace vacuum of the can through the inflowing air from the vacuum gauge itself are markedly seen (Table 1). This is in agreement with the statement by (Hans-Joachim Lango 1983) "the ordinary vacuum gauge always shows lower values than that actually in the headspace (before the perforation of the lid of the can)."

When the vacuum measured by the new method is less than 17 inches corresponding to 73°C, the can bulges on continued heating. Therefore, the method cannot be continued. However, the minimum vacuum for canned water is 15 inches as per CFTRI specifications and therefore, the product can should be rejected. If the simmering/boiling temperature is higher than 73°C, the can will remain normal without bulging.

The samples of 6 years old canned water with embossed code 070890 were tested and it was found that the cans started bulging beyond 56°C and 70°C. The cans were cooled and their vacuum was measured by vacuum gauge can tester to find that the values were 4 inches and 11 inches, respectively. This would confirm the dividing line of 73°C.

TABLE 1. VACUUM AND SIMMERING POINT OF CANS

Embossed can code	Simmering point, °C	Vapour pressure, mm	760 mm – V.P. mm	Vacuum in inches
140393	73	265.7	494 = 20°	17
180593	61	156	604 = 24°	24
140294	61	156	604 = 24°	24
180294	.65	187	573 = 23°	21
171094	62	164	596 = 24°	21
031294	57	130	630 = 25°	23
081293	60	149	611 = 24°	22
280394	65	167	573 = 23°	22
280394	80	355	405 = 16°	16
170993	56	136	624 = 25°	22

Source: Vapour pressure from "Hand Book of Chemistry and Physics", published by the Chemical Rubber Publishing Company, Cleveland, USA, 1962

Further work relating to a new method with respect to canned brine, canned vegetables in brine can lead to a field test for the measurement of vacuum inside such cans, which will be of much help to the Industry.

The author is grateful to Dr. M.N. Krishna Murthy for scrutiny and suggestions for improvement in the paper.

References

Hans-Joachim Lango (1983) Methods of Analysis for the Canning Industry. Food Trade Press, Orpington, England pp 250-252

Howard AJ (1949) Canning Technology p 182

Robert R, David H (1995) New Age International Limited, Publishers, Bangalore, pp 562-654

Received 22 September 1998; revised 30 July 1999; accepted 10 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 438-440

Non-refrigerated Storage of Tomatoes - Effect of HDPE Film Wrapping

ASHOK KUMAR, B.S. GHUMAN AND A.K. GUPTA*

Department of Processing and Agricultural Structures,
Punjab Agricultural University, Ludhiana-141 004, India.

Tomatoes at turning stage (the stage when the fruits start changing colour from greenish yellow to red) were plucked from the field and stored in evaporative cooling brick and sand store (ECBS). Tomatoes with wrapping in 0.01 mm (10 μm) thick HDPE film and without wrapping to extend their shelf life were kept in ECBS. Their storage behaviour was compared with tomatoes kept in open under ambient conditions by wrapping in HDPE film and without wrapping. Unwrapped tomatoes kept in open (35-40°C; 70-80% RH) were unacceptable after 3 days due to more than 40% weight loss and wrapped tomatoes after 10 days due to 10% weight loss. Tomatoes stored in ECBS gave better results, as temperature inside the store was 26-29°C and RH was 35 to 99%. The safe storage periods were found to be 8 and 25 days, respectively for wrapped and unwrapped tomatoes kept in ECBS. The effect of storage conditions on tomatoes turning red was also observed and maximum retarded turning was found to be in case of wrapped tomatoes in ECBS.

Keywords : Tomato, HDPE Film wrapping, Weight loss, Brick and sand store, Evaporative cooling, Non-refrigerated Storage.

The recent emphasis laid by the Government agencies on the diversification of crops has resulted in the increase in areas under cultivation of fruits and vegetables in the State of Punjab. High yielding cultivars of various vegetables have been introduced by the large scale tomato processing companies, resulting in increased tomato production in the State. During harvesting season usually May and June, the Punjab State faces a tomato glut in the markets, while at other times, the tomato prices are quite high. The tomato prices in the State vary from Rs. 2 to Rs. 30 per kg. (Singh et al. 1989). The environment temperatures during this glut period are generally higher than 40°C and tomato being highly perishable lasts only for 4-5 days. Post-harvest losses amount to about 35-40% of the total produce (Singh et al. 1989). Various low cost techniques have been suggested to enhance the storage life of perishables. Evaporative cooling is effective under high temperature and low humidity conditions (Hall 1961). Controlled atmosphere (CA) and modified atmosphere (MA) storage in which commodities are stored under reduced O_2 and increased CO_2 levels to reduce decay due to respiration are some other techniques. Modified atmosphere storage combined with low temperature storage extends the shelf life significantly (Anon 1969; Buescher 1975; Mansfield 1978; Singh et al. 1965). But MA and CA methods are quite costly and complicated to establish at retail outlet level. Seal packaging in plastic films retards the deterioration of many fruits and vegetables (Hardenberg 1971; Ben-Yehoshua 1985). In case, growers are able to store tomatoes without any significant losses for a few more weeks after the harvest, they can increase their profits as the tomato prices start rising from the last week of June onwards. The present study carried out on the storage of tomatoes signifies the effect of HDPE film wrapping during storage in a low cost evaporative cooling brick and sand store.

Storage : Tomatoes of "Rupali" cultivar manually plucked, when greenish yellow in colour, were used in this experiment. Tomatoes were washed thoroughly for the storage experiment carried out in the laboratory of the Department of Processing and Agricultural Structures, PAU, Ludhiana. Two storage conditions were selected - ambient and the evaporative cooling brick and sand (ECBS) store. Two sets of samples - unwrapped and individually wrapped in HDPE films (10 μm thickness) were prepared for each storage condition. The samples were loaded on 20 kg capacity wooden trays (120 x 40 x 8 cm) with wire mesh bottom. One set of unwrapped and wrapped tomatoes was stored under ambient conditions, while the other set was stored in a ECBS store. This store is a permanent structure (1.9 x 1.3 x 0.6 m) having double walls with a gap of 7.5 cm in between filled with sand, which is kept moist by pouring water twice a day. The water poured was sufficient enough to completely saturate the store. The top cover is a wooden frame covered by moist jute bags. The experiment was conducted in summer months (May and June). Dry and wet bulb

* *Corresponding Author*

TABLE 1. ENVIRONMENTAL TEMPERATURE AND RELATIVE HUMIDITY DURING STORAGE (WEEKLY AVERAGE)

Storage period, weeks	Environmental conditions: Ambient temperature and relative humidity		ECBS store	
	DBT, °C	RH, %	DBT, °C	RH, %
1	34.2	79.0	23.1	93.4
2	34.3	73.8	23.3	93.6
3	38.2	69.7	23.7	95.0
4	39.8	72.4	25.0	95.4
5	40.1	70.8	25.2	96.0
6	39.0	69.9	26.5	95.6
7	38.6	69.8	27.2	95.6
8	35.4	75.7	26.6	95.1
9	34.9	74.3	27.6	94.9
10	35.1	73.7	29.8	93.8
11	32.3	81.7	29.8	94.4
12	32.0	80.3	29.7	94.8
13	33.9	83.9	29.6	95.1
14	33.1	79.3	29.8	95.4

DBT = Dry Bulb Temperature; RH = Relative Humidity

thermometers were used for recording the ambient as well as ECBS storage conditions, thrice a day at an interval of 4 h. Relative humidity was calculated from wet bulb depression. Environmental temperature and relative humidity during storage are given in Table 1. Storage losses which mainly included the weight loss due to transpiration and rottening were recorded twice a week. Other observations included the TSS (%) and percentage of tomatoes turning red (Sandhu 1995). The results are presented in Tables 2, 3 and 4. The effect of the storage period on percentage total weight loss for various storage conditions is shown in Fig. 1.

Decay : The results shown in Table 2 for the ambient storage conditions clearly indicate the extended shelf life of HDPE film-wrapped tomatoes. The unwrapped tomatoes became completely rotton in two weeks, while it took 4 weeks for HDPE film-wrapped tomatoes to reach the same stage. Similar effect of HDPE film wrapping was evident as seen from Table 3 for ECBS stored tomatoes, which took 14 weeks to rot the entire lot compared with 8 weeks for the unwrapped ones. The curves in Fig. 1 show the overall storage behaviour under different storage conditions. There was a marked improvement in the storage period for both the samples stored in ECBS store. This is due to improved environment (reduced temperature and increased humidity) inside the brick and sand store compared with the ambient conditions. The average weekly ambient conditions recorded over the entire study period of 15 weeks gave dry bulb temperatures in the range from 32 to 40°C and RH from 60 to 84%. The environment inside the ECBS store during the same period was recorded as dry bulb temperatures in the range from 26 to 29°C and RH 95 to 97%. Thus, the ECBS store provides a 6 to 11°C drop in dry bulb temperature and 1 to 26% improvement in the RH. Considering 10% total weight loss as the acceptable level, the

TABLE 2. WEIGHT LOSS (%) OF TOMATOES STORED UNDER AMBIENT CONDITIONS

Storage period, weeks	Unwrapped L_1	Unwrapped L_2	Unwrapped Total	HDPE film-wrapped L_1	HDPE film-wrapped L_2	HDPE film-wrapped Total
0	-	-	-	-	-	-
1	6.1	70.0	76.1	1.6	1.2	2.8
2	7.8	90.3	98.1	3.8	12.6	16.4
3	-	-	-	7.2	36.1	43.3
4	-	-	-	14.7	85.3	100.0

L_1 = % Weight loss due to shrinkage
L_2 = % Weight loss due to rotting

TABLE 3. WEIGHT LOSS (%) OF TOMATOES STORED IN ECBS STORE

Srorage period, weeks	Unwrapped L_1	Unwrapped L_2	Unwrapped Total	HDPE film-wrapped L_1	HDPE film-wrapped L_2	HDPE film-wrapped Total
0	-	-	-	-	-	-
1	1.5	6.0	7.6	0.3	1.0	1.3
2	2.3	14.2	16.5	1.4	4.5	5.9
3	3.5	19.5	23.0	1.7	4.9	6.6
4	6.2	31.0	37.2	3.2	9.1	12.3
5	9.1	42.7	51.8	3.8	14.2	18.0
6	12.0	61.0	73.0	6.5	18.7	25.2
7	17.0	73.3	90.3	8.0	23.6	31.6
8	18.5	81.5	100.0	8.8	33.2	42.0
9	-	-	-	8.9	45.4	54.3
10	-	-	-	9.2	60.0	69.2
11	-	-	-	9.6	72.8	82.4
12	-	-	-	9.8	78.0	87.8
13	-	-	-	10.1	82.3	92.4
14	-	-	-	11.0	89.0	100.0

L_1 = % weight loss due to shrinkage
L_2 = % weight loss due to rotting

TABLE 4. PERCENTAGE TURNING OF TOMATOES UNDER VARIOUS STORAGE CONDITIONS

Storage period, weeks	Ambient conditions Unwrapped	Ambient conditions HDPE film-wrapped	ECBS-stored Unwrapped	ECBS-stored HDPE film-wrapped
1	19.0	21.0	15.0	0
2	-	31.0	65.0	35.0
3	-	78.0	92.0	70.0
4	-	-	100.0	85.0
5	-	-	-	98.0
6	-	-	-	100.0

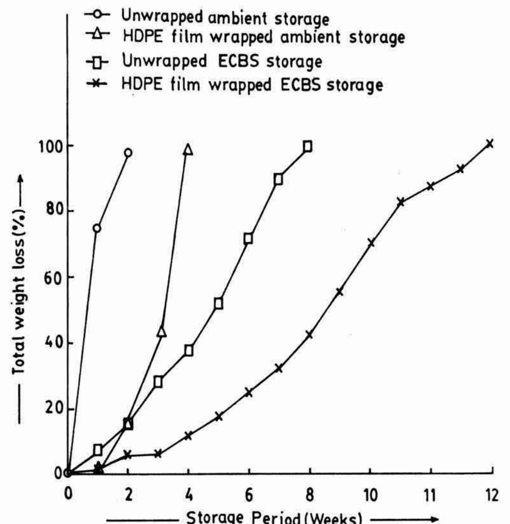


Fig. 1. Percentage total weight loss of tomatoes during storage under different conditions

unwrapped and HDPE film wrapped tomatoes under ambient conditions can be safely stored upto 4 and 12 days, whereas the storage periods in ECBS store for unwrapped and wrapped tomatoes are 8 and 25 days, respectively.

Though the HDPE film-wrapped samples stored at ambient conditions indicated improved storage period compared with unwrapped samples stored in ECBS store, the combination of both HDPE film wrapping and ECBS store environment provided an adequate storage upto about 4 weeks. Some suitable additional treatment for the reduction of rottening loss may be provided to further enhance the storage life.

Colour changes of tomatoes : The effect of storage conditions on turning of yellow tomatoes to red is shown in Table 4. The maximum retarded turning is in case of HDPE film-wrapped tomatoes stored in ECBS store. Thus, there is a clear relationship between the stage of harvest of tomatoes and the safe storage period. The effect of storage period and the mode of storage on the TSS was found to be insignificant. For most of the time under all the different storage conditions, the TSS was observed to be about 4.5%.

The results of this study on non-refrigerated storage of tomatoes have suggested a safe storage period of 4 weeks for HDPE film-wrapped tomatoes stored in ECBS store. Even ambient storage results for wrapped tomatoes have indicated a safe storage cum handling period upto 10 days. These results are quite encouraging for growers as well as large and small scale marketing, since it would enable the distribution of tomatoes to distant markets during off-season within the country and abroad. The evaporatively cooled brick and sand store being permanent and low cost structure appears to be quite promising for short duration storage of tomatoes during the summer months.

References

Anon (1969) Oxygen starved tomatoes stay fresher. Chem Engg News 47:53–66

Ben-Yehoshua S (1985) Individual seal packaging of fruits and vegetables in plastic film– a new post-harvest technique. Hort Sci 20(1):32–37

Buescher RW (1975) Influence of CO_2 on post-harvest ripening and deterioration of tomatoes. J Am Soc Hort Sci 104:545–547

Hall CB (1961) The effect of low storage temperature on the colour caroteniod pigments, shelf life and firmness of ripened tomatoes. Am Soc Hort Sci 78:480–487

Hardenburg RC (1971) Effect of in-package environment on keeping quality of fruits and vegetables. Hort Sci 6(2):198–201

Mansfield DH, Morris LL, Strand LL (1978) The potential for prolonged storage of tomatoes in controlled atmosphere. Proc. 3rd Tomato Quality Workshop, Adult Education Centre, University of Maryland, USA

Singh AK, Kashyap MM, Gupta AK (1985) Studies on tomatoes in CO_2 enriched atmosphere. Proc. ISAE, SJC, Vol III pp 25–29

Singh AK, Bhumbla VK, Gupta AK, Singh Y (1989) An overview on storage of tomatoes. Proc. ISAE, SJC, Processing Food Engg. pp 98–104

Sandhu BS (1995) Non-refrigerated storage of tomatoes. B.Tech. Dissertation, Department of Processing Agricultural Structures, Punjab Agricultural University, Ludhiana

Received 16 January 1998; revised 26 April 1999; accepted 10 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 441-443

Effect of Spices on Urinary Calcium and Serum Vitamin A Levels in Adult Women

B. PRAVEENA AND G. SAROJINI*

Post-Graduate and Research Centre, College of Home Science,
Andhra Pradesh Agricultural University, Hyderabad-500 030, India.

Seven healthy women volunteers were fed three experimental diets containing mixed spice powder at 0.5% level and red chilli powder at two levels i.e., at 1.25% and 2.5% each for a period of 7 days. The results showed significantly (F>0.01) lower levels of urinary calcium (107.96±6.88 mg/24 h) in subjects, fed diets containing mixed spice and higher levels (175.01±4.12 mg/24 h and 205.28±6.58 mg/24 h) on red chilli powder diet at 1.25 and 2.5% levels as compared to control diet without spice (133.50±8.18 mg/24 h). Similarly, serum vitamin A levels showed non-significant (F>0.01) higher levels (25.16±6.08 µg/dl and 26.28±6.89 µg/dl) with mixed spice diet at 0.5% and red chilli powder diet at 1.25% as compared to those obtained on control diet (22.71±6.30 µg/dl). However, the levels were lower with red chilli powder diet (18.30±5.39 µg/dl) at 2.5% compared to those obtained on control diet.

Keywords : Spices, Vitamin A, Urinary calcium, Red chillies, Mixed spices.

Spices are indispensable in our culinary practice and are reported to be good sources of dietary fibre, polysaccharides with high uronic acid residues, oxalates and phytates (Rama Sastry 1983; Gopalan et al. 1988), which interfere with the absorption of calcium in gastro-intestinal tract and reabsorption in the urinary tubule, which may potentially alter the calcium metabolism. In addition, spices are known to be good sources of β-carotene in the diets of rural familes (Thimmayamma et al. 1983; Madyastha 1983). Spices increase bile secretion and favourably enhance the absorption of fat soluble vitamins (Sambaiah and Srinivasan 1991). The aim of the present investigation was, therefore, to study the effect of spices on urinary calcium and serum vitamin A levels in adult women.

Seven healthy female volunteers aged between 20-23 years of homogenous group were selected for the study. A control (bland) diet, which was free of spices and three experimental diets were administered to each subject. Spices were incorporated equally in three meals per day. Balanced diets were formulated for each subject to provide 1800 Kcal, 50 g proteins, 30 g of fat, 500 mg of calcium, 2450 µg of β-carotene per day. Three experimental diets namely : a) mixed spice diet (containing a mixture of 10 spices with the following composition (for 100 g) black pepper-5 g, cardamom-5 g, coriander-5 g, caraway seeds-5 g, cloves-5 g, cinnamom-5 g, coriander-55 g, cumin-10 g, dry ginger-4 g, garlic-4 g, turmeric-2 g at 0.5% (4.65 g) level (for 7 days); b) red chilli powder diet at 1.25% (11.25 g) level (for 7 days) and c) red chilli powder diet at 2.5% (23.24 g) level (for 7 days) were fed to the subjects. Initially, the control diet was fed for a period of 7 days followed by mixed spice diet and then, the control diet so as to nullify the effect of previous experimental diet.

On the 8th day i.e., after the completion of each diet, 24 h urine was collected and the aliquots were estimated for calcium (Baginski et al. 1973). Venous blood (5 ml) was collected on the same day and the serum was analysed for vitamin A by HPLC (Esiong 1991). Statistical analysis was done using the method of Snedecor and Cochran (1967).

When mixed spice diet at 0.5% level was fed, the urinary calcium levels were significantly lower (107.96±6.88 mg/24 h) as compared to subjects fed on control diet (133.50±8.18 mg/24 h). These spices contain dietary fibre polysaccharides with high uronic acid residues (Uma Nageshwar Rao 1988), which are known to reduce the availability of calcium for absorption in small intestine by binding to the non-cellulosic fraction (James et al. 1978). Further, the phytates present in spices precipitate in the intestinal tract to form calcium phytate and thus, severely affect calcium absorption (Reinhold et al. 1981; Brooner 1988).

Contrary to the above findings, when diets containing red chilli powder diet at 1.25% and 2.5% levels were fed, the urinary calcium levels were significantly higher 175.01±4.12 mg/24 h and 205.28±6.58 mg/24 h as compared to control. This increase may be due to enhanced absorption of calcium in the gastrointestinal tract or decreased reabsorption from kidney tubules. Red chillis contain high amounts of lysine and arginine and these

* *Corresponding Author*

TABLE 1. EFFECT OF SPICES AND CORRELATION COEFFICIENT VALUES ON URINARY CALCIUM AND SERUM VITAMIN A LEVELS IN HUMAN SUBJECTS

Type of diet	Level of spice, g	Urinary calcium, mg/24h	'r' values of urinary calcium	Serum vitamin A, µg/dl	'r' values
Control diet (Bland)	Nil	133.50±8.18	ND	22.71±6.30	ND
Mixed spice powder diet (0.50%)	4.65	107.96±6.88	–0.384*	25.12±6.08	+0.976
Red chilli powder diet (1.25%)	11.62	175.01±4.12	+0.644*	26.28±6.89	+0.997
Red chilli powder diet (2.50%)	23.24	205.28±6.58	+0.751*	18.30±5.39	–0.942

* Figures carrying same superscripts are not signiicant (F>0.01)
* r values are not significant (r>0.05)

amino acids by forming intermediate carrier phosphate compounds enhance the active absorption of calcium (Wasserman et al. 1956).

There was a significant positive correlation between red chilli powder intake and urinary calcium levels. In contrast, a insignificant negative correlation was observed with mixed spice powder and urinary calcium (Table 1).

The serum vitamin A level showed a non-significant increase with mixed spice diet (25.12±6.08 µg/dl) compared to that of control (22.71±6.30 µg/dl). All the subjects had vitamin A levels well above the normal range (> 20 µg/dl) (Table 1). This increase in serum levels of vitamin A could be due to enhanced secretion of bile and bile acid levels, as red chillis are known to be potent stimulators of bile and bile acid secretion (Sambaiah and Satyanarayana 1991; Bhat et al. 1984). The deoxy colic acid present in bile acids aids in better absorption of carotenes (Drummond and McWater 1935).

A reverse trend was observed on the vitamin A level, when red chilli powder diet at 2.5% level was administered. Vitamin A levels in serum decreased to 18.30±5.39 µg/dl from that of control, but it was not significant. The decrease in vitamin A levels may be attributed to possible increase in acid secretion caused by gastric irritation by the red chilli at this concentration. The acid medium in gastro-intestinal tract provides unfavourable condition for the absorption of fat soluble substances (Jones 1978). The biliary effect has been nullified by increased acid secretion in the diet, as the levels increased from 1.25% to 2.50%. There was significant positive correlation between serum vitamin A levels and mixed spice powder at 0.5% and red chilli powder at 1.25% level. There was also significant negative correlation between red chilli powder diet at 2.5% level and serum vitamin A level.

The results of the present studies have shown that mixed spice diet decreased the absorption of calcium from gastro-intestinal tract but increased the absorption of vitamin A. The red chilli powder at two concentrations enhanced the absorption of calcium from gastro-intestitional tract. Red chilli powder at the low concentration (1.25%) increased its absorption of vitamin A but at higher levels lowered the absorption. Hence, it may be concluded that intake of spices might alter the micro-nutrient levels.

References

Baginski S, Marie SS, Clark WH, Zak B (1973) Direct micro determination of urinary calcium. Clin Chem Acta 46:49–55

Bhat BG, Srinivasan MR, Chandrasekhara N (1984) Influence of cumin, capsaicin and other spices on the composition and secretion of bile in rats. J Food Sci Technol 21: 225–227

Brooner F (1988) Gastro-intestinal absorption of calcium. J Physiol 83:236–237

Drummond JC, McWater RJ (1935) Effect of bile on absorption of carotenes. Int Rev Phy 57:315–319

Esiong T (1991) The analysis of carotenoids and retinoids. Food Chem 41:147–156

Gopalan C, Ramasastri BR, Balasubramanian SC (1988) Nutritive Value of Indian Foods. National Institute of Nutrition, Indian Council of Medical Research, India

James C, Branch WJ, Southgate DAI (1978) Calcium binding by dietary fibre. J Nutr 1:638–639

Jones M (1978) Regulations of hepatic biliary research. Ann Rev Physiol 41:63–67

Madhyastha MS (1983) Mycotoxic fungi and mycotoxins in major spices with special reference to piper nigrum. Ph.D. Thesis. Mysore University, Mysore, India

Ramasastry J (1983) Calcium, iron and oxalate content of some spices and condiments. Plant Foods Hum Nutr 33:11-15

Reinhold JC, Nasr K, Lanhimgarzadeh, A, Heda J, Yate H (1981) Effect of purified phytate rich bread on zinc, calcium phosphorus and nitrogen in man. Lancet 1:282–284

Sambaiah K, Satyanarayana MN (1991) Hypocholesterolemic effect of red pepper and capsaicin. Ind J Exp Biol 18:898–899

Sambaiah K, Srinivasan K (1991) Secretion and composition of bile in rats fed diets containing spices. J Food Sci Technol 28:35–38

Snedecor GW, Cochran WG (1967) Statistical Methods. The Iowa State University Press, Ames, Iowa

Thimmayamma BVS, Parvathi Rao, Radhaiah A (1983) Use of spices and condiments in the dietaries of urban and rural families. Ind J Nutr Diet 20:153–161

Uma Nageswar Rao (1988) Studies on Indian spices. Nutrient composition and influence on sorghum and legume protein utilisation. Ph.D. Thesis. Acharaya NG Ranga Agricultural University, Hyderabad, India

Wasserman RN, Comar CI, Mold MM (1956) The influence of amino acids and other organic compounds on the gastro–intestinal absorption of calcium and strontium in the oat. J Nutr 18:59–67

Received 29 May 1997; revised 23 July 1999; accepted 11 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 444-445

Spectrophotometric Methods for the Determination of Di-t-Butylhydroquinone in Oils

U. VIPLAVA PRASAD*, V. SRINIVASULU, P.Y. NAIDU AND G. VENKATESWARA RAO

Department of Organic Chemistry, Foods, Drugs and Water Analysis Laboratories
Andhra University, Visakhapatnam-530 003, India.

Three simple spectrophotometric methods have been developed for the determination of di-t-butyl hydroquinone in commercial samples and oils based on the formation of coloured species with Fe (III) - 1,10- orthophenanthrolein (method A) or Fe (III)-2,2'bipyridyl (method B) or Folin-Ciocalteu reagent (F-C) (method C).

Keywords : Spectrophotometry, Di-t-butyl hydroquinone, 1,10-ortho-phenanthroleine, o-Phosphoric acid, 2,2'-bipyridyl, Folin-Ciocalteu reagent.

Di-t-butyl hydroquinone (TBHQ) is an antioxidant permitted to be added to edible oils in a concentration of 0.02% either individually or in combination as stated in the prevention of Food Adulteration Act India (PFA Act 1954, Rules 1955 as amended up to 1984). Very few visible spectrophotometric methods have been reported for the estimation of TBHQ (Prasad et al. 1988; Sastry et al. 1993). The reducing character of TBHQ or the reduced products produced by it has not been exploited so far, for designing sensitive, accurate and flexible visible spectrophotometric methods for the determination of TBHQ in oils. Therefore, an attempt has been made to develop three visible spectrophotometric procedures by utilising reducing property of TBHQ (Fe (III)/PHEN, method A, Fe (III)/BPD, method B and F-C, method C). In methods A and B, TBHQ reduces the oxidant Fe (III) to the Fe (II) state. PHEN (λ_{max}: 490 nm) or BPD (λ_{max}: 525 nm) then forms a coloured complex with the Fe (II) (yellow and pink, respectively). In method C, the reduction of F-C reagent by TBHQ and the reduced species produced possess a characteristic intense blue colour (λ_{max}: 770 nm). These methods are sensitive and useful in the purity assays of TBHQ and its estimation in oil samples.

Instrumentation : All spectral and absorbance measurements were made on a Systronics model 106 digital spectrophotometer with 1 cm matched glass cells. pH measurements were made on a Elico LI-120 digital pH meter.

Preparation of reagents : All the reagents used were of AR/GR grade. Double distilled water was used for preparing solutions. Aqueous solutions of Fe (III) (0.54%, w/v), PHEN (0.2%, w/v) methanolic solution of BPD (0.2%, w/v), Na_2CO_3 (10%, w/v) and o-phosphoric acid (2.0% x 10^{-2}M) were prepared. F-C reagent supplied by M/s. Loba chemicals was used directly in the investigation. TBHQ (food grade, with 99.6% purity) was obtained from M/s polyscience.

Standard TBHQ solution : The standard solution was prepared by dissolving 100 mg TBHQ in 100 ml distilled water (I mg/ml). The working standard solutions for methods A, B and C were prepared with distilled water by suitable dilution.

Procedure for method A : Aliquots of the standard TBHQ solution (1.0-9.0 ml, 30 µg/ml) were transferred into a series of 25 ml graduated test tubes and diluted to 10 ml with distilled water. Then, 1.0 ml of ferric chloride (3.32 x 10^{-3}M), 2.0 ml of o-phenanthroleine (1.0 x 10^{-2}M) were added successively to each tube and kept in a boiling water bath for 10 min. The tubes were removed from the bath, cooled and 1.5 ml of o-phosphoric acid (2.0 x 10^{-2}M) solution was added, mixed well and diluted upto the 25 ml mark with distilled water. The absorbance of the coloured complex was measured at 490 nm against a reagent blank. The calibration graph was prepared by taking known amounts of TBHQ within the Beer's law limits and graph was plotted against concentration versus absorbance. The straight line passed through the origin works as the calibration graph. The amount of unknown TBHQ was calculated from its calibration graph.

Procedure for method B : To a series of 60.0 ml separating funnels containing TBHQ (0.5-4.5 ml, 50 µg/ml), 1.5 ml of ferric chloride solution, 2.0 ml of 2,2'-bipyridyl (0.2%) solution were added and the reaction mixture was kept aside for 5 min. Then, 6.0 ml of n-butanol was subsequently added and allowed to stand for 10 min after mixing. The absorbance of n-butanol layer was measured at 525 nm against a reagent blank. The amount of unknown

* *Corresponding Author*

TBHQ was calculated from its calibration graph as was done in procedure A using method B.

Procedure for method C : Aliquots of the standard TBHQ solution (0.5–3.5 ml, 50 μg/ml) were transferred into a series of 25 ml graduated test tubes and diluted to 10 ml with distilled water. Then, 2.0 ml of 2N F–C reagent and 7.5 ml of Na_2CO_3 (10%) solution were added and kept aside for 10 min. Finally, the solution was diluted to 25 ml with distilled water. The absorbance was measured at 770 nm against a similar reagent blank. The amount of the unknown TBHQ was calculated from its calibration graph as was done in procedure A using method C.

Determination of TBHQ in oils : Ten grams of oil (6 samples) containing TBHQ were initially dissolved in 100 ml of petroleum ether and extracted with four 20 ml portions of acetonitrile. The combined acetonitrile extract was evaporated to dryness and the residue was then dissolved in an appropriate volume of aqueous methanol (1:1 v/v). The recommended procedure was then followed for estimation of TBHQ.

A study of the effect of concentration of reagents and other parameters in each method with respect to maximum sensitivity and stability, minimum blank and obedience to Beer's law led to the procedures. The optical characteristics such as Beer's law limits, molar absorptivity and regression data obtained from linear least squares treatment such as slope, intercept and correlation coefficient are given in Table 1. The percent relative standard deviation and percent range of error obtained from six replicate samples are also given in Table 1.

TABLE 1. OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF PROPOSED METHODS FOR TBHQ

Parameters	Method A	Method B	Method C
λ_{max}	490 mnm	525 nm	770 nm
Beer's law limits, μg/ml	1.2–10.8	2.0–16.0	2.0–14.0
Molar absorptivity, $l.mole^{-1}cm^{-1}$	9.435×10^3	7.881×10^3	1.159×10^4
Sandell's sensitivity, $\mu g/cm^2/0.001$ absorbance unit	0.0266	0.0289	0.0189
Regression equation, A*			
Slope, b	0.0338	0.0340	0.0529
Intercept, a	0.0396	0.0089	0.0028
Correlation coefficient, r	0.9997	0.9996	0.9999
Relative standard deviation, %	0.8600	0.4800	0.5100
% Range of error** (confidence limits)			
0.05 level	0.9100	0.5100	0.5400
0.01 level	1.4200	0.7900	0.8400

* : a+ bC where C is the concentration in mg/ml
** : Six replicate samples

TABLE 2. RECOVERY OF ADDED ANTIOXIDANTS FROM EDIBLE OILS AND FATS

Sample	Antioxidant added, mg	Found by percent recovery proposed methods			Reference method*
		A	B	C	
Coconut oil	10	99.2	99.5	99.3	98.7
Groundnut oil	10	99.4	99.6	99.3	98.1
Sunflower oil	10	99.1	99.5	99.4	98.6

* Visible spectrophotometric method with metol – $KMnO_4$ (Prasad et al. 1988)

Comparison of the values of the recovery experiments of TBHQ in various oils with those from methods proposed and reported method revealed good recovery and accuracy (Table 2). The proposed methods are sensitive and have high accuracy. Since the three proposed methods are based on the reducing properties of TBHQ, other antioxidants with similar reducing properties such as butylated hydroxianisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) interfere with the determination of TBHQ by the proposed methods. The close similarity in the solubility properties of these antioxidants and TBHQ frustrated the attempts to remove the interferences by selective solvent extraction procedures. Preliminary separation by TLC was, therefore, necessary for the estimation of TBHQ in such samples. Good separation of PG and BHA from TBHQ was achieved using silica gel G–Kieselguhr (2:1) impregnated with EDTA as stationary phase and a mixture of chloroform/ xylene/methanol (8:2:1, v/v/v) as the mobile phase. The R_f values were found to be 0.37, 0.59 and 0.91 for TBHQ , PG and BHA, respectively, in the above system. Separation of BHA, BHT and TBHQ using silica gel as stationary phase and n–hexane/ acetic acid (6:1, v/v) as the solvent system has also been reported (Nakazato et al. 1980).

References

Nakazato M, Kan Muri M, Ariga T, Fujinuma K, Naoi Y (1980) Simultaneous determination of TBHQ, BHA and BHT in edible oils. Shokuhin eiseigaku Zasshi 21:64–69

Prasad UV, Divakar TE, Ram Reddy RV, Sastry CSP (1988) Spectrophotometric determination of TBHQ in oils. J Food Sci Technol 25:42–43

Sastry CSP, Gopala Rao S, Sastry BS (1993) New spectrophotometric methods for the determination of TBHQ. Food Chem 46:101–103

Received 17 September 1997; revised 14 July 1999; accepted 11 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 446-448

Effect of Grinding Time and Moisture on Size Reduction of *Makhana*

S.N. JHA*[1] AND B.B. VERMA[2]
[1]Dairy Engineering Division, [2]Dairy Technology Division,
National Dairy Research Institute, Karnal–132 001, India.

A study was conducted on grinding behaviour of *makhana* (popped *Euryale ferox*) by keeping the duration of grinding (25–150 sec) and moisture content of *makhana* (0.5–14% w.b.) as independent variables and the mass fraction retained on B.S. sieve (+4, +14, +20 and –20 fractions) as dependent parameters. Mass fraction retained on each sieve increased with grinding time except +4 fraction, which actually declined. The –20 fraction decreased with increase in moisture content of *makhana* and a consequent increase in +14 fraction was observed. The +14 fraction was found to be maximum at 14% moisture content of *makhana* and 1 min grinding time beyond which it declined continuously.

Keywords : Gorgon nut (*Euryale ferox*), *Makhana*, Grinding, Particle size, *Makhana kheer*, Pudding.

Makhana is a popped kernel of gorgon nut (*Euryale ferox*), which is cultivated in stagnant fresh water pools of the north and north-eastern States of the country. Its wild populations are also available in China, Japan, the former Soviet Union and North America (Jha et al. 1991). Processing of gorgon nut to get *makhana* is mainly concentrated in Darbhanga, Madhubani, Saharsa, Samastipur, Muzaffarpur and adjoining districts of north Bihar.

Makhana contains (g/100 g) 12.8 moisture, 76.9 carbohydrates, 9.7 proteins, 0.1 fat, 0.5 total minerals, 0.02 calcium, 0.9 phosphorus and 0.001 iron (Gopalan et al. 1987). *Makhana* is used in various forms of foods. The most widely accepted item is *kheer*, a milk-based pudding. Conventionally *makhana kheer* is prepared by the traditional method, which involves roasting of *makhana* in an open cast iron pan followed by manual grinding (hand-pounding) to smaller size and cooking in boiling milk to get a desired consistency. In absence of any standard technique, the conventional method of making *makhana kheer* results in wide variations in consistency and other sensory quality attributes. The variation in the quality of product is mainly due to use of unspecified means of size reduction (usually by hand-pounding), resulting in uncontrolled size distribution of *makhana* particles. The objectives of this study were to evaluate the effect of moisture content and grinding time on particle size distribution of ground *makhana* and to suggest suitable grinding time and moisture content of *makhana* for getting maximum percentage of uniform particle size.

Makhana procured from local market, was sorted into three grades (Jha and Prasad 1996). The grade I *makhana* was used in the experiment. The moisture content of *makhana* was determined by hot air oven method (Hall 1970). Samples of *makhana* with three moisture levels viz., 14% (as purchased), 7% (partially dried) and 0.5% (almost completely dried) were prepared by drying raw *makhana* in hot air oven. The prepared samples were stored in different desiccators partly filled with saturated salt solutions so as to equilibrate and maintain its moisture at desired level. Studies on size reduction of samples prepared as above were conducted in a domestic mixer/grinder (Make–Sumeet, model sp–16), using blade recommended for dry grinding. A sample of 50 g *makhana* was put into the grinding bowl and lid was closed. The speed controlling knob was kept at medium level and ground for desired period (30–150 sec). The grinding time was recorded with the help of a stop watch. The loss of moisture, if any, during the grinding process was not controlled. The ground *makhana* was passed through a set of sieves (BS sieve numbers 4,14,20) and a pan at the bottom. Materials retained (mass fraction) at each sieve were expressed as percentage of initial weight of the sample. Experiments were replicated thrice to minimise experimental error and average data were analysed. Mass fractions retained on each sieve were plotted against the grinding time and initial moisture content of *makhana*. Mass fractions retained on sieve numbers 4,12,30 and on pan were denoted as large, medium, small and fine particles, respectively.

In general, mass fraction of material retained on each sieve, except the +4 fraction, increased with increase in grinding time at each moisture level of *makhana* (Fig. 1-3), whereas, the amount of *makhana*

* Corresponding Author

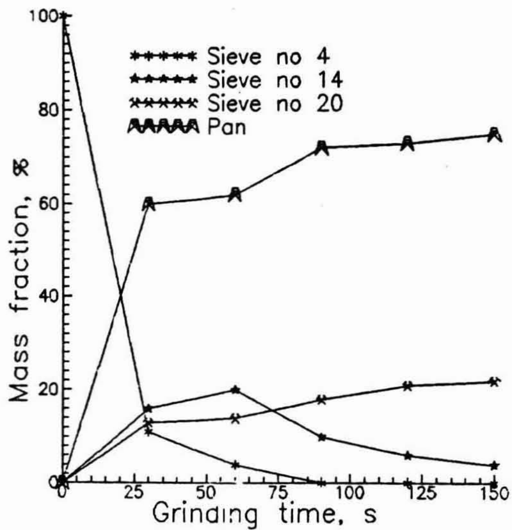


Fig. 1. Sieve analysis of grinding of *makhana* for various periods and moisture content at 0.5% (wb)

particles retained on sieve no. 4 (large size particles) decreased with increase in grinding time and being added to other particle sizes. This happens due to the fact that the size reduction of a batch of sample increases with increase in grinding time. Medium size particle, i.e. +14 fraction, increased with increase in grinding duration upto 60 sec and thereafter, particle size started decreasing. This may be due to decrease in amount of +4 fraction of material initially to medium size and subsequently getting further ground to smaller sizes.

The effect of moisture on mass fraction retention, illustrated in Fig. 4, indicated that moisture had practically no effect on +14 and +20 fractions upto a moisture content of 7%. Beyond this moisture evel, however, the +14 fraction increased, whereas +20 fraction decreased. Materials retained on pan (–20 fraction) and on sieve number 14 (+14 fraction), respectively decreased and increased almost linearly with moisture content for 1 min of grinding time (Fig. 4). Decrease in –20 fraction (fine particles), which consequently produces more medium size particles with increased in moisture, is due to the fact that *makhana* contains higher amount of starch i.e., 77%, (Jha and Prasad 1993), which gets gelatinised during popping operation of gorgon nut (Jha 1993). The gelatinised starch in popped *makhana* gets softened, resulting in reduction in its brittleness.

The study has revealed that the percentage of medium sized particles becomes maximum (66%)

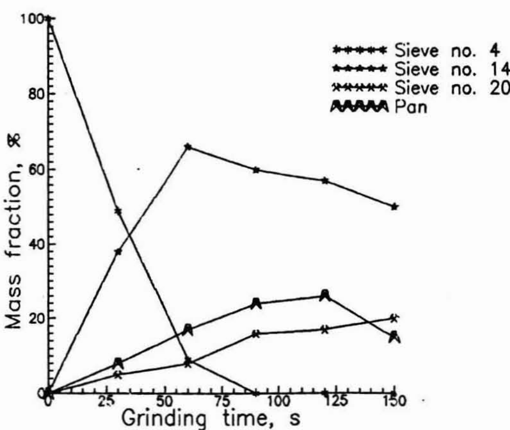


Fig. 3. Sieve analysis of grinding of *makhana* for various periods and moisture content at 14% (wb)

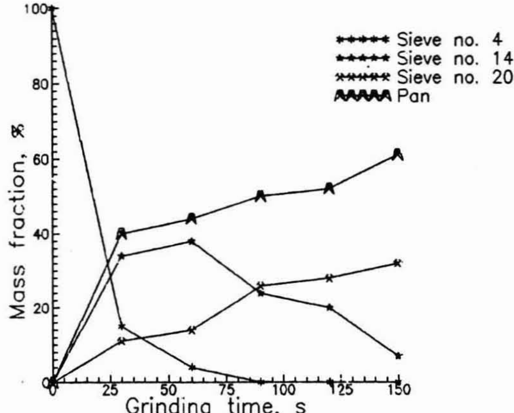


Fig. 2. Sieve analysis of grinding of *makhana* for various periods and moisture content at 7% (wb)

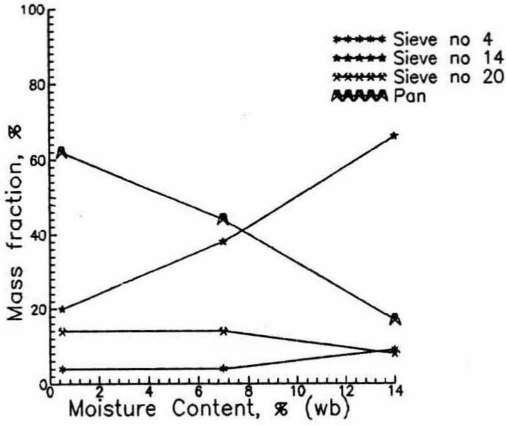


Fig. 4. Sieve analysis of grinding of *makhana* at various moisture content for 1 min

at 1 min of grinding time and a moisture content of 14% in *makhana*. This study has further indicated that a maximum amount of uniform medium size particles of *makhana* can be obtained, if it is ground in domestic grinder (make Sumeet, model sp-16) using dry grinding blade at medium speed for a period of 1 min. The study has also established that the need of roasting or frying of *makhana* merely for grinding purpose in traditional method for *makhana kheer* making can be dispensed with, as the normal moisture contents of market samples of *makhana* lie in the vicinity of 12-14% in almost all the seasons of the year and could be ground to desired particles size in domestic grinder easily.

References

Gopalan C, Ramasastri BV, Balasubramanian SC (1987) Nutritive Value of Indian Foods. National Institute of Nutrition, Hyderabad

Hall CW (1970) Drying Farm Crops. Lyall Book Depot Publication. Ludhiana

Jha SN (1993) Development of a processing machine for gorgon nut (*Euryale ferox*). Ph.D. Thesis. Indian Institute of Technology, Kharagpur

Jha SN, Prasad S (1993) Moisture diffusivity and thermal expansion of gorgon nut. J Food Sci Technol, 30(3):163-165

Jha SN, Prasad S (1996) Determination of processing conditions of gorgon nut (*Euryale ferox*). J Agric Engng Res 63: 103-112

Jha V, Barat GK, Jha UN (1991) Nutritional evaluation of *Euryale ferox* salisb. J Food Sci Technol, 28(5):320-328

Received 31 January 1998; revised 31 May 1999; accepted 12 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 449-452

Studies on Advanced Potato Hybrids for the Preparation of French Fries

R.S. MARWAHA*, DEVENDRA KUMAR AND P.C. GAUR

Central Potato Research Station, Jalandhar-144 003, India.

Tubers of seven advanced stage potato hybrids and three control varieties 'Kufri Bahar', 'Kufri Jyoti' and 'Kufri Lauvkar' were evaluated for the preparation of French fries and for important morphological and physico-chemical characteristics, influencing processing. Dry matter contents of the advanced hybrids varied from 17.8-22.5%, as against 18.0-20.5% in control varieties. The reducing sugar contents of the advanced hybrids were lower (104-180 mg/100 g fresh wt) than those of control varieties (216-245 mg/100 g fresh wt). Phenolic compounds and free amino acid contents were also lower in the advanced hybrids. Yield recovery on fresh weight basis ranged from 46.1-59.3% in advanced hybrids, as against 44.7-50.1% in control varieties. Based on colour, texture, flavour and yield of French fries, the advanced hybrids 'MP/90-83' and 'MP/91-G' were considered most suitable for processing into French fries.

Keywords : Potato processing, French fries, Potato varieties, Physico-chemical characteristics.

Potato processing is presently confined largely to the developed countries (Young 1981; Holm et al. 1994), whereas, it is still in its infancy in most of the developing countries including India. In India, due to increased urbanization, preference of new generation for easy-to-prepare and fast foods, rising per capita income, increase in the number of working women and expanding tourist trade, production of processed food products is likely to be accelerated. In this context, potato processing is one such area, where a rapid growth is expected. Among processed potato products, chips and French fries are the most popular forms. French fries are served in the restaurants or at fast food outlets and sometimes prepared fresh at home. With the entry of multinational companies in the processing sector, advancement in processing technology and improvement in storage and transportation facilities, the frozen French fries are likely to be introduced shortly in the market.

The yield and texture of the processed potato products are determined by the dry matter content of tubers, besides other factors such as contents of sugars, phenolic compounds and shape, size and colour of tubers (Santerre et al. 1985). The processing quality of French fries is largely determined by the colour after frying, which is closely correlated to reducing sugars, as these combine with free amino acids to cause non-enzymatic browning through Maillard reaction (Fuller and Hughes 1984; Roe et al. 1990). Excessive darkening and development of off-flavours due to high reducing sugar content are unacceptable for processed products (Pritchard and Adam 1994). Phenolic compounds are also important in processing, as they are responsible for enzymatic discoloration (Schaller and Amberger 1974) and after-cooking darkening in potatoes (Shaw and Booth 1983). Tuber characters influence the yield of French fries as peeling, trimming and cutting losses vary with the shape, size and depth of eyes of tubers, which ultimately influence the yield recovery.

The potato breeding programmes at the Central Potato Research Institute, Shimla was earlier geared to develop high yielding disease-resistant varieties. However, in the recent past, due to increasing demand for varieties suitable for processing, work was also started on developing hybrids suitable for processing purposes. Some of the advance stage hybrids from this programme were evaluated for various physical and chemical characteristics, which influence the processing quality, especially the French fries. Results of this study are presented in this communication.

Seven advanced hybrids viz., 'MP/90-74', 'MP/90-82', 'MP/90-83', 'MP/90-86', 'MP/90-94', 'MP/90-97' and 'MP/91-G' along with 3 control varieties viz., 'Kufri Bahar', 'Kufri Jyoti' and 'Kufri Lauvkar' were grown at the Central Potato Research Station, Modipuram during the period October, 1994 to January 1995 in a randomized block design with four replications. All the standard cultural practices were followed and the crop was harvested after 90 days.

French fries were prepared from large size (100-120 g) tubers in the first week of March and various chemical constituents were also determined simultaneously. Eight to ten tubers of each hybrid/variety were hand-peeled and strips of sizes 1x1 cm were cut using a vegetable cutter (Crystal make). The cut strips were dipped in cold water

* *Corresponding Author*

and washed thoroughly, air dried and immediately fried in groundnut oil at 180°C for 4–5 min. The fresh fried French fries were subjectively graded for colour, texture and flavour according to the slightly modified score sheet, used by Holm et al (1994). For determination of dry matter content, tubers were cut into small pieces and oven-dried to a constant weight at 70°C. Reducing sugars were determined according to the procedure of Nelson (1944). Total phenols and free amino acids were determined by the methods of Swain and Hillis (1959) and Moore (1968), respectively. Four replicates for each estimation were taken and the data were statistically analysed.

Morphological characters : Morphological characters such as shape and eye depth of the tubers of 7 hybrids along with 3 control varieties are given in Table 1. Hybrid 'MP/90–74' had oval shape and red skin colour, whereas, the remaining hybrids were oblong in shape and white skinned. All the hybrids, except 'MP/90–86' had fleet eyes. Since tubers of long oval or oblong shapes and shallow or fleet eyes are preferred (Sukumaran and Verma 1993) so as to produce fries of desirable length (6–7 cm) with minimum processing losses, all the hybrids except 'MP/90–74' and 'MP/90–86' were found suitable for the preparation of French fries.

Dry matter content of tuber and texture of French fries : The dry matter contents of tubers of the hybrids varied between 17.8% and 22.5%, as against the control varieties, which showed dry matter in the range of 18.0% to 20.5% (Table 2). Hybrids 'MP/90–74', 'MP/90–83' and 'MP/91–G' recorded more than 22% tuber dry matter, which was significantly higher than that (20.5%) observed in the best control variety 'Kufri Lauvkar'. Variations

TABLE 1. MORPHOLOGICAL CHARACTERS OF THE TUBERS OF SOME ADVANCED POTATO HYBRIDS USED FOR FRENCH FRIES

Cultivar	Shape of the tuber	Type of tuber eye	Colour of tuber skin
'MP/90–74'	Oval	Fleet	Red
'MP/90–82'	Oblong	Fleet	White
'MP/90–83'	Oblong	Fleet	White
'MP/90–86'	Oblong	Medium deep	White
'MP/90–94'	Oblong	Fleet	White
'MP/90–97'	Oblong	Fleet	White
'MP/91–G'	Oblong	Fleet	White
'Kufri Bahar'	Oval	Medium deep	White
'Kufri Jyoti'	Oval	Fleet	White
'Kufri Lauvkar'	Round	Medium deep	White

TABLE 2. IMPORTANT PHYSICO-CHEMICAL CHARACTERS OF THE TUBERS OF ADVANCED POTATO HYBRIDS USED FOR FRENCH FRIES

Cultivar	Dry matter, %	Reducing sugars*	Free amino acids**	Total phenols*
'MP/90–74'	22.2	122	56.1	31.9
'MP/90–82'	18.9	124	86.7	37.2
'MP/90–83'	22.1	108	82.8	30.1
'MP/90–86'	18.4	180	84.1	32.6
'MP/90–94'	21.5	104	70.2	25.6
'MP/90–97'	17.8	136	83.7	34.1
'MP/91–G'	22.5	112	66.9	27.1
'Kufri Bahar'	19.4	216	94.3	38.9
'Kufri Jyoti'	18.0	245	91.8	45.2
'Kufri Lauvkar'	20.5	228	89.2	40.2
CD (0.05)	1.2	11	2.4	1.6

* mg/100 g fresh weight
** mg N/100 g fresh weight

in tuber dry matter contents of varieties are also reported earlier (Marwaha and Raj Kumar 1987; Marwaha and Kang 1994). Potatoes having high dry matter contents are considered suitable for the preparation of French fries and chips. High tuber dry matter is also associated with mealiness, crispness, and reduced oil uptake in French fries (Brody 1969; Kirkpatrick et al. 1956). In the present investigation also, six advanced hybrids having dry matter contents above 18% produced fries of acceptable mealy to crispy texture, whereas, the hybrid 'MP/90–97' having low tuber dry matter (17.8%) produced greasy textured unacceptable fries (Tables 2 and 3).

Reducing sugars and free amino acids : Reducing sugar contents of potato tubers are of considerable importance in relation to processing, especially for fried products. Potatoes containing more than 0.5% reducing sugars on fresh weight basis are not considered suitable for french fries (Burton and Wilson 1978). All the advanced hybrids and the controls contained lower levels of reducing sugars than the prescribed limit. Hybrid 'MP/90–94' had the lowest level of reducing sugars (104 mg/100 g fresh wt), which is at par with 'MP/90–83' (108 mg/100 g fresh wt) and 'MP/91–G' (112 mg/100 g fresh wt). The lowest level of free amino acids was recorded in hybrid 'MP/90–74' (56.1 mg N/100 g fresh wt) followed by 'MP/91–G' (66.9 mg N/100 g fresh wt), 'MP/90–94' (70.2 mg N/100 g fresh wt) and 'MP/90–83' (82.8 mg N/100 g fresh wt). Among controls, the variety 'Kufri Lauvkar' had the lowest level (89.2 mg N/100 g fresh wt) of free amino acids.

TABLE 3. LOSSES DURING PREPARATION OF FRENCH FRIES, PROCESSING QUALITY AND YIELD OF FRIES PREPARED FROM ADVANCED POTATO HYBRIDS

Cultivar	Peeling loss, %	Trimming and cutting loss, %	Total losses during processing, %	Colour	Texture	Flavour	Yield of French fries, %
'MP/90-74'	12.7	2.1	14.8	VL	Mealy	Typical	53.3
'MP/90-82'	7.2	1.3	8.5	L	Mealy	Typical	55.7
'MP/90-83'	9.0	2.3	11.3	L	Mealy	Typical	59.3
'MP/90-86'	10.7	2.1	12.8	LB	Mealy	Typical	46.1
'MP/90-94'	7.3	2.1	9.4	VL	Crispy	Typical	54.0
'MP/90-97'	8.5	2.5	11.0	L	Greasy	Typical	55.6
'MP/91-G'	8.4	1.7	10.3	L	Crispy	Typical	58.3
'Kufri Bahar'	7.1	3.0	10.1	L	Mealy	Typical	50.1
'Kufri Jyoti'	10.4	3.5	13.9	I	Mealy	Bitter	44.7
'Kufri Lauvkar'	9.2	5.9	15.1	L	Mealy	Typical	49.7
CD (0.05)	0.8	0.3	1.1	-	-	-	3.2

VL = Very light; L = Light; LB = Light brown; I = Inconsistant
Peeling loss is the weight of skin removed during peeling
Trimming loss includes the weight of residual skin, eyes and discoloured area removed from peeled potatoes
Cutting loss includes the weight of slivers (thin slices) and nubbins (short or broken pieces) separated from the product after cutting of french fry strips

Total phenols : Phenolic compounds have been associated with enzymic discoloration, which occurs due to the oxidation of these compounds by polyphenoloxidase (Schaller and Amberger 1974). Levels of total phenols in all the advanced hybrids were significantly lower than those in the best control variety 'Kufri Bahar'. None of the hybrids showed enzymic discoloration during processing. The lowest level of phenols was recorded in 'MP/90-94' (25.6 mg/100 g fresh wt) followed by 'MP/91-G' (27.1 mg/100 g fresh wt), and 'MP/90-83' (30.1 mg/100 g fresh wt). The highest total phenols content was recorded in the variety 'Kufri Jyoti' (45.2 mg/100 g fresh wt), which also showed enzymic discoloration in the strips prior to frying.

Losses during processing for French fries : Peeling losses in the advanced hybrids varied between 7.2% and 12.7%, whereas, the losses in control varieties were between 7.1% and 10.4% (Table 3). Among advanced hybrids, 'MP/90-97' showed maximum trimming and cutting loss (2.5%), which was significantly lower than all the control varieties. The minimum total losses during processing were recorded for the hybrids 'MP/90-82' and 'MP/90-94'. The hybrids 'MP/91-G' and 'MP/90-83' also showed significantly lower total losses than the popular processing varieties 'Kufri Jyoti' and 'Kufri Lauvkar'.

Colour, flavour and yield of French fries : The colour of French fries prepared from all the advanced hybrids varied from very light, light to light brown, all within acceptable range with typical pleasing flavour (Table 3). Among control varieties, 'Kufri Jyoti' produced fries of inconsistent colour with bitter flavour, which were unacceptable. None of the cultivars produced dark coloured fries. All the hybrids except 'MP/90-86' gave significantly higher yield of French fries than the best control variety 'Kufri Bahar'. Yield recovery of French fries on fresh weight basic ranged from 44.7 to 59.3% being the highest in 'MP/90-83' (59.3%), followed by 'MP/91-G' (58.3%). Hybrid 'MP/90-97', although, produced high yield of French fries (55.6%), the quality was not acceptable due to greasy texture.

The above results suggested the suitability of 5 advanced hybrids viz., 'MP/90-74', 'MP/90-82', 'MP/90-83', 'MP/90-94' and 'MP/91-G' for processing into French fries. However, based on tuber morphological characters, tuber dry matter content and colour, texture, flavour and yield of French fries, 2 advanced hybrids viz., 'MP/90-83' and 'MP/91-G' were most suitable for processing into French fries.

The authors thank the Director, Central Potato Research Institute, Shimla for providing necessary facilities and to Dr. N.P. Sukumaran, Head, Division of Crop Physiology and Biochemistry, Central Potato Research Institute, Shimla for Interest in the work. Thanks are also due to Mr. Raj Kumar for help in statistical analysis of data.

References

Brody J (1969) Pointers on potatoes. Food Engng 41:124-132

Burton WG, Wilson AR (1978) The sugar content and sprout growth of tubers of potato cultivar. Record, grown in different localities, when stored at 10, 2 and 20°C. Potato Res 21:145–150

Fuller TJ, Hughes JC (1984) Factors influencing the relationships between reducing sugars and fry colour of potato tubers of cv. Record. J Food Technol 19:455–467

Holm ET, Orr PH, Johansen RH (1994) Sensory evaluation as a tool in breeding potatoes for French fries and flakes. Am Potato J 71:1–14

Kirkpatrick ME, Heinze PH, Craft CC, Mountjoy BM, Falatko CE (1956) French-frying quality of potatoes as influenced by cooking methods, storage conditions and specific gravity of tubers. USDA Tech Bulletin p 1142

Marwaha RS, Raj Kumar (1987) Relationship between specific gravity and dry matter content of potato tubers. Inter J Trop Agri 5:227–230

Marwaha RS, Kang GS (1994) Evaluation of exotic potato germplasm under short days for processing and yield. In: Sekhawat GS, Khurana SMP, Pandey SK, Chandla VK (eds), Potato-Present and Future. Indian Potato Association, Shimla, India, pp 23–26

Moore J (1968) Amino acid analysis. Aqueous dimethyl sulfoxide as solvent for the ninhydrin reaction. J Biol Chem 243:6281–6283

Nelson N (1944) A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem 153: 375–380

Pritchard MK, Adam LR (1994) Relationships between fry colour and sugar concentration in stored Russet Burbank and Shepody potatoes. Am Potato J 71:59–68

Roe MA, Faulks RM, Belsten JL (1990) Role of reducing sugars and amino acids in fry colour of chips from potatoes grown under different regimes. J Sci Food Agric 52:207–214

Santerre CR, Cash JN, Chase RW (1986) Influence of cultivar, harvest-date and soil nitrogen on sucrose, specific gravity and storage stability of potatoes grown in Michigan. Am Potato J 63:99–110

Schaller K, Amberger A (1974) Relationships between enzymic browning of potatoes and several constituents of the tuber. Qual Plant Foods Hum Nutr 24:183–190

Shaw R, Booth R (1983) Potato tubers. In: Simple Processing of Dehydrated Potatoes and Potato Starch. International Potato Centre, Lima, Peru, pp 9–14

Sukumaran NP, Verma SC (1993) Storage and Processing. In: Chadha KL, Grewal JS (eds), Advances in Horticulture, Vol 7, Malhotra Publishing House, New Delhi, India, pp 720–721

Swain T, Hillis WE (1959) The phenolic constituents of *Prunus domestica* 1. The quantitative analysis of phenolic constituents. J Sci Food Agric 10:63–68

Young NA (1981) Potato Products: Production and Markets in the European communities. In: Commission of the European Communities Information on Agriculture, No. 75. Office for Official Publications of the European Communities, Luxembourg

Received 25 March 1998; revised 15 May 1999; accepted 12 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 453-456

Zinc, Calcium and Iron Availability Using Molar Ratios in Processed and Cooked Wheat Products

HARPREET K. GREWAL, CHARANJEET K. HIRA* AND B.L. KAWATRA

Department of Foods and Nutrition,
Punjab Agricultural University, Ludhiana-141 004, India.

Molar ratios for availability of zinc, calcium and iron were calculated from various processed and cooked samples of wheat. Phytate: zinc molar ratio was more than 20 for all processed wheat samples. Phytate x calcium: zinc molar ratio was more than 50 mM/100g in refined flour and semolina and less than this value in rest of the samples. The phytate: calcium, phytate: iron and iron: zinc molar ratios in all processed wheat samples were more than the desired ratios. In cooked products of whole and pearled wheat flour, the phytate: zinc ratio below 20 and phytate x calcium: zinc ratio above 50 were observed in *chapati* and *parantha*, but not in *pooris*. In all refined flour products, the phytate: zinc, phytate x calcium: zinc, phytate: calcium and phytate: iron ratios were found to be lower compared to whole and pearled wheat flour products.

Keywords : Zinc availability, Iron availability, Calcium availability, Molar ratios, Wheat.

Some minerals are essential nutrients, as they are components of many enzyme systems. Iron is an important element that is incorporated in the haemoglobin molecule and plays an important role in the transport of oxygen. Zinc acts as a constituent of number of enzymes. Calcium is required for formation and maintenance of skeleton, normal contraction of muscle, heart, nervous activity and blood clotting. Wheat being the major staple food of India contributes almost one third of calcium and more than two thirds of iron required by adult humans in low socio-economic groups of population in northern India (Hira et al. 1993). Wheat bran contains 70 to 80% of the total phosphorus of the whole wheat kernel, 80 to 90% of which is phytate phosphorus (O'Dell et al. 1972). Studies indicate that phytic acid forms complexes with trivalent cations and protein and is associated with reduced mineral bioavailability (Honig et al. 1984). Solubility of phytic acid not only depends upon pH but also on molar ratios of minerals to phytic acid (Grynspan and Cheryan 1983; Fordyce et al. 1987; Simpson et al. 1981). Fordyce et al (1987) observed that phytate : zinc and phytate x calcium: zinc molar ratios were useful for predicting zinc bioavailability. But, Bindra et al (1986) have suggested that phytate x calcium: zinc ratio is a better predictor of zinc bioavailability than the phytate: zinc ratio.

In India, wheat is consumed in various forms such as *chapatis, pooris*, porridge, bread, biscuits, and cakes. Wheat is consumed as a staple and contributes to mineral consumption significantly. In the present study, processed wheat samples and cooked wheat products were tested for phytate zinc, phytate x calcium: zinc, phytate: calcium, phytate: iron and iron: zinc ratio to evaluate mineral availability.

The bulk sample of wheat (*Triticum aestivum*) var. 'HD 2329' was procured from Department of Plant Breeding, Punjab Agricultural University, Ludhiana. Wheat grains were cleaned, washed, dried and freed of any extraneous substances. These wheat grains were processed for whole wheat flour, pearled wheat flour, refined flour, semolina and broken wheat in the laboratory. From these processed wheat samples, different products were prepared using various cooking methods. *Chapatis* (unleavened bread), *paranthas* (shallow-fried unleavened bread) and *pooris* (deep-fried unleavened bread) were prepared from whole and pearled wheat flour using standard methods. Fermented dough, bread and *bhatura* (deep-fried fermented dough) were prepared from refined flour. The fermented dough was prepared using compressed yeast (3.0 g), sugar (2.5 g), salt (1.0 g) and fat (2.5 g) per 100 g refined flour. This dough was kept in the incubator for fermentation at 30°C for a period of 3 h. Semolina *halwa* and broken wheat porridge were prepared by roasting in fat and cooking in water.

All the processed wheat samples and cooked wheat products were dried (60±2°C) and used for analysis. All the analyses were carried out in triplicate. The calcium, iron and zinc were estimated using atomic absorption spectrophotometer after diacid (HNO_3:$HClO_3$::5:1) digestion, by following the method of Piper (1950). Phytin phosphorus was estimated after extraction with 0.2N HCl, following the colorimetric method of Haug and Lantzsch

* *Corresponding Author*

(1983). The factor for converting the phytin phosphorus to phytate was 3.55 as suggested by Tangkongchitr et al (1981). The millimoles of phytic acid, zinc, calcium and iron were calculated by dividing milligrams of phytic acid by 660 (molecular weight of the phytate ion), milligrams of zinc by 65.4 (atomic weight of zinc), milligrams of calcium by 40 (atomic weight of calcium) and the milligrams of iron by 65.8 (atomic weight of iron). The molar ratio of phytic acid to zinc, calcium and iron were then calculated by dividing millimoles of phytic acid by millimoles of zinc, calcium and iron.

Processing of wheat into pearled wheat flour, refined flour and semolina significantly ($p<0.001$) reduced the phytin phosphorus contents by 5.06, 31.64 and 30.38%, respectively (Table 1) due to removal of bran, as phytate phosphorus is concentrated in aleurone and other outer layers of the grain, which contain much dietary fibre. The zinc, calcium and iron contents in refined flour decreased significantly ($p<0.001$) by 64.15, 23.85 and 46.51% and in semolina by 75.47, 38.72 and 57.16%, respectively when compared to the respective values in whole wheat flour. Thus, the phytate, zinc, calcium and iron contents were found to be maximum in whole wheat flour, followed by pearled wheat flour and minimum in refined flour and semolina.

The phytate: zinc, phytate: calcium; phytate x calcium: zinc; phytate: iron and iron: zinc ratios were found to be the highest in semolina followed by refined flour, pearled wheat flour and whole wheat flour. High ratios on refining are due to the fact that removal of zinc, calcium and iron was more than that of phytate. Phytate: zinc molar ratio of 10 or less is usually associated with adequate zinc bioavailability in animals and ratio above 20 is associated with clinical or chemical evidence of zinc deficiency in rats (Davies and Olpin 1979). In the present study, all the processed samples had ratios more than 20 showing them as poor sources of available zinc. Phytate x calcium: zinc molar ratio above 50 mM/100g may be of concern for poor zinc status in humans (Fordyce et al. 1987). The results of the present study revealed that phytate x calcium: zinc molar ratio were high only

TABLE 1. PHYTATE: ZINC, PHYTATE X CALCIUM: ZINC, PHYTATE: CALCIUM, PHYTATE: IRON AND IRON: ZINC RATIOS OF PROCESSED WHEAT SAMPLES AND COOKED WHEAT PRODUCTS

Product	Phytate, mM	Zinc, mM	Calcium, mM	Iron, mM	Phytate, zinc	Phytate: calcium: zinc	Phytate: calcium	Phytate: iron	Iron: zinc
Processed wheat samples									
Whole wheat flour	0.850^{a}	0.040^{a}	2.12^{a}	0.156^{a}	21.00	44.49	0.400	5.45	3.85
Pearled wheat flour	0.807^{b}	0.033^{a}	1.95^{b}	0.121^{b}	24.45	47.68	0.414	6.67	3.67
Refined flour	0.581^{c}	0.014^{b}	1.61^{c}	0.083^{c}	41.50	66.81	0.360	7.00	5.93
Semolina	0.592^{c}	0.010^{b}	1.30^{d}	0.067^{d}	59.20	76.96	0.455	8.83	6.70
Broken wheat	0.850^{a}	0.039^{a}	2.09^{a}	0.156^{a}	21.79	45.55	0.407	5.45	4.00
Cooked wheat products									
Whole wheat flour									
Chapati	0.774^{a}	0.056^{a}	2.40^{a}	0.190^{a}	13.82	33.17	0.322	4.07	3.39
Parantha	0.772^{a}	0.047^{b}	2.21^{b}	0.161^{b}	16.42	36.30	0.349	4.79	3.42
Poori	0.710^{b}	0.029^{c}	2.12^{c}	0.132^{c}	24.48	51.90	0.335	5.37	4.55
Pearled wheat flour									
Chapati	0.745^{a}	0.045^{a}	2.05^{a}	0.167^{a}	16.55	33.94	0.363	4.46	3.71
Parantha	0.710^{b}	0.040^{a}	1.96^{b}	0.126^{b}	17.75	34.79	0.362	5.63	3.15
Poori	0.688^{c}	0.022^{b}	1.82^{c}	0.108^{c}	31.27	56.91	0.378	4.37	4.91
Refined flour									
Fermented dough	0.350^{a}	0.024^{a}	2.75^{a}	0.144^{a}	14.58	40.10	0.127	2.43	6.00
Bread	0.344^{a}	0.071^{b}	2.58^{b}	0.157^{b}	4.85	12.50	0.133	2.19	2.21
Bhatura	0.339^{a}	0.016^{a}	2.48^{c}	0.156^{b}	18.83	46.71	0.137	2.17	8.67
Semolina									
Samolina *halwa*	0.473	0.014	1.93	0.070	33.78	65.21	0.245	6.75	5.00
Broken wheat									
Porridge in water	0.559	0.047	2.32	0.158	11.89	27.59	0.241	3.54	3.36
Porridge in milk	0.602	0.054	14.50	0.160	11.15	161.65	0.050	3.76	2.96

Values with different superscripts indicated that these were significantly ($p<0.001$) different from one another

in refined flour and semolina and rest of the samples had low ratios. The phytate: calcium ratio in all processed wheat samples was found to be greater than 0.2, which is undesirable for good Ca availability (Morris and Ellis 1985). Rao and Rao (1983) found that when phytate: iron ratio was 3:1, more than 90% of iron was present in soluble form. The presence of calcium ions decreased the iron solubilising ability of phytate, thus phytate alone did not affect the iron availability in foods, but it is the relative concentration of phytate binding minerals like calcium, magnesium, zinc and their interaction with iron: phytate complex. The phytate: iron ratio of all the processed wheat samples was more than 3:1, indicating poor iron availability. The iron: zinc ratio in all processed samples was found to be greater than 2:1. Solomons (1986) found the competitive inhibition of zinc uptake by excess iron in ratio of 2:1 or greater, when the total amount of ionic species, greater than 25 mg appeared to have a measurable effect on human zinc nutriture.

In whole and pearled wheat flour products, phytin phosphorus contents in *chapatis*, *paranthas* and *pooris* got reduced by 8.86, 9.18 and 16.45% in whole wheat flour and 7.66, 12 and 14.66% in pearled wheat flour. This reduction in phytin phosphorus content might be due to its breakdown on heating. The phytin phosphorus contents of *chapatis* and *paranthas* differed significantly ($p<0.001$) from *pooris*, but non-significant differences were observed between *chapatis* and *paranthas*. Among refined flour products, phytin phosphorus in bread got decreased by 39.81% when compared to refined flour. Nearly same per centage of reduction was observed in fermented dough and *bhatura*. In semolina *halwa*, phytin phosphorus content got decreased by 20% compared to raw semolina. In wheat porridge and porridge with milk, the phytin phosphorus content got decreased by 34.17 and 29.11%.

In whole and pearled wheat flour products, calcium, zinc and iron contents were found to be maximum in *chapatis* followed by *paranthas* and *pooris* due to fat incorporation in the latter products. Inspite of high phytate content in *chapatis* the phytate: zinc, phytate x calcium: zinc, phytate: iron and iron: zinc ratios were found to be lowest in *chapatis* due to proportionately higher mineral content. These low ratios indicated better availability of zinc and iron from *chapatis* in both whole and pearled wheat flour. The phytate: zinc ratio below 20 and phytate x calcium: zinc above 50 was observed only in *chapatis* and *paranthas* but not in *pooris*. The phytate: zinc and phytate x calcium: zinc molar ratio in *chapatis* and phytate x calcium: zinc molar ratio in *chapatis* and *paranthas* was found to be comparable with the ratio reported by Hira and Kaur (1993). In *chapatis* and *paranthas* of both whole and pearled wheat flour, phytate: zinc, phytate x calcium: zinc and iron: zinc ratios were lower than the corresponding flours due to decrease in phytate content during cooking.

Among refined flour products, the iron and zinc contents were found to be maximum in bread, while the calcium content was maximum in fermented dough due to addition of yeast, salt and during preparation and cooking. The phytate: zinc, phytate x calcium: zinc, phytate: calcium, phytate: iron ratios were found to be lower in all refined flour products, compared to all other cooked products, indicating better availability of these minerals. This was primarily due to breakdown of phytate during the process of fermentation. But the iron: zinc ratio was found to be lower only in bread but was higher in fermented dough and *bhatura* than the other products. Among refined flour products, lowest ratio of phytate: zinc, phytate x calcium: zinc, phytate: calcium and iron: zinc ratio were observed in bread. This low ratio in bread might be due to high zinc uptake from the transite on which they are baked. The amount of zinc in the transite was unknown but was believed to be the contamination point. In semolina *halwa*, phytate: zinc, phytate x calcium: zinc, phytate: calcium, phytate: iron and iron: zinc ratios were found to be higher when compared with the desired values for ratios. In broken wheat products, phytate: zinc ratio was found to be lower than 20 mM, while phytate x calcium: zinc ratio was higher in porridge with milk and lower in porridge with water. The higher ratio was due to high calcium content of milk. Among all cooked products, wheat porridge with milk had better availability of calcium, followed by refined flour products.

It may be concluded from the results that zinc bioavailability could be maximum from whole wheat flour, followed by broken wheat and pearled wheat flour as indicated by phytate: zinc and phytate x calcium: zinc ratios obtained from these cereals. Among whole and pearled wheat flour products, *chapati* was a better source of available zinc. All refined flour products had better availability of iron and zinc. Among them, bread was a good source of available minerals. Calcium availability was maximum from refined flour products and porridge with milk.

References

Bindra GS, Gibson RS, Thompson LU (1986) Phytate x calcium/zinc ratios in Asian immigrant lacto-ovo-vegetarian diets and their relationship to zinc nutriture. Nutr Res 6: 475–483

Davies NT, Olpin SE (1979) Studies on phytate: Zn molar contents in diets as a determinant of zinc availability to young rats. Brit J Nutri 41:591–603

Fordyce EJ, Forbes RM, Robbins KR, Erdman JW (1987) Phytate x calcium/zinc molar ratios: Are they predictive of zinc bioavailability. J Food Sci 52:440–444

Grynspan F, Cheryan M (1983) Calcium phytate-Effects of pH and molar ratio on *in vitro* solubility. J Am Oil Chem Soc 60:1761–1765

Haug W, Lantzsch HJ (1983) Sensitive method for the rapid determination of phytate in cereals and cereal products. J Sci Food Agric 34:1423–1426

Hira CK, Sadana BK, Grover K, Kochar A (1993) Nutrient adequacy of rural adult males in Ludhiana district of Punjab. Ecol Food Nutr 29:147–157

Hira CK, Kaur AP (1993) Phytate/zinc and phytate x calcium/zinc ratios of common cereals, legumes and their combinations. J Food Sci Technol 30:213–215

Honig DH, Wolf WJ, Rachis JJ (1984) Phytic acid and phosphorus content of various soybean protein fractions. Cereal Chem 61:523–525

Morris ER, Ellis R (1985) Bioavailability of dietary calcium. Effect of phytate on adult men consuming non-vegetarian diets. In Nutritional Bioavailability of calcium. American Chemical Society, Washington DC pp 63–72

O'Dell BL, Burpo CE, Savage JE (1972) Evaluation of zinc availability in foodstuffs of plant and animal origin. J Nutr 102:653–660

Piper CS (1950) Soil and plant analysis. Interscience Publication Inc New York p 212

Rao KS, Rao BSN (1983) Studies on iron chelation by phytate and the influence of other mineral ions on it. Nutr Rep Int 28:771–775

Simpson KM, Morris ER, Cook JD (1981) The inhibitory effect of bran on iron absorption in man. Am J Clin Nutr 34:1469–1478

Solomons NW (1986) Competitive interaction of iron and zinc in the diet: Consequences for human nutrition. J Nutr 116:927–935

Tangkongchitr U, Seib PA, Hoseney RC (1981) Phytic acid, II. Its fate during breadmaking. Cereal Chem 58:229–234

Received 25 March 1998; revised 4 May 1999; accepted 12 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 457-458

Nutritional Evaluation of Vegetable *Colocasia* Grown in Himachal Pradesh

R.A.K. AGGARWAL*, P.S. ARYA AND N.C. MAHAJAN
Department of Chemistry and Biochemistry,
HP Krishi Vishvavidyalaya, Palampur–176 062, India.

Colocasia has the capability of growing under varied climatic conditions. It is a widely grown vegetable in almost all parts in Himachal Pradesh. The versatility of this plant is reflected by the fact that not only the rhizome but also the stem and leaves are used as vegetable. The nutritional quality of *Colocasia* and *Alocasia* grown in different regions of Himachal Pradesh was assessed. This will help in developing the varieties of *Colocasia* with better nutritional quality. The results showed that carbohydrates ranged from 5.51 to 9.87% and constituted the major source of dietary energy in all the samples. Fat content varied from 0.34 to 0.61% and proteins 1.11 to 1.64% in edible portion of the tuber. All samples were fairly rich in magnesium and potassium.

Keywords : *Colocasia, Alocasia,* Nutritional evaluation.

Colocasia esculanta and *Alocasia* are perennial tuberous plants with large heart shape leaf blades borne on long petioles of 1.5 to 7.0 feet high arising from a group of underground corms. The corms vary greatly in size and shape and the central ones are big and conspicuous. *Colocasia* in its various forms is a crop of warm humid region and grows on all kinds of soils but thrives best in deep, well drained and well manured loam soil. It can also be cultivated in dry regions under irrigation (The Wealth of India 1950). All parts of *Colocasia* (leaves, stem, tuber) are edible and rich in carbohydrates, proteins and minerals. Steamed corms constitute a high energy food and its flour can be prepared from raw or pre-cooked tubers for preparing soups and gruels. It is excellent for gravies and puddings and is not glutenous like wheat flour. Young leaves and stalks are edible and can be cooked and used like spinach. The stalks are also used for the preparation of nuggets. In view of its versatile acceptance for edible purposes, an attempt was made to collect its germplasm from different parts of Himachal Pradesh and its nutritional quality was assessed. *Colocasia* and *Alocasia* tuber samples were collected from different parts of Himachal Pradesh, directly from the field at the time of harvest. The samples were analyzed in laboratory for different dietary parameters according to the methods of AOAC (1990). Minerals were analyzed using Atomic Absorption Spectrophotometer (Gabriels and Cottenie 1976). The results showed that carbohydrates were the major constituents in *Colocasia* (Table 1). The maximum levels of carbohydrates in *Colocasia* (9.87%) and *Alocasia*

TABLE 1. LEVELS OF VARIOUS DIETARY CONSTITUENTS OF *COLOCASIA* AND *ALOCASIA* IN HIMACHAL PRADESH. (VALUES ARE GIVEN IN PERCENT OF EDIBLE FRESH WEIGHT BASIS).

	g/100 g							mg/100 g						
Name of Place	Moisture, %	Crude proteins	Fat	Carbo-hydrates	Silica	Ash	Crude fibre	Ca	Mg	Na	K	Fe	Zn	Cu
Colocasia														
Shimla	86.82	1.20	0.42	7.82	0.33	1.10	1.17	1.72	16.81	1.41	525.4	0.069	0.030	0.000
Solan	87.09	1.12	0.42	7.93	0.48	0.86	0.88	2.49	10.06	2.55	574.6	0.000	0.080	0.000
Sarahan	89.79	1.22	0.43	5.92	0.31	0.84	1.02	2.82	35.83	1.63	542.9	0.217	0.100	0.016
Renuka	90.00	1.53	0.34	5.51	0.33	0.93	1.13	1.27	11.96	1.63	366.2	0.098	0.040	0.000
Panjahal	83.93	1.64	0.61	9.87	0.82	1.11	0.90	2.39	50.39	2.85	526.4	0.285	0.080	0.085
Alocasia														
Shimla	86.22	1.26	0.42	7.88	0.45	0.65	0.58	2.23	29.43	3.18	454.4	0.136	0.030	0.000
Sarahan	84.62	1.16	0.48	9.72	1.03	1.04	1.28	2.63	38.03	3.46	516.5	0.138	0.000	0.000
Panjahal	87.88	1.11	0.48	7.33	0.25	0.88	1.45	3.50	58.55	2.26	395.0	0.169	0.000	0.000
Mandi	88.54	1.27	0.35	6.54	0.48	1.05	1.10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. = Not done, Each value is mean of three determinations

* *Corresponding Author*

(9.72%) were found in samples from the regions of Panjahl and Sarahan, while minimum levels of 5.92% and 5.51% were observed in *Colocasia* of Sarahan and Renuka regions, respectively. The levels of carbohydrates in other samples did not differ widely (6.8 - 7.5%). The crude protein (1.64%) and fat (0.61%) were maximum in *Colocasia* samples of Panjahal, while the values in the samples from other places ranged from 1.11 to 1.53% and 0.34 to 0.48% for protein and fat, respectively. The crude fibre was 1.45%, a highest level in *Alocasia* samples from Panjahal and minimum was 0.58% in samples from Shimla. The *Colocasia* samples from Solan and Panjahal also had lower crude fibre contents of 0.88% and 0.90%, respectively. Table 1 also presents data on the selected mineral contents of *Colocasia* and *Alocasia*. Both the roots have shown to contain fair amounts of minerals such as potassium, magnesium, sodium and iron. Further, the results showed that there was a degree of variation in the mineral contents of samples collected from different regions of Himachal Pradesh. However, because of its versatile acceptance and its vegetative output coupled with the presence of carbohydrates and minerals, there is scope for developing these crops in a big way for a possible introduction in Indian dietary.

The authors thank Prof. S.D. Sankhyan, Department of Soil Science, Himachal Pradesh Krishi Vishvavidyalaya, Palampur for help in analysis of samples for minerals using Atomic Absorption Spectrophotometer.

References

AOAC (1990) Official Methods of Analysis. Association of Official Analytical Chemists, Washington DC

Gabriels R, Cottenie A (1976) Direct reading emission spectrophotometric determination of major and trace elements in plants, soils and soil extracts. Lab Practice 25(12):835–838

The Wealth of India (1950) Council of Scientific and Industrial Research, New Delhi, Vol. II, pp 309-312

Received 25 March 1998; revised 8 May 1999; accepted 12 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 459-462

Physico-chemical, Microbiological and Sensory Characteristics of Washed Fish Mince Prepared from Some Selected Species of Fish

FEMEENA HASSAN* AND SALEENA MATHEW
School of Industrial Fisheries, Cochin University of Science and Technology, Fine Arts Avenue, Kochi-682 016, India.

Physico-chemical, microbiological and sensory characteristics of washed fish mince prepared from *Cyprinus carpio*, *Oreochromis mossambicus*, *Nemipterus japonicus* and *Scoliodon sorrakowah* were determined using standard procedures. All species studied showed that the minces prepared were suitable for product development. Since the consumer acceptability varies with species, separate procedures should be adopted for each species, while standardising methodology for surimi preparation in commercial establishments.

Keywords : Surimi, Compressibility, Sensory evaluation, Rheological property.

Throughout the world, fish mince has attracted considerable attention from food manufacturers. Its unique texture forming ability made it an excellent base to manufacture a variety of seafood products. Minced fish is mechanically separated flesh that has not been washed and is not as stable as fish fillets on storage because of tissue disruption and enzyme release during mincing (Nakayama and Yamamoto 1977; Lee 1984; Regenstein 1986). Water washing can improve quality and functional characteristics of minced fish. By repeated washing of minced meat, most of odour imparting compounds, pigments, water soluble proteins and undesirable, materials are removed and a translucent, bland material - surimi - is obtained. Surimi provides greater opportunities for product diversification and has better storage stability than unwashed mince.

Numerous biochemical reactions continue to take place in the fish muscle even after death. Biochemical and microbiological changes affect the composition of meat. To produce a finished product of high quality, it is important to protect and monitor the integrity of the product. Biochemical composition of mince also varies with species. Before selecting fish for producing mince to prepare a product, it is very essential to know the characteristics of mince. The present study was, therefore, undertaken to assess the physico-chemical, microbiological and sensory characteristics of washed fish mince prepared from some selected species of fish.

For preparing surimi, a fresh water , a brackish water and two marine fish were used. The fresh water fish used was common carp (*Cyprinus carpio*). It was collected from M/s Pookote Fish farm, Trichur, India. The brackish water fish used was Tilapia (*Oreochromis mossambicus*) and was collected from Matsyafed Fish Farm, Narakkal, Kochi. The marine fish used were kilimeen (*Nemipterus japonicus*) and shark (*Scoliodon sorrakowah*). Both were collected from Kochi Fisheries Harbour. The fishes were immediately chilled in ice at 1:1 ratio and brought to the processing hall without delay.

Preparation of surimi : In the processing hall, the fishes were beheaded and eviscerated. Inner lining of the peritoneum was removed and washed with potable water at 10°C. If the fishes were more than 20 cm in length, they were filleted. This was fed to a Baader 694 meat-bone separator (Nasan, Nova Scotia Corporation, New York) equipped with a 5-mm diameter perforated drum. The minced meat coming out of the machine was collected in polythene bags. These bags with mince were put in vessels containing ice cubes, so that mince will always remain at a temperature below 10°C.

Washing of mince was carried out at 10°C using potable water. The volume of water for each washing was five times that of mince. Three washing cycles were followed. For the last washing, 0.3% sodium chloride solution was used to ease the removal of water in the further processing steps. During washing, agitation was done at a low speed of 300 rpm using a paddle type stirrer. The washed mince was subjected to partial dewatering by covering with cheese cloth and then squeezed in a screw press, until the mince just began to come out. Then, this partially dehydrated

* *Corresponding Author; Present address: Krishi Vigyan Kendra, Indian Institute of Spices Research, Peruvannamuzhi (P.O.) . Kozhikode, Kerala, India.*

meat was mixed with 4% sucrose, 4% sorbitol and 0.3% sodium tri poly phosphate in a silent cutter for 2 min at 15–18°C. This washed mince - surimi - was packed in polythene bags, frozen at -40°C for 90 min and stored at -20°C temperature. Samples were withdrawn at regular intervals for conducting various analyses. Each analysis was carried out in triplicate.

Composition and quality attributes of surimi gels : Moisture, protein and non-protein nitrogen contents of the samples were estimated according to AOAC (1984) methods. The pH was determined with the help of pH meter. Trimethyl amine and total volatile nitrogen contents were estimated by micro- diffusion method of Conway (Conway 1950). The method of Pope and Stevens (1939), using 5% trichloroacetic acid extract of the sample determined alpha amino nitrogen. Compressibility of the samples was determined as follows: Surimi (80 g) was mixed with 2% NaCl and homogenized for 3 min in a homogeniser with cutting blades. The resulting paste was stuffed in a cylindrical pre-fabricated mould (2cm ϕ x 6cm) and steam, cooked for 20 min. The sample was taken out from the mould and tested in the Universal Testing Machine (Model; Zwick 1484) as per standard procedure. A similar sample prepared as above was used to conduct Folding test. For this, the sample was cut into a slice of 3-mm thickness. The cut slice was folded into half and again into quarter. Then, the pieces were examined for signs of structural failure (cracks). The minimum amount of folding required to produce a crack in the gel determined the score of the test. Scores were assigned as per the standard method (Suzuki 1980). Expressible moisture of sample was determined by pressing one gram of sample between two filter papers under constant pressure (10 kg/cm^2) for 10 sec. The weight difference of the sample before and after pressing when expressed in percentage gave expressible moisture.

Microbiological parameters, total plate count (TPC) per g. *E. coli*/g, *Staphylococcus*/g, *Salmonella* g, *Vibrio*/g were determined as per the procedure given by US Food and Drug Administration (1984).

For conducting organoleptic studies, samples were steam-cooked for 20 min in pre-fabricated cylindrical stainless steel moulds (40 mm ϕ x 5 mm). A sensory panel consisting of 10 judges was formed. The samples were coded and presented to the judges. The panelists were instructed to give appropriate scores to the sensory attributes of appearance, colour, odour, texture and overall acceptability of the products in the score sheet given to them.

The results were analyzed statistically (Snedcor and Cochran 1967).

The chemical characteristics of washed mince of fishes studied are shown in Table 1. *Nemipterus* showed high moisture content compared to surimi of other species (82%). Shark showed the least moisture content (75.12%). Similar results were reported by Poulter and Trevino (1983); Babbit (1986); Hastings et al (1990); Spencer et al (1992); Yean (1993) and Saeki et al (1992). Of the four species of fishes studied, the highest protein content was found in shark (17.01%) and lowest in *Nemipterus* (15.75%). On studying the protein content in different species of fishes, Babbit (1986), Chang-Lee et al (1990), Poulter et al (1981) and Yean (1993) reported similar values. The

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF WASHED FISH MINCE

Characteristics	Common carp	*Tilapia*	Shark	*Nemipterus*
*** Chemical**				
Moisture, %	80.12 ± 0.44	80.40 ± 0.23	75.12 ± 0.34	82.00 ± 0.18
Total proteins, g/100 g	16.38 ± 0.11	16.57 ± 0.23	17.01 ± 0.31	15.75 ± 0.24
Non-protein nitrogen, g/100g	0.17 ± 0.03	0.20 ± 0.006	0.33 ± 0.05	0.22 ± 0.06
pH	6.81 ± 0.10	6.86 ± 0.12	6.72 ± 0.09	6.8 ± 0.11
TMA, mgN/100g meat	ND	1.8 ± 0.02	4.0 ± 0.04	2.1 ± 0.02
TVN,mgN/100g meat	4.5 ± 0.06	2.7 ± 0.03	6.2 ± 0.04	8.0 ± 0.04
Alpha amino nitrogen, mgN/100g meat	42.2 ± 0.23	33.9 ± 0.35	16.8 ± 0.68	27.2 ± 0.52
**** Physical**				
Compressibility,Nm	3.48 ± 0.11	2.6 ± 0.05	3.6 ± 0.01	3.0 ± 0.06
Folding test score	4.98 ± 0.55	3.8 ± 0.36	5.0 ± 0.01	4.4 ± 0.12
Expressible water, %	22.00 ± 0.56	36.0 ± 0.68	38.0 ± 0.47	45.0 ± 0.96

*mean of 3 replicates
**mean of 10 x 3 replicates

non-protein nitrogen contents in the samples studied ranged between 0.17 and 0.33 g/ 100g wet meat. The lower values may be due to the repeated washing. The washing step during surimi preparation will remove most of soluble nitrogenous compounds.

Table 1 shows that the pH values range between 6.72 and 6.86. Poulter et al (1981) have reported similar values. They obtained pH values of 6.5, 6.4, 6.5 and 6.9 for the surimi of Gulf croaker, Bronze-stripped grand, Orange mouth *corina* and *Caboi cuchos*, respectively. After studying the pH value of surimi of whitehake, herring and Atlantic mackerel, Spencer et al (1992) reported the values of 6.6, 7.2, and 6.9, respectively. In Salmon surimi, Saeki et al (1992) reported a value of 6.9.

Lee (1992) reported that rheological property of surimi was affected by muscle pH. The strength of surimi gel is dependent on the pH value of comminuted fish meat. The optimum pH varies with species, formulation of surimi and ingredients added. The optimum pH was found to be in the range of 6.0 to 7.0.

Trimethylaminoxide (TMAO) is a natural component in muscle and many organs of marine fish, but is completely lacking or is present in very small amounts in freshwater fish (Dyer 1952). Microorganisms invade the fish flesh and reduce TMAO to TMA as spoilage advances. The TMA content is often used as an indicator for decomposition in fish. According to Babbit (1986), TMA has been linked to fishy odour and flavour. The TMA content seems to be less in surimi compared to fish fillets, since it is water soluble. Table 1 shows the contents of TMA, TVN and alpha amino nitrogen of the washed mince in the species of fishes studied. For each study, the contents were found to be different.

Table 1 shows the physical properties of washed fish mince also. Gel forming ability of surimi is the most important functional requirement, imposing good quality to surimi-based products. In processed meat products, gelation of muscle protein contributed desirable texture and stabilization of fat and water. Gel forming ability varies with species (Table 1). This may be due to the consequence of intrinsic difference in myosin, initial pH, protein extractability and functional properties of muscle proteins in different species. Folding test, in turn, depends on gel forming property or gel strength. The folding test score was found to be maximum for shark. Compressibility also was found to be maximum for shark. The least expressible water was in common carp and the highest was in *Nemipterus*, which may be due to the peculiarity of meat.

The flesh of fish caught from sea is sterile. After death, bacterial attack from surface, gut and gill increases the microbial load of fish muscle. So, the environment has got a role in contributing to the bacterial load of muscle. During processing of surimi due to beheading, gutting and washing, there is a reduction in bacterial load, but at the same time, the increased handling will increase the number of bacteria in the surimi.

Table 2 shows the incidence of bacteria studied. TPC/g ranged from 5.5×10^4 to 3.8×10^5. The least count was reported in common carp. Raccach et al (1978) reported that aerobic plate count (APC) of mechanically deboned cod, pollock and whiting ranged from 4.7×10^5/g to 7.0×10^5/g. Blackwood (1974) found that there was a bacterial load of 10^8/ g of meat for the minced fish samples. In the present study, though there was no incidence of *Salmonella* and *Vibrio* in any of the samples studied, there was incidence of *E. coli* in the samples of *Tilapia* and incidence of *Staphylococcus* in common carp, *Tilapia* and *Nemipterus*. While preparing mince, utmost care should be taken to prevent the incidence of pathogenic bacteria, since mince is meant for human consumption.

Sensory evaluation is the oldest and still the most widespread means of evaluating the acceptability and edibility of fish. The mean sensory scores for different attributes studied for washed mince of different species are presented in Table 2.

TABLE 2. MICROBIOLOGICAL AND SENSORY PROPERTIES OF WASHED SURIMI

Characteristics	Common carp	*Tilapia*	Shark	*Nemipterus*
***Microbiological**				
TPC/g	5.5×10^4	3.4×10^5	3.8×10^5	1.1×10^5
E. coli/g	ND	4.0	ND	ND
Staphylococcus/g	5.0	6.0	ND	4.0
Salmonella/g	ND	ND	ND	ND
Vibrio/g	ND	ND	ND	ND
****Sensory attributes**				
Appearance	8.6	8.4	8.2	8.3
Colour	8.8	8.1	8.7	8.8
Odour	8.2	8.4	8.4	8.6
Texture	7.6	7.6	8.3	7.8
Overall acceptability	8.0	7.8	8.0	7.8

*mean of three replicates
**mean of 10 x 3 replicates

It was observed that there was a variation in acceptability with species. So, while standardising methodology for the production of surimi in commercial preparation, separate procedures should be adopted for each species to get better acceptability. To improve the organoleptic qualities, methods can be adopted by incorporating some ingredients or by altering the existing technology, but at the same time attention has to be given to retain the physico-chemical and microbiological parameters within the acceptable limit.

References

AOAC (1984) Official Methods of Analysis 14th edn. Association of Official Analytical Chemists, Washington DC

Babbit JK (1986) Suitability of seafood species as raw material. Food Technol 40:97-100

Blackwood CM (1974) Utilization of mechanically separated fish flesh-Canadian experience. In: Kreuzer R (ed.) FAO Conference on Fishery Products, Tokyo, Publishers. Fishing News (Books) Ltd., Surrey, England.

Chang-Lee MV, Lampila LE, Crawford DL (1990) Yield and composition of surimi from Pacific whiting (*Meluccinus productus*) and the effect of various additives on the gel strength. J Food Sci 44(4):1087-1092

Conway ED (1950) Microdiffusion analysis and volumetric error. 3rd edn. Crossby, Lockwood and Sons Ltd., London

Dyer WJ, French HV, Snow JM (1950) Proteins in fish muscle. Extraction of protein fractions in fresh fish. J Fish Res Board Can 7:585-593

Hastings RJ, Keay JN, Young KW (1990) The properties of surimi and kamaboko gels from nine British species of fish. Int J Food Sci Technol 25(3):281-294

Lee CM (1984) Surimi processing technol. Food Technol 38: 69-70

Lee CM (1992) Factors affecting physical properties of fish protein gel. In: Advances in Seafood Biochemistry and Quality. George J, Flick JR, Martin RE (eds) Technomic Publishing Co. Inc., USA

Nakayama T, Yamamoto M (1977) Physical, chemical and sensory evaluations of frozen stored deboned (minced) fish flesh. J Food Sci 42(4):900-945

Pope CG, Stevens MF (1939) The determination of amino nitrogen using a copper method. Biochem J 33:1070-1074

Poulter NH, Trevino JE (1983) Acceptability of a canned paste product based on some Gulf of California shrimp by-catch. J Food Technol 18:405-409

Raccah M, Baker RC (1978) Microbial properties of mechanically deboned fish flesh. J Food Sci 43:1675-1677

Regenstein JM (1986) The potential for minced fish. Food Technol 40:101-106

Seaki H, Shoji T, Hirata F, Nanaka M, Arai K (1992) Effect of calcium chloride on gel forming ability and cross linking reaction of myosin heavy chain in salt ground meat of tuna, carp and walleye pollock. Nippon Suisan Gakkaishi, 58:2137-2146

Snedecor GW, Cochran WG (1967) Statistical Methods. 6th edn. Iowa State University, Iowa.

Spencer KE, Hotten C, Ablett RF, Bligh EG (1992) Gelling and storage performance of surimi from a range of Atlantic Canadian species. In: Advances in Seafood Biochemistry Composition and Quality pp 199-211

Suzuki T (1981) Fish and Krill Processing Technology. Applied Science Publ. Ltd. London

USFDA (1984) Bacteriological Analytical Manual. 6th edn. Association of Official Analytical Chemists, London

Yean YS (1981) The quality of surimi made from threadfin bream stored on ice for different periods. Int. J Food Sci Technol 28:343-346

Received 30 April 1998; revised 31 May 1999; accepted 12 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 463-465

Comparison of Mutton, Rabbit and Their Combination of Meats for Sausage Processing

A.R. SEN

Central Sheep and Wool Research Institute, Avikanagar–304 501, India.

The physico-chemical, nutritional and sensory properties of rabbit, mutton and their combination (50:50) were compared for sausage processing. Mutton produced significantly (P<0.05) less stable emulsion than did either rabbit or combination of meats. The water holding capacity (WHC) of rabbit meat was superior (P<0.05) to that of mutton, while its fat content was lower (P<0.05), as compared to mutton or combination meat sausage. The mutton sausage was darker red and firmer (P<0.05) than the rabbit sausage confirmed by Hunterlab and Instron readings. Sensory evaluation for flavour and juiciness of rabbit sausages was comparable to combination meats and slightly inferior to those of mutton. However, overall palatability scores were higher (P<0.05) in mutton sausage.

Keywords : Mutton, Rabbit, Emulsion, Sausage, Texture, Sensory characteristics.

Meat from culled sheep is tougher and poor in palatability due to higher collagen content, its cross linkages and objectionable odour (Locker 1980). On other hand, rabbit meat has very good nutritional value being comparatively high in proteins, low in fat, calories and sodium (Sunki et al. 1978). However, due to pet appearance, rabbit meat is not so popular throughout the country. Hence, for effective utilization of culled sheep and rabbit, the right choice would be to convert them into value-added, ready-to-eat meat products.

A number of researchers have worked out the properties of sausage emulsions and the products prepared from chicken (Hargus et al. 1970; Schnell et al. 1972), chicken and rabbit (Baker et al. 1972; Whiting and Jenkins 1981), mutton (Marshall et al. 1977) and chicken, pork and rabbit (Mendiratta and Panda 1992). The combination of meats was also studied for various products such as frankfurters (Bushway et al. 1988) and patties (Anjaneyulu et al. 1990), whereas combinations of mutton and rabbit have not been studied. Therefore, the purpose of the present study was to examine the nutritional, physico-chemical and sensory properties of sausages prepared from mutton, rabbit and their combination of meats.

Sausage formulation and processing : Malpura sheep and White Giant rabbits were slaughtered by traditional Halal method and the carcasses were manually deboned within 3 h post-mortem. The meat was trimmed of excess fat and connective tissue. All the materials were frozen/stored at –10°C and used for the study after partial thawing at 5°C for 15–20 h. Meat was cut into small cubes and coarsely minced by a mincer of 8 mm plate. Meat emulsion was prepared for each treatment in a bowl chopper. Combination formulation contained 50% each of mutton and rabbit meat.

Sausage was prepared by standard commercial methods. The lean meat was pre-mixed with salt 1.75%, phosphate 0.5%, ice flakes 10% and sodium nitrate 100 ppm for 2 h and chopping was continued, until a stable emulsion was formed. The emulsion was stuffed by hand-operated sausage filler into sheep casings prepared in the laboratory. The prepared sausage was linked and cooked for 25 min in a 90°C water bath. The prepared sausage was showered with cold water for 20 min and then refrigerated at 4°C for later sensory evaluation (one day after preparation).

Physico-chemical analysis : Stability of meat emulsion was determined as per the procedure of Towensend et al (1968) with some modifications. About 25 g of sample was placed in polyethylene bags and the bags with samples were weighed and sealed. These bags were immersed in a water bath at 80°C for 20 min. The bags were, then, removed from the water bath, cut open and cook fluids (fat, water and solids) were drained. The cooked samples were weighed and loss of weight after cooking was calculated and expressed in percentage as index of emulsion stability.

The raw meat and emulsion pH values were recorded using digital pH meter by homogenizing 10 g of sample with 50 ml distilled water for 1 min. Shear force value of sausages was determined using Warner Bratzler Shear force press (GR Electric Manufacturing Co. USA). A press method was used for the estimation of water holding capacity (WHC) of raw meat and sausage (Trout 1988). One gram of finely ground meat was placed

* *Corresponding Author*

between two filter papers. The filter papers and samples were then placed between two plexiglass plates and pressure was applied for 1 min. The moisture absorbed by the filter papers after removal of the tissue residue was taken as a measure of water holding capacity. The proximate analyses of raw meat and sausage samples was carried out by AOAC (1984) methods.

Instrumental evaluation of colour and texture: Hunter lightness(L), redness (a) and yellowness (b) of sausages were recorded, using a computerised colour matching system (Jaypak 4802), the illuminant chosen was D65. The firmness of sausage was measured on the computerised Instron system (ID:4465–H1864, UK). The peak force (maximum force in mN required to penetrate 2.5 cm slice of sausage) was recorded in these samples as a measure of firmness or hardness (Bushway et al. 1988).

Sensory evaluation : The sausages were deep-fat-fried, cut into 2 cm length pieces and randomly offered to the sensory panel, comprising 7 members. The panelists were asked to score the sausage in 6–point Hedonic scale for the sensory attributes.

Statistical analysis : The data were subjected to analysis of variance (Snedecor and Cochran 1968) and significant differences were compared by Duncan's multiple range test (Duncan 1955).

Sausage yield (Table 1) was higher ($P<0.05$) in combination meat sausage formulation as compared to sheep and rabbit meat sausages. This was also confirmed by emulsion stability test. The amount of exudate in stability test indicated that mutton had less ($P<0.05$) stable emulsion than either rabbit or combination of meats. Rabbit emulsion was smooth and fine in texture, while the mutton was noticeably coarser and thicker. Such differences could be attributed to higher connective tissue of mutton, as also observed by Whiting and Jenkins (1981). The pH values of raw and emulsion of sheep and rabbit meat were similar. The texture of mutton sausage was, however, significantly ($P<0.05$) better, as indicated by shear force value than the sausage of other formulations. The results are in agreement with the observations of Anjaneyulu et al (1990). The WHC% was higher ($P<0.05$) in raw rabbit meat as compared to mutton. whereas the WHCs of prepared sausages from sheep, rabbit and combination of meats were similar.

The sausage of rabbit meat formulation was lower ($P<0.05$) in fat content than the mutton sausage reflecting the differences in intramuscular fat between rabbit and red meat (Table 1). Similar

TABLE 1. EFFECT OF COMBINATION OF MEATS ON QUALITY CHARACTERISTICS OF SAUSAGE

		Mutton	Rabbit	Mutton+Rabbit
Cooking yield, %		94.89 ± 0.47^{b}	94.79 ± 0.39^{b}	96.24 ± 0.05^{a}
Emulsion stability, %		3.38 ± 0.14^{a}	2.51 ± 0.21^{b}	2.73 ± 0.07^{b}
Emulsion pH	Raw	5.77 ± 0.05	5.91 ± 0.01	5.88 ± 0.04
	Product	6.21 ± 0.06	6.21 ± 0.01	6.22 ± 0.03
WHC, %	Raw	65.98 ± 1.13^{b}	71.40 ± 0.42^{a}	71.15 ± 0.77^{a}
	Product	56.01 ± 0.52	61.07 ± 1.99	56.44 ± 1.94
Moisture, %	Raw	72.43 ± 0.84	73.48 ± 0.41	73.91 ± 0.67
	Product	64.30 ± 0.35^{b}	65.44 ± 0.32^{a}	64.22 ± 0.38^{b}
Fat, %	Raw	4.51 ± 0.20^{a}	1.21 ± 0.11^{c}	2.22 ± 0.29^{b}
	Product	10.05 ± 0.34^{a}	6.55 ± 0.52^{b}	9.33 ± 0.34^{a}
Proteins, %	Raw	19.86 ± 0.12^{b}	23.13 ± 0.51^{a}	20.62 ± 0.73^{b}
	Product	12.08 ± 0.11^{c}	14.32 ± 0.06^{a}	13.55 ± 0.16^{b}
Hunterlab				
L		40.19 ± 0.55^{b}	46.39 ± 0.25^{a}	46.66 ± 0.24^{a}
a		7.38 ± 0.11^{b}	5.57 ± 0.09^{a}	6.53 ± 0.15^{b}
b		11.86 ± 0.24	13.55 ± 0.16	11.28 ± 0.19
Colour		4.83 ± 0.31^{a}	4.33 ± 0.33^{ab}	3.83 ± 0.31^{b}
Flavour		4.66 ± 0.21	4.00 ± 0.51	4.33 ± 0.49
Juiciness		4.33 ± 0.33	3.83 ± 0.48	3.83 ± 0.31
Tenderness		4.66 ± 0.33^{a}	4.00 ± 0.36^{b}	3.83 ± 0.17^{b}
Overall palatability		4.66 ± 0.35^{a}	4.00 ± 0.36^{b}	3.83 ± 0.34^{b}
Peak force (mN/2.5 cm)		2076.56 ±166.33^{a}	1851.25 ±80.70^{b}	1912.68 ±115.12^{b}

Means with the different superscripts in rows differ significantly ($P<0.05$)

observations were also made by Bushway et al (1988) in frankfurters prepared from mutton and fowl. The protein contents in raw meat and product of rabbit were higher (P<0.05) as compared to mutton and combination of meat sausage formulation, indicating the superiority of rabbit meat in emulsion-based comminuted products.

Sausages prepared from rabbit meat were light coloured (higher 'L' value) than those of mutton and combination meat sausage (Table 1). The mutton sausage was much redder ('a' value-7.38) than rabbit sausage ('a' value-5.57), which can be explained by the fact that rabbit muscle has lower myoglobin than red muscle meats (Lawrie 1979). The higher (P<0.05) 'b' values signified that the rabbit sausage was yellower in colour as compared to mutton and combination meat sausages, which is not a preferred characteristic of sausages.

The colour score (Table 1) was higher (P<0.05) for mutton sausage, which was corroborated by Hunterlab colorimeter readings. The flavour and juiciness scores also demonstrated that the mutton sausages were preferred over those made with rabbits. Mutton sausage received higher (P<0.05) texture score than that of rabbit and combination meat sausage. As observed earlier, texture of raw mutton emulsion was coarser and this probably influenced the panelists' evaluation of the finished sausage. Overall palatability scores were higher (P<0.05) in mutton sausage than other two formulations. The mutton sausage was firmer (P<0.05) than the rabbits and combination meat sausage, as was indicated from the Instron peak force.

This study has demonstrated that mutton and rabbit, which have lesser economic value as fresh meat, can be effectively used to manufacture acceptable processed meat products such as sausages. It was also evident that the sausage prepared from mutton was superior in quality attributes than those of rabbit meat.

The author acknowledges the facilities provided by the Director, CSWRI, Avikanagar (Rajasthan) and thanks Mr. M. Nassimuddin, Meat Technology Laboratory and Mr. Gulab Chand for technical assistance.

References

Anjaneyulu ASR, Kondaiah N, Salahuddin Mir, Panda B (1990) Quality of patties from chicken, mutton and combination of meats. J Food Sci Technol 27:184–185

AOAC (1984) Official Methods of Analysis. 14th edn. Association of Official Analytical Chemists, Washington DC

Baker RC, Darfler JM, Vadehra DV (1972) Acceptability of frankfurters made from chicken, rabbit, beef and pork. Poult Sci 51:1210–1213

Bushway AA, Leconte NB, Work TM, True RH (1988) Characteristics of frankfurters prepared from mutton and fowl. J Food Sci 53:67–69

Duncan DB (1955) Multiple Range and Multiple 'F' tests. Biometrics 11:1–42

Hargus GL, Froning GW, Mebus CA, Neelakantan S, Hartung TE (1970) The effect of processing variables on stability and protein extractability of turkey meat emulsion. J Food Sci 35:688–690

Lawrie RA (1979) Meat Science. 3rd edn. Pergamon Press, New York

Locker RH (1980) Cured lamb. In: Developments in Meat Science Vol. 1. Lawrie RA (ed) Applied Science Publishers Ltd., London p 181

Marshall WH, Smith GC, Dutson TR (1977) Mechanically deboned goat, mutton and pork in frankfurters. J Food Sci 42:193–196

Mendiratta SK, Panda PC (1992) Comparison of functional properties of chicken, pork and rabbit meats in different combinations in relation to yield while preparing sausage. Ind Vet J 69:241–245

Schnell PG, Nath KR, Darfler JM, Vadehra DV, Baker RC (1972) Physical and functional properties of hand deboned poultry meat as used in the manufacture of frankfurters. Poult Sci 52:1363–1367

Snedecor GW, Cochran WG (1968) Statistical Methods. Oxford and IBH Publishing Co. Calcutta

Sunki GR, Annapureddy R, Rao DR (1978) Microbial, biochemical and organoleptic changes in ground rabbit meat stored at 5 to 7°C. J Animal Sci 48:584–588

Towensend WE, Witnaeur LP, Riloff JA, Swift CE (1968) Comminuted meat emulsions. Differential thermal analysis of fat transition. Food Technol 22:319–323

Trout GR (1988) Techniques for measuring water binding capacity in muscle foods. A review of methodology. Meat Sci 23:235–252

Whiting RC, Jenkins RK (1981) Comparison of rabbit, beef and chicken meats for functional properties and frankfurter processing. J Food Sci 48:1693–1696

Received 30 April 1998; revised 1 June 1999; accepted 12 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 466-468

Post-harvest Biochemical Changes in Mature Oil Palm (*Elaeis guineensis*) Fruit Pericarp During Storage

SALINI BHASKER, G.L. MINIL KUMAR AND C. MOHANKUMAR*
Plant Biochemistry Unit, Department of Botany,
University College, Thiruvananthapuram - 695 034, India.

Activities of peroxidase (POD), polyphenol oxidase (PPO) and lipoxygenase (LOX) in the fruit pericarp were assayed and quantified. The nature of these enzymes during storage under various conditions viz., ambient temperature (31°C), -10°C, 15°C and 50°C was examined. The results showed that the enzyme activities had enhanced during storage at room temperature and reduced to a lower level at 50°C. At cold conditions, the activities of POD, PPO and LOX were found to be decreasing. It was also observed that POD and LOX were extremely active in the epicarp as compared to mesocarp.

Keywords : Oil palm fruit, Epicarp, Mesocarp, Pericarp, Post-harvest changes, Storage, Enzymes.

The fruit of oil palm is a 'drupe' consisting of an outer soft epicarp and middle fibrous fleshy mesocarp, together called the pericarp with a hard shell enclosing the endosperm. Oil palm (*Elaeis guineensis*) has three varieties in which 'tenera' is the main cultivar because of its high yielding potential. From the commercial angle, the mesocarp of oil palm fruit has been studied intensively, since it is the source of palm oil. Excessive production of free fatty acids during handling and bruising is a major problem associated with harvesting and processing of oil palm fruits (Arumughan et al. 1989). It has been reported that lipase of oil palm fruit mesocarp was the key enzyme responsible for the quality deterioration of palm oil (Mohankumar et al. 1990; Henderson and Osborne 1991). Very few studies have been conducted to understand the biochemical changes of structural and soluble non-lipid constituents of oil palm fruit mesocarp (Mohankumar et al. 1994; Salini Bhasker et al. 1997). Being a tropical fruit, other physiologically active enzymes present in the fruit tissues of oil palm have not be investigated in spite of their physiological role in the quality of palm oil. Therefore, in the present study, an attempt was made to find out the post-harvest physiological changes in pericarp of oil palm fruit during storage under different conditions.

Plant material : Mature sound oil palm fruits were collected from identified healthy palms of 'tenera' variety from Central Plantation Crops Research Institute (CPCRI), Palode, Thiruvananthapuram. The fruits were stored for 5 days under following conditions: ambient condition (31°C), incubator at 50°C, cold chamber at 15°C and freezer (-10°C). Fruit samples from the storage conditions were drawn everyday for the enzyme assay studies. The regions of the fruit tissues viz., outer epicarp and middle mesocarp were used for the assay.

Assay of enzymes : Peroxidase (POD), polyphenol oxidase (PPO) and lipoxygenase (LOX) were assayed by spectrophotometric methods. Absorbance was made against enzyme blanks containing all the reactants except substrate. Enzyme activity was measured as units per gram tissue. LOX, POD and PPO were extracted and assayed according to the methods of Chen and Whitaker (1986), Golíber (1989) and Pilar Cano et al (1995), respectively. Known quantities of epicarp and mesocarp tissues were chopped and homogenised in phosphate buffer at 4°C. The slurry was centrifuged at 15000 **g** and the supernatant fraction was used as the enzyme source for the assay.

Peroxidase activity was determined by measuring the rate of increase in absorbance at 470 nm. The assay mixture contained 20 mM guaiacol solution, 10 mM H_2O_2 and enzyme extract. Activity of PPO was determined by reacting the enzyme with catechol as substrate at 420 nm. Linoleic acid was used as the substrate for the assay of LOX and increase in absorbance was recorded at 234 nm. The *in vivo* lipase activity related to the formation of free fatty acids was estimated by the method of Arumughan et al (1989).

Distribution of peroxidase (POD), lipoxygenase (LOX) and polyphenol oxidase (PPO) in the epicarp and mesocarp : Oil palm fruit requires 180 days after pollination to reach full maturity. The fruit

* *Corresponding Author*

TABLE 1. DISTRIBUTION OF POD, PPO AND LOX IN THE EPICARP AND MESOCARP OF OIL PALM FRUIT DURING STORAGE

Storage condition	Parts of the fruit	Activity of POD Units/g tissue					Activity of PPO Units/g tissue					Activity of LOX Units/g tissue					*In vivo* lipase activity % of FFA released*				
		Days of storage					Days of storage					Days of storage					Days of storage				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Room temp.	Epicarp	3.25	19.00	10.25	9.75	9.75	2.25	3.00	1.75	1.75	1.75	30.5	31.5	33.0	34.5	35.0					
31°C	Mesocarp	1.80	1.10	1.10	0.60	0.30	22.25	15.90	4.75	3.6	3.0	10.0	8.0	10.0	12.0	12.0	35.0	36.0	36.5	37.0	40.0
50°C	Epicarp	3.50	3.25	2.00	2.50	2.00	2.00	6.00	1.75	1.25	0.5	3.5	3.5	2.5	2.5	2.5					
	Mexoxcarp	1.40	0.50	0.40	0.25	0.20	20.00	34.00	9.10	7.9	1.5	5.0	8.0	7.0	4.0	4.0	4.5	3.0	2.5	1.8	1.2
15°C	Epicarp	4.75	3.00	2.75	2.50	2.20	1.50	4.75	1.50	1.5	1.5	3.5	6.0	8.0	10.0	12.0					
	Mesocarp	1.60	1.20	0.80	0.75	0.60	17.10	22.10	3.50	3.5	4.0	5.0	3.0	2.0	2.0	2.0	35.0	35.0	35.0	35.0	36.5
-10°C	Epicarp	3.50	8.00	6.25	6.00	6.00	2.50	1.00	0.75	0.75	0.75	4.5	6.0	10.5	22.5	24.0					
	Mesocarp	1.30	1.20	1.00	1.00	0.60	17.00	19.40	2.50	1.3	1.3	5.0	9.0	9.0	10.0	12.0	35.0	37.0	38.0	39.0	39.0

* The whole fruit tissue (Epicarp + Mesocarp) is used for the assay

tissue pericarp has an outer skin or epicarp and middle fleshy mesocarp. Table 1 shows the activity of POD, PPO and LOX distributed at the epicarp and mesocarp regions of the fruit during storage. It is clear from the Table that the activity of POD and LOX was higher in the epicarp region than in the mesocarp region. Under ambient condition (31°C), the activity of POD in epicarp showed an initial steep increase and then a gradual attainment of a steady level, whereas, the activity of lipoxygenase did not show any remarkable change from its maximum level of activity (31 units/g tissue). At 50°C, the activity of POD showed a gradual reduction to a minimum of 2 units /g tissue. Activity of lipoxygenase also exhibited a steep reduction during storage at 50°C. A similar level of reduction in activity of LOX was also observed in fruit stored at cold conditions. Activity of POD in the epicarp showed a moderate level of increase at -10°C.

Activity of peroxidase in the mesocarp exhibited a gradual reduction during storage under conditions such as 31°C, 50°C, 15°C and -10°C. But under the same conditions, lipoxygenase activity showed remarkable variations. A reduction in activity of lipoxygenase was observed in the mesocarp of fruits stored at 50°C and 15°C. Under freezing conditions, lipoxygenase exhibited a sharp increase in activity. Unlike POD and LOX, the activity of PPO was higher in the mesocarp than in epicarp (Table 1). During storage under the above conditions, the activity of PPO in the mesocarp showed a rate of gradual reduction.

Activity of POD, PPO and LOX in the fruit pericarp : The pericarp region of oil palm fruit includes the whole fleshy portion including the outer skin epicarp. It was observed that the three enzymes were active in the pericarp of oil palm fruit at the day of harvest. During storage at ambient condition, it was found that LOX was extremely active in the pericarp, while PPO and POD exhibited a reduction towards the fifth day. However, a steep increase in activity of POD was observed on the second day. The three enzymes PPO, POD and LOX showed losses in activities to a minimum level at 50°C. Activities of LOX and PPO exhibited marked losses on the first day itself and retained the tendency of reduction on consecutive days of storage, whereas the activity of POD was increased initially to a minimum on the fifth day. At cold (15°C) and freezing (-10°C) conditions, the activities of the three enzymes were seen decreasing to a lower level except for LOX at -10°C. Under freezing

conditions, it was found that lipoxygenase activity was increasing after a steep reduction on the first day.

Since the fruit pericarp is the ultimate reserve of lipids, quality deterioration of palm oil can mainly be attributed to the lipolytic enzyme lipase, which is responsible for the formation of free fatty acids (FFA). So, in the present study, the free fatty acids released in the fruit *in vivo* were also estimated to understand the stability of lipase activity *in vivo*. When the fresh fruit was homogenised at 31°C, permitting the lipase to act on endogenous lipids, the free fatty acids rose to 35%. It was noticed that the FFA content remained at a higher level during subsequent days of storage. A reduction in the formation of FFA was noticed in fruit pericarp stored at 50°C for five days. On the fifth day, the fruit pericarp showed a level of 1.2% FFA only. The results obtained from the present study are comparable with earlier reports regarding the formation of free fatty acids (Mohankumar et al. 1990). However, biochemical evidence for the presence of LOX, PPO and POD in oil palm fruit pericarp has not been reported before. It was also observed that the moisture level in the pericarp of fruit after 5 days storage at 50°C was reduced from 28% to 3%.

It is clear from the present study that oil palm fruits stored at hot conditions (50°C) have shown lesser activities of enzymes than at ambient, cold and freezing conditions. Since the fruit enzymes–lipase, peroxidase, polyphenol oxidase and lipoxygenase showed manageable levels of activities at 50°C, it could be considered as an optimum temperature for the post-harvest processing of fruits. However, further research needs to be carried out to design the processing technique for maintaining the quality of the oil.

The authors are grateful to STEC, Government of Kerala for supporting the research. The authors also thank Dr. C. Arumughan, RRL(T) and Dr. R.S.N. Pillai, CPCRI, Palode for help rendered during this work.

References

Arumughan C, Sundaresan A, Prasad KVSV, Damodaran AD. Nampoodiri KUK (1989) Studies of the extraction and evaluation of raw palm oil for edible use. J Food Sci Technol 26:277–282

Arumughan C, Mohankumar C, Kaleysa Raj R (1989) Histochemical Investigation of Oil Palm Fruit Lipase. Proceedings PORIM pp 92–100

Chen AO, Whitaker JR (1986) Purification and characterization of lipoxygenase from immature English peas. J Agric Food Chem 42:2286–2290

Goliber TE (1989) Gravitational stress and lignification in aerial vs. submerged shoots of *Hippuris vulgaris*. Physiolgia Plantarum 75:335–361

Henderson J, Osborne DJ (1991) Lipase activity in ripening and mature fruit of the oil palm. Stability *in vivo* and *in vitro*. Phytochem 30:1073

Mohankumar C, Arumughan C, Kaleysa Raj R (1990) Histological localisation of oil palm fruit lipase. J Am Oil Chem Soc 67:665–668

Mohankumar C, Arumughan C, Kaleysa Raj R (1994) Histochemical changes in mesocarp of oil palm (*Elaeis guineensis*) fruit during Development. J Food Sci Technol 31:19–21

Pilar Cano, Begona De Ancos, Gloria Lobo (1995) Peroxidase and polyphenol - oxidase activities in papaya during post-harvesting, ripening and after freezing/thawing. J Food Sci 60:815–817

Salini Bhasker, Mohankumar C, Pillai RSN (1997) Early identification of oil palm (*Elaeis guineensis*) varieties - dura, tenera and pisifera. Proceedings of 9th Kerala Science Congress pp 121–124

Received 5 June 1998; revised 15 May 1999; accepted 12 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 469-471

Keeping Quality of Salt Cured Shark *Carcharhinus sorrah*

A. TAMIL SELVI*, P. JEYACHANDRAN, G. INDRA JASMINE, S.A. SHANMUGAM AND S. KANNAPPAN

Department of Industrial Fish Processing Technology, Fisheries College and Research Institute, Tuticorin–628 008, India.

Fresh wholesome shark *Carcharhinus sorrah* were aseptically dressed, analyzed for proximate composition, salted in 3:1 ratio and kept at room temperature (32°C) for 48 h after which the dressed fish was washed in potable water, dried under sun for 72 h, packed in High Density Polyethylene (HDPE) oven sacks and stored at room temperature (32°C) for 120 days. Physico-chemical, biochemical and sensory characteristics were studied on samples drawn at 15 days interval. Protein contents increased from 29 to 43 g %, while urea levels reduced from 1.51 to 1.13 mg %. Peroxide value analysis showed significant increase from 3.17 to 38.86 meqO_2/kg fat. Other quality indices have been found within the allowable limits. The samples kept well throughout the storage.

Keywords : Shark, *Carcharhinus sorrah*, Proximate composition, Salting, Keeping quality, Organoleptic evaluation.

Seafoods are known to be potential sources of proteins, fats, amino acids, minerals and small amounts of carbohydrates (Gordievskaya 1973). Among seafoods, *Elasmobranchs*, particularly shark has the outstanding feature that every part of it can be utilised (Shenoy and Dey 1984). In addition, shark is also known for its medicinal value. Smaller sharks are more preferred for edible purposes because these cannot yield suitable hide and fins of commercial value (Shenoy and Dey 1984). Sanitary conditions prevailing in the shark curing yards are alarmingly poor owing to inadequate potable water supply, sand contamination, poor packaging, etc. The salt curing is done by unscientific methods with poor quality salt and unhygienic conditions. As a result, the products are grossly contaminated with dirt, sand, microbes and insect infestation and have only limited shelf life (Govindan 1985). The present study was undertaken to assess the shelf life and suggest ways and means to produce good quality salt cured fish, which is in great demand.

Fresh and wholesome sharks, *Carcharhinus sorrah* caught by trawl nets were procured from Tuticorin, Tamil Nadu, India, fishing harbour, iced immediately and brought to the laboratory. The sharks were washed with 5 ppm chlorinated water, beheaded, degutted, filleted, scored and cleaned in 5 ppm chlorinated water. One part of fresh shark fish was then analyzed for proximate composition. The dressed sharks were dry-salted at 3:1 ratio (fish:salt), cured for 48 h in plastic trays, covered with polythene sheets. The samples were washed in 10% brine solution and sun-dried for 72 h. on iron meshed racks. Finally, the shark fillets were packed in HDPE oven sacks lined inside with LDPE and stored at room temperature (32°C). Samples were analyzed once in 15 days for quality parameters.

Proximate composition : Sodium chloride and water activity by Volhard's method and (AOAC 1980; Doe et al. 1983), trimethylamine (TMA–N) and total volatile base nitrogen (TVB–N) by Convvay micro diffusion method, Beatty and Gibbons (1937), alpha amino nitrogen (AAN) and free fatty acids (FFA) by Pope and Stevens (1939), peroxide value (AOAC 1975), urea content (AOAC 1970), total plates counts (TPC), fungal content (TFC) halophilic count (THC) (APHA 1976) were determined. Sensory evaluation was carried out both in uncooked and cooked (boiled in hot potable water for 10 min) based on 9–point Hedonic scale, with scores such as like extremely 9; like very much 8; like moderately 7; like slightly 6; neither like nor dislike 5; dislike slightly 4; dislike moderately 3; dislike very much 2; and dislike extremely 1.

Proximate composition of fresh shark fish : The moisture content of the raw shark fish *Carcharhinus sorrah* was 78.37%, coinciding with the value (76.0%) reported for Requiem shark muscle (Watabe et al. 1983). The protein content of fresh shark (21.0%) and fat content (0.16%) are in accordance with Gordievskaya (1971). However, Chari (1948) has reported a fat content of 1.44% in *Carcharhinus laticudus* from west coast of India. The initial NPN content of shark on wwb was found to be 2.8 mg%, whereas Watabe et al (1983) have reported 11.2 mg% of NPN. According to Govindan (1985), the

* *Corresponding Author; Present Address: Human Resource Development, Central Food Technological Research Institute, Mysore–570 013, India.*

NPN constitutes 30% of the total nitrogen, in the case of Elasmobranches, which works out to be 3.8 to 4.4% of the wet weight of the muscle. The initial urea content of the shark (wwb) was found to be 1.68 mg%, which was in agreement with the value reported by Solanki and Venkataraman (1978).

Storage studies: In salt-cured shark fish, the moisture content decreased to 32.57% from 48.03% in 120 days of storage. This is in accordance with Velankar (1952). There was an apparent increase in the NaCl content of salt-cured shark fish from 16.9 to 22.0%. Jinadatharayaq and Vernekar (1979) have reported a salt content between 20–28% in salt-cured shark fish. The BIS has specified a maximum moisture content of 40% on wwb and NaCl content on 25.30% on dwb for dry-salted shark fish (BIS 1962). The water activity of cured shark decreased from 0.77 to 0.75 in first fifteen days and remained stable thereafter. Ramachandran and Solanki (1992) have reported a water activity of 0.77 in salt-cured shark fish with a moisture content of 55.97%. Both TMA–N and TVB–N values of cured shark increased with an increase in storage period. Chari (1948) reported a TVB–N content of 99.4 mg% in salt-cured shark fish. It was found that the cured fish samples with TVB–N values <200 mg% had good organoleptic rating (Venkataraman and Vasavan 1959) as observed in this study with cured shark. Free fatty acids increased from 0.237 – 23.61%, similarly, peroxide value also increased from 3.17 to 38.86 meqO_2/kg fat at the end of 120 days. Alpha amino nitrogen content decreased from 29.16 to 14.72 mg%. Sibsankar Gupta and Govindan (1975) reported free amino acid nitrogen content of 47.0 mg% in fresh shark meat, while Solanki and Venkataraman (1978) reported alpha– amino nitrogen content of 140 mg% in the stored fresh fillets of shark fish. Urea content in cured shark decreased from 1.51 to 1.13% during storage. The major demerit associated with sharks and rays is the presence of urea, which limits their commercial utilisation. Hence, removal of urea from the meat becomes a major step in the utilisation of their meat. Kandoran et al (1965) observed a decrease in urea content of 6.2 mg% (dwb) in shark fish on sating, desalting and resalting. The TPC and TFC levels of salt-cured shark fish showed, gradual increase during the storage period. Fungal contents varied from 1.30 to 2.32 log cfu/g and halophilic content increased marginally up to 45 days and then showed a decrease (Table 1).

Organoleptic evaluation : The organoleptic scores decreased with the period of 120 days. The samples however, were in acceptable condition at the end of 120 days, as judged by the panelists

TABLE 1. CHANGES IN PHYSICO CHEMICAL, MICROBIAL AND OVERALL SENSORY CHARACTERISTICS OF SALT CURED SHARK FISH

	Storage period, days								
Characteristic	0	15	30	45	60	75	90	105	120
Moisture, g%	48.03±0.64	46.17±0.19	44.06±1.43	42.44±1.43	40.04±0.83	38.60±0.33	36.72±0.14	33.94±0.19	32.57±0.35
Protein, g%	29.27±0.33	30.32±0.61	31.50±0.50	32.26±0.47	34.19±0.00	37.44±0.14	39.13±0.93	41.40±0.87	43.85±0.04
Crude fat g%	0.38±0.00	0.42±0.02	0.45±0.03	0.47±0.01	0.49±0.00	0.51±0.31	0.53±0.01	0.55±0.01	0.56±0.02
NaCl g%	16.94±0.48	19.23±0.21	20.84±0.07	21.25±0.61	21.93±0.03	22.00±0.08	22.10±0.12	22.37±0.14	22.67±0.01
Water activity, a_w	0.77	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
TMA–N, mg%	08.50±0.33	11.12±0.73	13.49±0.90	15.35±0.27	17.53±0.15	19.18±0.63	21.33±0.73	23.81±0.24	25.17±0.44
TVB–N, mg%	19.50±0.33	22.33±0.41	26.17±0.83	31.50±0.65	58.33±0.94	64.66±0.44	78.50±0.52	85.33±0.47	89.73±0.43
FFA, mg%	0.237±0.10	1.980±0.09	3.270±0.09	9.020±0.05	9.840±0.05	11.47±0.08	17.35±0.51	21.91±0.03	23.61±0.10
Peroxide value, meq O_2/kg fat	3.17±0.45	9.60±0.30	11.52±0.40	21.69±0.25	27.18±0.25	29.00±0.11	31.52±0.45	35.81±0.31	38.86±0.39
AAN, mg%	29.16±1.16	27.79±0.46	25.54±0.01	23.55±0.23	20.76±0.46	18.03±0.70	17.31±0.81	16.51±0.11	14.72±0.24
NPN, mg%	2.38±0.02	2.31±0.05	2.26±0.04	2.05±0.04	1.88±0.04	1.83±0.00	1.70±0.02	1.68±0.01	1.65±0.02
Urea, mg%	1.51±0.28	1.43±0.06	1.34±0.05	1.33±0.00	1.31±0.50	1.30±0.46	1.21±0.02	1.17±0.03	1.13±0.01
TPC, log cfu/g	2.70	2.93	3.00	3.17	3.36	3.78	3.04	4.23	4.46
TFC, log cfu/g	ND	ND	1.30	1.95	2.41	2.11	2.20	2.23	2.32
Halophiles, log cfu/g	ND	1.30	1.69	1.95	0.84	0.47	ND	ND	ND
					Organoleptic scores				
Uncooked	8.90	8.60	8.00	6.80	6.50	6.30	6.00	5.90	5.50
Cooked	8.90	8.80	7.80	6.40	6.20	6.10	5.80	4.90	4.50

ND: Not detected; TMA–N: Trimethylamine Nitrogen; FFA: Free Fatty Acid; NPN: Non Protein Nitrogen; TPC: Total Plate Count; TFC: Total Fungal Count; AAN: Alpha Amino Nitrogen; Values are expressed on wet weight basis, Values are means of triplicate determination.

on quality attributes on a 9-point Hedonic scale. (Table 1). Cured shark fish can be produced by salting (3:1 ratio) and curing for 48h, later washing and sun-drying for 72 h at less than 48% moisture content. The quality parameters were at the acceptable level as suggested by BIS. The urea content also reduced through salting preservation. Therefore, hygienic preservation of salt-cured shark would result in better quality product having a shelf life of about 4 months and improve the commercial utilisation of sharks.

The authors thank the Dean, Fisheries College and Research Institute, Tuticorin for permission to conduct this experiment.

References

AOAC (1970) Official Methods of Analysis, 11th edn. Association of Official Analytical Chemists, Washington, DC

AOAC (1975) Official Methods of Analysis, 12th edn, Association of Official Analytical Chemists, Washington, DC

AOAC (1980) Official Methods of Analysis, 13th edn, Association of Official Analytical Chemists, Washington, DC

APHA (1976) Compendium of Methods for the Microbiological Examination of Foods In: Speck MC (ed.) American Public Health Association, New York

Beatty SA, Gibbons NE (1937) The measurement of spoilage in fish. J Biol Bd Can 3(1):77-91

BIS (1962) Specifications for the Salt used for Fish Curing, (IS-594) Bureau of Indian Standards, New Delhi

Chari ST (1948) Nutritive value of some of the west coast marine food fishes of the Madras Province. Ind J Med Res 36(3):253-258

Doe PE, Poulter RG, Curran CA (1983) Determination of water activity and shelf life of salted dried fish products. In: James D (ed) The Production and Storage of Dried Fish, FAO Fish Report 279:202-208

Gordievskaya VS (1973) Shark fish in the food industry. Israel Programme for scientific translation. IPST Cat. No. 60 802 available from NTIS, Springfield, USA p 26

Govindan TK (1985) In: Fish Processing Technology, Oxford and IBH Publishing Co., New Delhi, p 252

Jinadatharaya YA, Vernekar VS (1979) Modern method for salt curing and drying shark fish. Seafood Exp J 11(1):48-50

Kandoran MK, Govindan TK, Suryanarayana Rao SV (1965) Some aspects of curing of sharks and rays. Fish Technol 2:193-197

Pope CG, Stevens MF (1939) The determination of amino nitrogen using a copper method. Biochem J 33:1070-1074

Ramachandran A, Solanki KK (1992) Water activity and shelf life of semi-dried salted shark fillets. In: FAO Fisheries Report, No. 470, Suppl. Rome: pp 247-256

Shenoy AS, Dey UK (1984) Shark and its utility. Sea Food Exp J 16(1):5-11

Sibsankar Gupta, Govindan TK (1975) The free alpha amino and nitrogen contents of some common food fishes of Kakinada region. Andhra Pradesh, Fish Technol 12(2):151-155

Solanki KK, Venkataraman R (1978) Ice storage characteristics of fresh and dried shark fillets. Fish Technol 15(1):7-11

Velankar NK (1952) Moisture, salt, trimethylamine and volatile nitrogen contents and bacterial counts of salt cured marine fish. J Sci Ind Res 24(8):359-364

Venkataraman R, Vasavan AG (1959) Investigation on the quality of salted fish sold in the market. Madras Fisheries Station Report and Year Book, 1955-56, pp 261-265

Watabe S, Yoshilivo Ochiai, Satoshi Kanoh, Kanehisa Hishimoto (1983) Proximate composition of Requiem shark muscle. Bull Jap Soc Sci Fish 49(2):265-268

Received 1 November 1998; revised 20 May 1999; accepted 12 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 472-474

Polyaromatic Hydrocarbons in Fresh Marine Fin and Shell Fishes

S. KANNAPPAN*, G. INDRA JASMINE, P. JEYACHANDRAN AND A. TAMILSELVI
Department of Industrial Fish Processing Technology,
Fisheries College and Research Institute, Tuticorin–628 008, India.

Different varieties of fresh marine fin and shell fishes from the same catch of Tuticorin waters were analyzed for polyaromatic hydrocarbons [PAHs] by HPLC. The results showed that chrysene followed by dibenzanthracene was present in the majority of the samples analyzed. Benzo (a) pyrene was not detected in the fishes.

Keywords : Fishes, Crustaceans, Molluscs, Chrysene, Dibenzanthracene, Benzo (a) pyrene.

Polyaromatic hydrocarbons (PAHs) are organic compounds containing two or more fused benezene rings, which may or may not have substitute groups attached to one or more rings. These PAHs are mainly formed by incomplete combustion of organic materials, automobile exhausts and by anthropongenic waste combustion as in residential heating and in industrial processes (especially cooking and thermal power plants). So far, more than 100 PAHs have been identified from different samples (Grimmer and Bohnkey 1975) among which few may produce different types of cancer (Sivasamy et al. 1990).

Though fishes are practically free from any contamination, PAHs persist in the aquatic environment as a result of dumping of industrial and domestic effluents (Hoffman et al. 1984) and other contaminated atmospheric particle, indicating a correlation between PAHs and level of pollution in the area (Frease and Windsor 1991). Petroleum pollutions of seas could pose a cancer risk to man through contamination of fisheries resources with carcinogenic PAHs (Sullivan 1974). PAHs have also been found in phytoplankton, plant leaves, vegetables, river sediments, suspended solids and worms (Smith et al. 1985; Lake et al. 1979). These aqueous biota serve as a food and PAHs source for all aquatic animals (Andelman and Suess 1970). Epidemiological studies have unequivocally established a relationship between the occurrence of PAHs and different types of cancer. Few of these groups of compounds are however, competitive inhibitors. Very few studies have been done on the occurrence of PAHs in Indian fishes. Hence, the present study was undertaken to determine the presence of selective PAHs in fresh marine fin fishes, crustaceans and molluscs.

* *Corresponding Author : Present address: Division of Microbiology, Defence Food Research Laboratory, Siddarthanagar, Mysore–570 011, India.*

Estimation of polyaromatic hydrocarbons (PAHs): Different varieties of fresh fishes from the same catch were procured from the Tuticorin landing center. Chrysene (CRY), dibenzanthracene (DBA) and benzo (a) pyrene (BAP) obtained from Sigma chemicals, USA were used as standards. Solvents like dichloromethane, cyclohexane and acetonitrile used for the extraction were of HPLC grade. A standard HPLC Shimadzu (Japan) consisting of LC6A single piston pump, Rheodyne simple injector, CTO–6A column oven and SCL–6B system controller and SPD - 6A spectrophotometric detectors were used for the analysis of PAHs. A stainless steel column shim pack CLC–ODS (6 mm id x 15 cm) was used for effective separation. The data were stored and processed with Shimadzu integrated data processor, C–R4A chromatopac. A solvent system of 100% acetonitrile pumped at the rate of 1 ml/min was used.

Sample extraction : Triplicate samples of 0.5 g of each of the fresh marine fin and shell fishes were aseptically homogenized with 10 ml distilled water in a homongeniser (Sivasamy et al. 1990). Then, the samples were extracted thrice with dichloromethane (40 ml) in a separating funnel. The extracts were pooled and then, 5 g of anhydrous sodium sulphate were added to absorb excess water. The clear supernatant was removed, concentrated to 5 ml under vacuum at 60°C in a rotary evaporator, 40 ml of cyclohexane was added again and concentrated to a minimum volume under vacuum. This was then passed through a silica gel (BDH, activated at 110°C for 5 h.) column (0.8 cm, id–13.5 cm). The eluent was mixed with 1 g of anhydrous sodium sulphate and the supernatant was collected and evaporated to dryness at 60°C in a rotary evaporator under vacuum. The residue was then dissolved in 3 ml of acetonitrile

and filtered (0.5 μ) through a membrane filter.

Sample dilution : The resultant extract was diluted to 5 ml with acetonitrile, transferred to a screw capped test tube labelled and stored in refrigerator. From this, solution of 5 μl was taken, diluted to 100 μl with acetonitrile and 20 μl of this was injected into HPLC column for identification and quantification (Panalacs 1976).

Standard : Five mg of each chrysene, dibenzanthracene and benzo (a) pyrene were dissolved separately in 25 ml of acetonitrile and stored in refrigerator until use. An aliquot (5 μl) was mixed and made upto 300 μl with acetronitrile and 10 μl of this was injected. A ternary gradient system of 100% acetronitrile was pumped at a flow rate of 1 ml/min. The elute was detected by UV spectrophotometric detector at 254 nm with a sensitivity of 0.005 absorbance units full scale. The selected PAHs like chrysene, dibenzanthracene and benzo (a) pyrene were identified by comparing retention time of separate peaks with reference standards and later quantified.

Parameters under ternary gradient in SCL-6B system controller (HPLC) are: T: Flow = 1 ml/min; B: Conc = 100%; C: Conc. = 0%; B: Curve = 0%; C: Curve = 94 Kgf/cm^2; Absorbance = 0.005 in SPD 6A; Wavelength = 254 nm; Oven temperature = 35°C and Pump"B" = 100% acetonitrile.

The results of PAHs are summarised in Table 1. The highest concentration of chrysene (1.18 μg/g) was found in *Sardinella gibbosa* (clupeid) and the lowest (0.01 μg/g) in *Leiognathus bindus* (Silver bellies). These values were lower than the values reported by Cocchieri et al (1990), who found 13 μg/kg of CRY (WWB) in razor fish (*Eusis sihqua*). Moreover, sardinella species being a plankton feeder, there may be ample chance for PAHs to have derived from their feed, wherein specific PAHs upto 2 μg/kg have been reported in polluted waters. Anchovies (*Engraulis mystax*) tested during the study had very less chrysene (0.03 μg/g), which was much lower than the value reported by Cocchieri et al (1990). In ribbon fish (*Trichurus lepturus*), the chrysene content was reported to be 0.61 μg/g, which was lesser (18.57 μg/g) than the value reported by Sivaswamy et al (1990) in salted dried *Trichurus lepturus*.

Benzo (a) pyrene was not detected in the fresh marine fin and shell fishes analyzed. Among shell fishes, the highest value (9.98 μg/g) of chrysene was observed in crab (*Portunus pelagicus*) and lowest in white shrimp (*Penaeus indicus*) (0.07 μg/g). Chrysene was significantly higher (0.11 μg/g) than dibenzanthracene (0.02 μg/g) in *Loligo indica* (squid). All the three PAHs have not been detected in fresh *Sardinella longiceps* (oil sardine) and *Lactarius lactarius* (big jawed jumper). *Crassostrea madrasensis* (edible oyster) showed the maximum levels of dibenzanthracene (9.27 μg/g) and chrysene (0.93 μg/g). These values were much lower than the values reported by Cocchieri et al (1990) and Cahnmann et al (1957) in common mussel and edible cockle (13 μg/g CRY-wwb, 20 μg/g DBA-wwb, 40 μg/g CRY-wwb and 8 μg/g DBA-wwb). Oysters are of sedentary nature and they live in shallow water, where they are frequently exposed to floating pollutants and are able to take up more PAHs from the surroundings.

TABLE 1. POLYAROMATIC HYDROCARBONS IN FRESH MARINE FIN AND SHELL FISHES (μg/g-WET WEIGHT BASIS)

Fin fishes	Chrysene (CRY)	Dibenzanthracene (DBA)
Engraulis mystax (Anchoviella)	0.03 ± 0.005	0.18 ± 0.050
Sardinella gibbosa (Clupeid)	1.18 ± 0.010	-
Rastrelliger kanagurta (Indian mackerel)	0.61 ± 0.050	0.10 ± 0.050
Arius dussumieri (Marine cat fish)	0.04 ± 0.010	0.25 ± 0.020
Shillago sihama (Indian whiting)	0.55 ± 0.050	1.17 ± 0.005
Leiognathus bindus (Silver belly)	0.01 ± 0.001	0.07 ± 0.005
Trichiurus lepturus (Ribbon fish)	0.61 ± 0.210	0.19 ± 0.050
Shell fishes		
Penaeus indicus (White shrimp)	0.07 ± 0.005	-
Penaeus monodon (Tiger shrimp)	0.45 ± 0.100	-
Portunus pelagicus (Crab)	9.98 ± 0.550	-
Loligo indica (Squid)	0.11 ± 0.040	0.02 ± 0.030
Crassostrea madrasensis (Edible oyster)	0.93 ± 0.040	9.27 ± 0.500

♦ CRY and DBA were not detected in *Lactarius lactarius* and *Sardinella fimbriata*.
♦ BAP was not detected in the fishes analyzed

World Health Organisation and Federal Republic of Germany, Austria and Poland recommended the allowable level of PAHs in drinking water and meat products to be 0.2-3 μg/g. In this study, highly carcinogenic and mutagenic benzo(a)pyrene was not reported but mild carcinogens like chrysene and dibenzanthracene were observed. Certain suggestions like waste water treatment with chemicals, microbes and

tubifex worms, depuration of shell fishes, treating the fishes in running water, etc will help in reducing the occurrence of these chemicals in aquatic environments.

The authors thank the Dean, Fisheries College and Research Institute, Tuticorin for permission for conducting this experiment. Financial assistance provided by ICAR, New Delhi is gratefully acknowledged.

References

Andelman JB, Suess MJ (1970) PAHs in Water Environment. .Bull, WHO 43: 479-508

Cahnmann HJ, Kuratsune M (1957) Determination of PAHs in oysters collected in polluted water. Anal Chem 29: 1312-1317

Cocchieri M, Arnese A, Anna Maria M (1990) Polycyclic aromatic hydrocarbon In: marine organisms from Italian Mediterranean coasts. Mar Poll Bull 21: 15-18

Frease RA, Windsor JG (1991) Behaviour of selected polycyclic aromatic hydrocarbons associated with stablised oil and coal ash artificial reef. Mar Poll Bull 22(1) 15-19

Grimmer G, Bohnke H (1975) Polycyclic aromatic hydrocarbon profile analysis of high-protein foods, oils and fats by Gas Chromatography. J Assoc Off Anal Chem 59: 725-733

Hoffman EJ, Mills GL, Latimer JS, Quinn JG (1984) Urban run off as a source of PAHs to coastal waters. Environ Sci Technol 18: 580-587

Lake JL, Norwood C, Dimock C, Bowen R (1979) Origins of polycyclic aromatic hydrocarbons in estuarine sediments. Geochim Cosmochim Acta 43: 1847-1857

Panalacs T (1976) Determination and identification of PAHs in smoked charboiled food products by HPLC and GC. J Environ Sci Health Bull 12: 2199-2215

Sivasamy SN, Balachandran B, Sivarama Krishnan VM (1990) Poly nuclear aromatic hydrocarbons in South Indian diet. Current Sci 59: 280-281

Smith JD, Hauser JY, Bagg J (1985) Polycyclic aromatic hydrocarbons in sediments of the Great Barrier Reef region. Australia Mar Poll Bull 16: 110-114

Sullivan JB (1974) Marine pollutions by carcinogenic hydrocarbons. National Bureau of Standards (Washington DC), Special Publication 409: 261-263

Received 5 November 1998; revised 6 May 1999; accepted 12 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 475-478

Development of Pulverised Starter for *Kinema* Production

JYOTI PRAKASH TAMANG

Microbiology Research Laboratory, Department of Botany,
Sikkim Government College, Gangtok, Sikkim 737 102, India.

Kinema is a traditional fermented soybean food common to the Eastern Himalayas. Pulverised starter using selected strain of *Bacillus subtilis* KK2:B10 (MTCC 2756), previously isolated from traditionally prepared *kinema*, was developed for *kinema* production. *Kinema* prepared by *Bacillus subtilis* KK2:B10 strain, which was grown and harvested in soybean extract broth, was dried in an oven at 70°C for 10 h and ground aseptically. The 1% of pulverised starter was added aseptically to steamed soybeans and fermented at 40°C for 20 h under 85% relative humidity to get *kinema*. Load of *Bacillus subtilis* in pulverised starter kept in room temperature was constantly maintained at ~10^9 cfu/g, even tested upto 6 months. The consumers' preference trials showed that *kinema* prepared by using pulverised starter was organoleptically more acceptable than market *kinema*. Water-soluble nitrogen and formol nitrogen to total nitrogen contents were higher in *kinema* prepared by using pulverised starter than market *kinema*. Application of inexpensive and ready-to-use pulverised starter may be appropriate for *kinema* processing at household level.

Keywords : *Kinema*, *Bacillus subtilis* KK2:B10, Soybean extract broth, Pulverised starter.

Kinema is a soybean-based fermented sticky, slightly alkaline food with a typical flavour consumed as curry in the Darjeeling hills and Sikkim in India, eastern Nepal and Bhutan. During *kinema* preparation, overnight-soaked whole soybeans are cooked, soybean extract is drained off, cracked moderately, the grits are then placed in a basket lined with fern (*Athyrium* sp.) leaves and left to ferment naturally for 1-3 days (Tamang et al. 1988). Daily per capita consumption of *kinema* was 3.3 g in the Darjeeling hills with annual home production of 829 tonnes, and 2.2 g with annual production of 326 tonnes in Sikkim, respectively during 1997-98 (Yonzan and Tamang 1998). *Kinema* is similar to other fermented soybean products such as *akhoni* of Nagaland, *troombai* of Meghalaya, *hawaijar* of Manipur and *bekang-um* of Mizoram (Tamang 1996) and *natto* of Japan and *thua-nao* of Thailand (Nikkuni 1997).

Bacillus subtlis, Enterococcus faecium, Candida parapsilosis and *Geotrichum candidum* are associated with *kinema* (Sarkar et al. 1994). However, *Bacilus subtilis* was found to be a sole organism in fermentation of soybeans to *kinema* (Sarkar and Tamang 1994; Tamang 1995). Strain of *Bacillus subtilis* KK2:B10 was selected as the starter culture (Tamang and Nikkuni 1996) and fermentation conditions for laboratory-scale production of *kinema* were optimised (Tamang and Nikkuni 1998). Selling of *kinema* in local market provides income generation to some rural women and the trade protected as hereditary right which passes from mother to daughter (Tamang 1998). The aim of the present work was to develop an inexpensive and ready-to-use pulverised starter for *kinema* production in order to upgrade the traditional process.

Soybean samples : Small (~6mm) with smooth yellow seed coat and dark brown hilum 'local yellow' variety of soybean [*Glycine max* (L.) Merrill] was purchased from Gangtok market.

Microorganism : *Bacillus subtilis* KK-2:B10 [MTCC* 2756 (*Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India) was isolated from naturally fermented *kinema* samples, identified as *Bacillus subtilis* (Ehrenberg) Cohn and was selected as best starter cultures for laboratory scale *kinema* production (Tamang and Nikkuni 1996).

Sample collection : *Kinema* samples were collected from Gangtok and Rongli markets in Sikkim aseptically in pre-sterile bags, which were kept in an ice-box and transported immediately to the laboratory for analysis.

Soybean extract broth : Overnight soaked soybeans (100 g) were autoclaved in a beaker containing 200 ml tap water at 121°C for 30 min. Sediment of soybean extract collected in beaker was filtered using Whatman filter paper No. 1. The final pH of filtered soybean extract was adjusted to 7.0 with 1 N NaOH, using pH-meter (Systronics 335, India).

Laboratory-scale kinema *preparation* : Soybeans were cleaned, washed and soaked in tap water overnight at room temperature. Soaked soybeans were autoclaved at 121°C for 30 min and inoculated with cell suspension of *Bacillus subtilis* KK2:B10, harvested in nutrient broth (HiMedia M002), phytone-sucrose broth (phytone peptone 10.0 g, sucrose

10.0 g, sodium chloride 10.0 g, agar 20.0 g, distilled water 1 L, pH 7.0), soybean extract broth and soybean extract-sucrose broth (in soybean-extract, added: sucrose 1.0%, NaCl 0.5%, pH 7.0) at 37°C for 18 h, respectively, at 10^4 cfu/g of cooked soybeans, while the temperature of soybeans was above 80°C. Inoculated soybeans were put into pre-sterile petri-dish (outer lid was replaced by perforated polythene film), and incubated at 40°C for 20 h under 85% relative humidity.

Microbial analysis : Culture of *Bacillus subtilis* KK-2B10 was transferred onto the nutrient broth, phytone broth, soybean extract broth and soybean extract-sucrose broth, separately and incubated at 37°C for 18 h to employ as purified *kinema* starter. Decimal dilution series were prepared in sterile physiological saline (0.85% w/v sodium chloride in water) and 1 ml of appropriate diluted suspension was mixed with molten tryptone soya agar (HiMedia M424) and incubated at 37°C for 24 h. Colonies appeared were counted as colony forming unit per ml (cfu/ml). For viability test, 10 g pulverised starter was mixed with 90 ml of sterile physiological saline for 10 min and decimal series were prepared as described above. The total viable counts of *Bacillus subtilis* in pulverised starter were determined every month till 6 months.

Sensory evaluation : The sensory attributes of *kinema* fermented by *Bacillus subtilis* KK2:B10, harvested in nutrient broth, phytone-sucrose broth, soybean extract and soybean extract-sucrose broth, respectively, were evaluated for flavour, taste, stickiness, texture and colour after sampling by a panel of 15 trained judges, using a 100-point score card as described by Sarkar and Tamang (1994).

Consumer preference trial : Market samples of *kinema* as well as *kinema* prepared by using pulverized starter were served to 100 consumers representing different ethnic groups of people of the Sikkim Himalayas. The 9-point Hedonic scale (IS 1971) used in this study ranged from 'dislike extremely' (score 1) to 'like extremely' (score 9).

Chemical analysis : Total nitrogen and water-soluble nitrogen of samples were determined by micro-Kjeldahl method (AOAC 1990). Formol nitrogen of sample was determined by formaldehyde titration as described by Tamang and Nikkuni (1996). Homogenized samples were mixed with distilled water and centrifuged at 20,000 x **g** for 15 min, 10 ml supernatant was mixed with 20 ml of previously neutralized formaldehyde and titrated against 0.1 N NaOH, using an auto-titrator (TOA, TSB-10a, Tokyo, Japan).

Statistical analysis : Data obtained were analyzed by determining errors of the mean and analysis of variance, using the least square design (Snedecor and Cochran 1989) and SALS software package (version 2.5).

Nutrient broth is conventionally used for development of *Bacillus subtilis (natto)* spores as starter cultures for commercial production of *natto* in Japan (Sulistyo et al. 1988). Nutrient broth is composed of expensive beef extract, which is not acceptable to the majority of the Hindu population in the Sikkim Himalayas, if one introduced the purified starter culture for *kinema* production. Moreover, the soybean extract after cooking soybeans is discarded during *kinema* preparation. Instead of discarding the soybean extract, an attempt was made to develop it as an economical soybean extract broth for production of *B. subtilis* spores. The load of *Bacillus subtilis* KK2:B10 was significantly ($P<0.05$) higher in soybean extract broth (3.2×10^8 cfu/ml) as compared to nutrient broth (0.4×10^8 cfu/ml) and phytone-sucrose broth (0.2×10^8 cfu/ml). However, there was no significant difference ($P<0.05$) in load of *B. subtilis* harvested in soybean extract as compared to soybean extract-sucrose broth. *Kinema* prepared by starter culture harvested in soybean extract broth had significantly ($P<0.05$) higher scores in all sensory attributes than those of *kinema* starter harvested in other broth media (Table 1). Hence, soybean extract after adjusting pH to 7.0 was selected as an inexpensive

TABLE 1. SENSORY SCORES OF *KINEMA* PRODUCED BY *B. SUBTILIS* KK2:B10, HARVESTED IN DIFFERENT BROTH MEDIA

Attributes	Broth			
	NB	PB	SE	SES
Flavour (20)	11.7^{bc} (11.0–12.2)	10.0^{c} (9.0–11.0)	16.2^{a} (15.5–17.0)	13.0^{b} (12.0–14.0)
Taste (20)	11.7^{b} (10.0–14.0)	10.0^{b} (9.0–11.0)	17.1^{a} (16.2–18.0)	12.0^{b} (11.0–13.0)
Stickiness (20)	12.1^{bc} (11.0–13.4)	10.0^{c} (9.0–11.0)	17.0^{a} (16.0–18.0)	13.0^{b} (12.0–14.0)
Texture (20)	11.7^{b} (10.0–14.0)	10.3^{b} (9.0–12.0)	17.2^{a} (16.5–18.0)	13.0^{b} (12.0–14.0)
Colour (20)	15.0^{b} (14.0–16.0)	12.3^{c} (12.0–13.0)	17.3^{a} (17.0–18.0)	14.3^{b} (14.0–15.0)
Total (100)	66.2^{b} (60.5–70.1)	48.4^{c} (45.0–52.4)	84.8^{a} (82.0–87.0)	65.3^{b} (60.5–69.4)

NB, nutrient broth; PB, phytone extract-sucrose broth; SES, soybean extract-sucrose; SE, soybean extract. Data represent the means of three replications. Ranges are given in parentheses. Values bearing different superscripts in each row differ significantly ($P<0.05$)

broth medium for producing *B. subtilis* spores for pure culture fermentation of *kinema* at the laboratory scale.

Ready-to-use starter culture for *kinema* production was prepared (Fig. 1). *Kinema* prepared by using *B. subtilis* KK2:B10 strain, which was harvested in soybean extract broth, was dried in an oven at 70°C for 10 h and ground aseptically to make pulverised starter. The 1% of pulverised starter (instead of *B. subtilis*, as in Fig. 1) was added aseptically to autoclaved soybeans and fermented to get *kinema* (Fig. 1, Stage A). The total viable counts of *B. subtilis* in pulverised starter were found constantly maintained at the level of ~10^9 cfu/g till 6 months. This was due to survival of endospores of *B. subtilis* for longer period at room temperature. No other microorganisms were recovered from pulverised starter kept in pre-sterile polythene bags at room temperature.

The consumers' preference trials showed that *kinema* prepared by using pulverised starter under optimized conditions was more acceptable than market *kinema*. Market *kinema* was liked extremely (score, 9) by 15%, very much (score, 8) by 30% and moderately (score, 7) by 55%, while *kinema*

Yellow-seeded soybean (100 g)
↓
Soaked in water overnight
↓
Drained the water
↓
Autoclaved at 1 kg/cm² for 30 min
| ←added *B. subtilis* KK-2B10 (10^4 cfu/g), harvested in soybean extract broth
↓
Incubated at 40°C for 20 h under 85% relative humidity
↓
Kinema (~230 g) **Stage A**
↓
Oven dried at 70°C for 10 h
↓
Ground aseptically
↓
Pulverised starter **Stage B**
↓
Pack in pre-sterile polythene bag

Fig. 1. Flow sheet of pulverised *kinema* starter production

TABLE 2. WATER-SOLUBLE NITROGEN AND FORMOL NITROGEN CONTENTS OF *KINEMA*

Parameter	Cooked soybean	Gangtok *kinema*[a]	Rongli *kinema*[b]	Laboratory-*kinema*[c]
Water-soluble nitrogen, % of TN	15.4 (12.2–17.0)	62.0 (54.0–68.0)	60.2 (57.0–64.0)	73.0 (68.0–78.0)
Formol nitrogen, % of TN	1.5 (1.2–1.7)	6.0 (4.0–7.2)	7.0 (4.0–8.7)	10.5 (8.5–12.5)

Data represent the means of four replications. Ranges are given in parentheses. TN, total nitrogen; [ab] *kinema* collected from Gangtok and Rongli markets in Sikkim, respectively; [c] *kinema* prepared by using pulverised starter

prepared by using pulverised starter was liked extremely by 30%, very much by 40% and moderately by 30% of the consumers. Water soluble nitrogen and formol nitrogen contents were higher in *kinema* prepared by using pulverised starter than market *kinema* (Table 2.). Increased water soluble nitrogen in *kinema* helps in digestibility and high amount of formol nitrogen, which contains free amino acid supplements that impart better taste to *kinema* (Nikkuni et al. 1995).

Application of ready-to-use pulverized starter may appear appropriate in *kinema* production at household level, since it is cost-effective. *Kinema* prepared by using pulverised starter had more advantages over traditional method due to shorter fermentation time that eliminates the chance of growth of contaminants, hygienic conditions, maintaining consistency with better quality and flavour.

The support of grant (ICA No: 95/026) from the United Nations University-Kirin Brewery Co. Ltd. of Japan is thankfully acknowledged.

References

AOAC (1990) Official Methods of Analysis, 15th edn. Association of Official Analytical Chemists, Virginia, USA

IS (1971) Indian Standard Guide for Sensory Evaluation of Foods. Part II. Methods and Evaluation Cards, IS:6273. Indian Standards Institution, New Delhi

Nikkuni S (1997) *Natto, kinema* and *thua-nao* : traditional non-salted fermented soybean foods in Asia. Farming Japan 31(4): 27-36

Nikkuni S, Karki TB, Vilku KS, Suzuki T, Shindoh K, Suzuki C, Okada N (1995) Mineral and amino acid contents of *kinema*, a fermented soybean food prepared in Nepal. Food Sci Technol Int 1 (2) : 107-111

Sarkar PK, Tamang JP (1994) The influence of process variables and inoculum composition on the sensory quality of *kinema*. Food Microbiol 11: 317-325

Sarkar PK, Tamang JP, Cook PE, Owens JD (1994) *Kinema* - a traditional soybean fermented food : proximate composition and microflora. Food Microbiol 11:47-55

Snedecor GW, Cochran WG (1989) Statistical Methods. 8th edn. Iowa State University Press, Ames, Iowa, USA

Sulistyo J, Taya N, Funane K, Kiuchi K (1988) Production of *natto* starter. Nippon Shokuhin Kogyo Gakkaishi 35(4):278-283

Tamang JP (1995) Study of traditional fermented foods production in the Darjeeling hills and Sikkim, with emphasis on *kinema*. The United Nations University Post-doctoral Fellowship Dissertation, National Food Research Institute, Tsukuba, Japan

Tamang JP (1996) Fermented soybean products in India. In: Buchanan A (ed) Proceedings of the Second International Soybean Processing and Utilization Conference, Bangkok, Thailand, pp 189-193

Tamang JP (1998) Indigenous fermented foods of the Sikkim Himalaya: socio-economical prospective. In: Rai SC, Sundriyal RC, Sharma, E (eds) Sikkim Perspectives for Planning and Development, Sikkim Science Society-Bishen Singh Mahendra Pal Singh, Dehra Dun, pp 513-523

Tamang JP, Nikkuni, S (1996) Selection of starter culture for production of *kinema*, a fermented soybean food of the Himalaya. World J Microbiol Biotechnol 12: 629-635

Tamang JP, Nikkuni, S (1998) Effect of temperatures during pure culture fermentation of *kinema*. World J Microbiol Biotechnol 14: 847-850

Tamang JP, Sarkar PK, Hesseltine CW (1988) Traditional fermented foods and beverages of Darjeeling hills and Sikkim - A review. J Sci Food Agric 44: 375-385

Yonzan H, Tamang JP (1998) Consumption pattern of traditional fermented foods in the Sikkim Himalayas. J Hill Research 11(1) : 112-115

Received 23 December 1998; revised 20 April 1999; accepted 12 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 479-482

Physico-Chemical Changes in Insect Infested Wheat Stored in Different Storage Structures

R. SAMUELS AND R. MODGIL*

Department of Food Science and Nutrition,
College of Home Science, HP Krishi Vishvavidyalaya, Palampur - 176 062, India.

Effect of insect infestation on nutritional quality of wheat stored in jute bags, peru, polythene bags and metal bins was studied by following changes in physical properties (weight, per cent damage and density), proximate composition, energy value, non-protein nitrogen (NPN), uric acid, true proteins, feed efficiency ratio (FER) and protein efficiency ratio (PER). With increase in storage period, significant decreases in weight, density, crude fat, calorific value and true proteins were observed, whereas moisture, ash, crude proteins, crude fibre, non-protein nitrogen and uric acid increased significantly ($P<0.05$). Changes were maximum in wheat, stored in jute bag and minimum in wheat stored in metal bin. Nature of packaging was found to play an important role in determining the extent of infestation damage.

Keywords : True protein, Uric acid, Non-protein nitrogen, Calories, Protein efficiency ratio, Feed efficiency ratio, Storage structures, Infestation, Physico-chemical properties.

Wheat is the second most important crop grown in the world (Bajaj - 1990; FAO 1986) and is accorded a premier place among cereals because of the vast acreage devoted to its cultivation and high nutritive value. The wheat after harvesting is stored by farmers, traders and government agencies in various storage systems. During storage, both qualitative and quantitative losses are inflicted by insects (Aujla et al. 1990; Lal 1990). Infested grains contain different stages of living insects, their body fragments and excreta (Sharma et al. 1979). Insect fragments being hard particles might contribute to digestive disturbances (Pingale et al. 1954). No detailed information is available on the proximate principles, calorific value, uric acid, feed efficiency ratio and protein efficiency ratio of insect infested wheat stored in jute bags, peru, polythene bags and metal bins. Therefore, in the present study, an attempt has been made to determine the effect of insect infestation on the physico-chemical changes in wheat, stored in different storage structures.

Wheat ("Sonalika" variety) was procured from the Seed Production Unit of the Department of Plant Breeding, College of Agriculture, Himachal Pradesh Krishi Vishvavidyalaya, Palampur. Peru is a storage structure made by local bamboo workers using bamboo strips. Jute bags, metal bins and polythene bags were purchased from the local market. Wheat was cleaned manually to get rid of the dust and other foreign materials. Wheat samples weighing 1500 g each in triplicate were stored for 6 months in jute bags, Peru, metal bins and polythene bags of 2 kg capacity. The grains were observed for insect infestation at monthly intervals. After every month, samples along with the containers were deep-frozen for 72 h. to kill the larval, pupal and adult insects. Insect debris and frass were removed manually and samples were stored in refrigerator, till further analysis. One thousand grains in triplicate were taken from each package and observed for per cent damage, which was calculated by noting the number of damaged grains. Difference between initial and final weight gave the percent weight loss. Weight volume ratio (density) was determined by noting the change in water level after adding one hundred grains (weighed). Two hundred grains were ground in Willy Mill and kept in air tight plastic containers, till further chemical analysis. Proximate composition was determined according to AOAC (1990) method. The nitrogen content was estimated by micro-Kjeldhal method (AOAC 1990) and it was multiplied by the factor 5.70 to obtain crude protein value. Non-protein nitrogen was estimated by Pellet and Young (1980) method. True protein was calculated by the following formula :

True proteins = Crude proteins – Non-protein nitrogen.

Uric acid was extracted as per AOAC (1990) method and estimated by the method of O'Ser (1971). Energy value was determined by chromic oxide method of O'Shea and Maguire (1962).

Animal experiment : Sixty male albino Wistar weanling rats aged 21 days and weighing 25±5g strain were obtained from the Germ Free Small Animal House of the College of Veterinary Sciences, CCS Haryana University, Hisar. Rats were divided into 6 groups of 10 rats each. One control group

* *Corresponding Author*

TABLE 1. FER AND PER OF INFESTED WHEAT STORED IN DIFFERENT STORAGE SYSTEMS

Attributes/Ingredient	Casein	Wheat	Wheat stored in: Jute bag	Peru	Metal bin	Polythene bag	CD (p<0.05)
Casein	13.00	-	-	-	-	-	-
Wheat	-	68.68	47.91	47.43	57.04	51.73	
Groundnut	10.00	6.31	5.73	5.72	5.87	5.87	-
Cellulose	5.00	3.85	2.64	3.05	3.53	3.90	-
Starch	66.98	11.14	33.70	33.78	23.54	29.05	-
** Feed consumed	155.67	186.72	186.97	176.85	214.73	173.00	6.03
** Protein consumed	15.57	18.67	18.69	17.68	21.47	17.30	0.40
** Weight gain	55.38	46.80	19.83	20.16	41.67	21.02	1.62
** FER	0.35	0.25	0.10	0.11	0.19	0.12	0.02
** PER	3.55	2.51	1.06	1.14	1.94	1.21	0.20

** Values are mean of 10 rats

was fed casein diet and another group was fed uninfested wheat. The remaining 4 groups were fed wheat, stored for 6 months in jute bags, peru, metal bins and polythene bags. Rats were caged individually in polypropylene metabolic cages. Water was given *ad libitum* for 28 days. All the diets contained (g/100g diet) sucrose 10, groundnut oil 10, mineral mixture 4, vitamin mixture 1, cellulose 5 and choline chloride 0.02, in addition to specific amounts of wheat to provide 10 g proteins. Diets were made to 100 g with starch (Table 1). The composition of mineral and vitamin mixtures was as recommended by NAS Committee (1972). The ingredients were mixed throughly to ensure uniform distribution and passed thorough 40 mesh sieve. Feed Efficiency Ratio (FER) and Protein Efficiency Ratio (PER) were determined by the method of Chapman et al (1959). The data were subjected to statistical analysis of variance in completely randomised design (Snedecor and Cochran 1968).

Weight of wheat stored in different storage structures (Table 2) decreased gradually as the storage progressed till 6 months. Maximum loss was observed in jute bags (45%) and minimum (6.73%) was observed in the grains stored in metal bins after six months of storage. Due to multiple insect attack of [*Sitophilus oryzae* Linnaeus (Rice weevil), *Tripoleum castaneum* (Herbest) (Rust red flour bettle), *Sitotrogan cerealell* Olivier (Angoumous grain moth)] percentage of damaged grains increased with increase in storage period. Damage was maximum in grains stored in jute bags (52%) and minimum in grains stored in metal bins (32.35%) after 6 months of storage. Significant changes in

TABLE 2. PHYSICO-CHEMICAL COMPOSITION OF WHEAT STORED IN DIFFERENT STORAGE STRUCTURES

Attributes/ Storage	Storage period, 2 months					4 months					6 months				
	JB	Peru	M.B.	PoB	CD (P<0.5)	JB	Peru	M.B.	PoB	CD (P<0.5)	JB	Peru	M.B.	PoB	CD (P<0.5)
Weight, g	1475	1470	1520	1487	15.0	1225	1250	·1455	1392	37.0	825	975	1399	1100	25.0
Damage, %	11.66	9.66	3.60	4.00	3.76	21.66	20.00	11.73	16.89	6.35	52.00	48.00	1921	32.35	3.35
Density, g/m	1.17	1.19	1.22	1.21	0.02	1.10	1.11	1.17	1.15	0.02	0.72	0.82	0.99	0.85	0.09
Moisture, %	14.25	14.32	12.67	13.69	0.11	16.75	16.23	13.11	15.22	0.23	19.32	18.57	13.31	17.71	0.07
Ash, %	1.74	1.72	1.63	1.73	0.04	2.25	2.19	1.82	1.91	0.05	3.02	2.90	2.08	2.43	0.05
Crude Protein, %	15.22	15.22	14.61	14.69	0.07	19.47	19.27	16.31	17.05	0.10	26.67	24.08	17.53	20.33	0.11
Crude fat, %	1.84	1.85	1.81	1.71	0.41	1.59	1.64	1.74	1.58	0.04	1.42	1.44	1.63	1.49	0.02
Energy, Kcal	320	319	322	321	0.61	262	266	305	273	0.49	167	199	286	211	0.46
NPN, %	0.21	0.21	0.03	0.07	0.02	1.17	1.12	0.40	0.71	0.02	3.01	2.49	0.77	1.81	1.10
True protein, %	14.00	14.02	14.42	14.22	0.03	12.78	12.88	13.98	12.96	0.05	9.69	9.88	13.12	10.01	1.20
Uric Acid, mg100g	205	175	152	200	11.46	375	275	241	254	76.42	515	505	259	368	33.62

JB - Jute Bag; MB - Metalbin; PoB - Polythene Bag

The figures are means by triplicate analysis

Initial values for weight, damage (%), density, moisture (%), ash, crude fibre, crude fat energy (Kcal), NPN, true protein and uric acid are : 1500, 0, 1.25, 12.60, 1.60, 14.56, 1.92, 321, 0.69, 14.41 and 0, respectively

the weight volume ratio of wheat stored in different storage structures were observed. Maximum weight volume ratio (density) was observed in wheat stored in metal bins and minimum in wheat stored in jute bags. No significant difference was observed in wheat stored in Peru and polythene bags after 6 months of storage. Initial increases in weight and density might be due to the presence of larva and pupa stages of insects. Afterwards, adult insects emerged out after boring the grains (Pingale 1954). With increase in storage period, per cent damage increased. This was due to the higher moisture contents in jute bags and Peru, because they absorbed more moisture from the atmosphere and conditions became more favourable for insect attack.

With increase in storage period significant ($P<0.05$) increases in moisture, ash, crude fibre and crude proteins were observed in all storage systems. Increases in these parameters were maximum in wheat stored in jute bags and minimum in wheat stored in metal bins (Table 2). Increase in moisture might be due to increased insect population, increased insect metabolism, seed respiration and activity (Ovchrov 1977). Increase was more in jute bags and Peru due to higher insect attack. Increases in all other parameters might be due to consumption of endosperm by insects, as a result, husk portion rich in ash and fibre was left behind (Girish et al. 1977).

Increased insect infestation resulted in increases in crude proteins, non-protein nitrogen and uric acid, which might be due to higher insect population, insect excreta and insect body fragments present in the grains. *Sitophilus oryzae* L. bores grains and leaves its excreta (Harcharan Singh 1984) inside which might contribute to increases in these parameters. Uric acid is the end product of protein metabolism in insects. Manifold increase in uric acid in wheat stored in jute bags and Peru are of significance importance from hygienic, nutrition and acceptability points of view (Swaminathan 1977; Passmore and Eastwood 1987). Significant decreases in crude fat, true proteins and energy were observed with increase in storage period in the wheat stored in jute bags, *Peru*, metal bins and polythene bags. Energy (Table 2) losses were maximum in wheat stored in jute bags and minimum in wheat stored in metal bins. The decrease in energy and crude fat might be due to consumption of endosperm and germ portions by insects, which otherwise contribute as major sources of carbohydrates and fat in wheat (Singh et al. 1990).

Due to poor quality of insect infested wheat stored in jute bags, Peru, metal bins and polythene bags, feed efficiency ratio and protein efficiency ratio decreased significantly ($p<0.05$) (Table 1). Decrease was less in wheat stored in metal bins, when compared with the other three storage structures. Weight gain was maximum in casein fed group, followed by uninfested group and wheat stored in metal bins. Weight gain was minimum in other three storage systems. The poor growth rate might be due to poor quality of insect infested wheat stored in different storage structures. Similar results have been reported by Daniel et al (1977). *In vitro* studies on insect infested wheat stored in jute bags, Peru, metal bins and polythene bags showed increases in crude protein contents, while the *in vivo* studies showed adverse effect of insect infestation on protein quality of wheat.

Storage systems play an important role in safe storage of wheat. Wheat stored in improper storage structures damaged both qualitatively and quantitatively due to insect infestation and the gets damaged grains become unfit for human consumption.

References

AOAC (1990) Approved Methods of Analysis. Association of Official Analytical Chemists, Washington DC

Aujla P, Oberoi K, Jindal U, Kaur SC (1990) An analysis of qualitative losses in wheat grains during storage. Bull Grain Technol 28(3): 244-249

Bajaj, YPS (1990) Wheat. Narosa Publishing House, 6, Community Centre. Punchsheel Park, New Delhi, India

Chapman DG, Castillo R, Campbell JA (1959). Evaluation of protein in foods. Canad J Biochem Physi 37: 679-685

Daniel VA, Rajan P, Sanjeevarayappa KV, Srinivasan K, Swaminathan M (1977) Effect of insect infestation on the chemical composition and protein efficiency ratio of the proteins of Kaffir corn and greengram. Ind J Dietet 14: 38-42

FAO (1986) Production Year Book. Vol 42, FAO, Rome

Girish GK, Tripathi BP, Tomer RPS, Krishnamurthy, K (1974) Studies on the assessment of losses. Bull Grain Tech 12(30): 199-210

Harcharan Singh (1984) Household and kitchen garden pests. Principles and practices. Kalyani Publishers, 24, Daryaganj, New Delhi, p 273

Lal S (1990) Studies on quantitative and qualitative losses in wheat due to insects at farm level storage. Bull Grain Tech 28: 210, 219

National Academy of Sciences (1972). Recommended levels of vitamin mixture for rats (BARR Committee on Animal Nutrition. Washington DC

Oser BL (1971) Hawk's Physiological Chemistry. Tata McGraw Hill Ltd. New Delhi

O'Shea J, Maguire MP (1962). Determination of calorific value of feed stuff by chromic acid oxidation. J Sci Food Agric 13(1): 530

Ovcharov KE (1977). Physiological Basis of Seed Germination. Amerind Pub. Co. Pvt. Ltd. New Delhi. pp 35-48

Passmore R, Eastwood MA (1986) Human Nutrition and Dietetics. Churchill Livingstone, Medical Division of Longmans Group, U.K. Ltd. Edinburgh. pp 183-185

Pellet LP, Young VR (1980) Nutritional Evaluation of Protein Foods. UN University Publication

Pingale SV, Narayana Rao M, Swaminathan M (1954) Effect of insect infestation on stored grain. I. Studies on soft wheat. J Sci Food Agric 5: 51-54

Snedecor W, Cochran WG (1968). Statistical Methods, 6th edn. Oxford and IBH Pub., New Delhi

Sharma SS, Thapar VK, Simwat GS (1979). Biochemical losses in stored wheat due to infestation of some stored grains insect pest. Bull Grain Technol 17(2): 144-147

Singh K, Agarwal NS, Girish GK (1972) Studies on the loss in weight caused by *Sitophilus oryzae* L. to various high yielding varieties of wheat. Bull Grain Technol 10(4) : 271-275

Swaminathan M (1977) Effect of insect infestation on weight loss, hygienic condition, acceptability and nutritive value of food grains. Ind J Nutr Dietet 14: 205-215

Received 7 June 1997; revised 11 July 1999; accepted 27 August 1999

J. Food Sci. Technol., 1999, Vol. 36, No. 5, 483-486

THE RETURN OF ω3 FATTY ACIDS INTO THE FOOD SUPPLY 1. LAND-BASED ANIMAL FOOD PRODUCTS AND THEIR HEALTH EFFECTS. Editor - A.P. Simopoulos. Publisher - S. Karger, P.O. Box CH-4009 Basel, Switzerland. pp 240, 1998, Price. US$ 215.00.

Ever since it came to the notice of nutritionists and medical professionals that eskimos have a lower incidence of cardiovascular diseases owing to the consumption of marine foods rich in ω3 fatty acids, serious efforts have been made to understand the underlying mechanisms for health benefits of these speciality lipids. Several studies on human volunteers and experimental animals have repeatedly demonstrated many useful facets of ω3 fatty acids. At the same time, it was realised that the sources of ω3 fatty acids available for human consumption are very limited. Eventhough the agricultural practices significantly improved the availability of food, they changed the quality of food to a great extent. When man was hunting animals for food, the meat of the animals in the wild had less fat, less satrated fat and more polyunsaturated fat particularly that of ω3 fatty acids. The ratio of ω6 to ω3 fatty acids prior to agricultural revolution was less than 2. However, the agricultural revolution changed the food habits of domestic animals reared for meat purposes. This increased the ω6 fatty acids but decreased the ω3 fatty acids content of the meat. Similar changes occurred in the composition of poultry, eggs and aquaculture fishes. As a result, human beings are deprived of ω3 fatty acid intake in their diets. With emerging data from many reliable studies, highlighting the importance of ω3 fatty acids in human diet, a search began for finding alternate sources to derive these fatty acids particularly from land-based animal food products, which are easily accessible to a majority of population in the world. The developments in this area formed the focal theme for the 1st International Conference on 'The Return of ω3 Fatty Acids into the Food Supply : I. Land-Based Animal Food Products and Their Health Effects' held at the Natcher Conference Center, National Institutes of Health in Bethesda, MD., on September 18-19, 1997. The conference was organized and sponsored by a number of institutions and associations and attended by scientists from International community. The proceedings of this conference was brought out by KARGER and ably edited by Dr. Artemis P. Simopoulos.

The proceedings in the book is divided into four parts. The first section deals with ω3 fatty acids and its implications on health and on selected diseases. Simopoulos, while reviewing the evolutionary aspects of ω3 fatty acids in the diet tracing the history from paleolithic diets to current food habits, has strongly recommended that the time has now come to return the ω3 fatty acids into the food supply, where it is currently at a very low level. Eaton and his colleagues have also expressed similar sentiments. They also felt that the current intake of essential fatty acids clearly differs from that of our ancestors in that the humans during pre-agricultural periods consumed ω6 and ω3 fatty acids in equal proportion, which has later on tilted in favour of ω6 PUFA. Hence, the balance between these essential fatty acids should be restored. The anti-arrhythmic action of ω3 fatty acids has been examined by Alexander Leaf and his group. They have made a cautious prediction that there exists a basic control on the function of the heart by dietary fatty acids. However, they have strongly felt that a careful, prospective, double blind, randomized placebo-controlled clinical trials should be done to find an answer to their prediction. Bruce Watkins has reviewed the regulatory effects of polyunsaturated fatty acids on bone modelling and cartilage functions and concluded that ω3 fatty acids directly enhanced osteoblastic bone formation and also upregulated IGF-1 anabolic action on bone. He has further predicted that dietary fatty acids and antioxidants might attenuate osteoclastic activity to reduce the severity of osteolytic diseases of the bone and joints. Dennis Hoffman and David Birch have concentrated on the docosahexaenoic acid status in red blood cells of patients with Retinitis Pigmentosa in an ongoing clinical trial. Retinitis Pigmentosa is a genetic disorder with errors in essential fatty acid metabolism. It was anticipated that the findings of this trial with docosahexaenoic acid might help in future research efforts to control the progression of this disease.

The second part of the proceeding deals with ω3 fatty acids in land-based animal food products. Barclay and his group have explored the possibility of using dried algal cultures of Schizochytrium as a source of docosahexaenoic acid in feeds for shrimp, swine, beef cattle, dairy cattle and laying hens. Substantial assimilation of docosahexaenoic acid took place after feeding algal supplements in the food. Similar findings were made by Sim and Van Elswyk et al, who have shown the beneficial effects of designer eggs, containing ω3 fatty acids in reducing the risk factors for cardivascular

diseases. Kyle and Arterburn have summarised results of various clinical studies using single cell oil sources such as microalgae. They have also highlighted its usefulness as a supplement to provide docosahexaenoic acid in infant foods. They have also shown its usefulness in treating the clinical conditions of hypodocosahexaenemia. Howe has summarised the results of studies on the enrichment of pork with ω3 fatty acids. Mandell et al have summarised results on enriching beef with ω3 fatty acids by varied approaches. These two along with poultry are important dietary sources for vast majority of people in North America and Europe. Hence, these modified meat may form useful sources for ω3 fatty acids in these continents. Holub's group emphasised the need for enriching milk with docosahexaenoic acid. Frank Born in his essay discussed various strategies to pass on the benefits of extensive research work on ω3 fatty acids to consumers for health promotion. He concluded the essay on an optimistic note saying that more and more people would become sensitized to what and how much they eat, creating an increasingly favourable environment for products compatible with a healthy life style.

The third part of the proceedings deals with companion animal nutrition. The two articles in this section by Hayek and Reinhart and by Bibus and Stitt examine the essentiality of ω3 fatty acids in cats and dogs. These studies on companion animal nutrition highlights the need for fortifying pet foods with ω3 fatty acids.

The final section in this proceeding deals with the scientific and policy aspects about ω3 fatty acids. Newton has discussed the global food fortification perspectives of long chain ω3 fatty acids. He has built up his arguements by discussing evolution of the human diet, maternal nutrition, importance of long chain PUFA in human life cycle and discussed the recommended intake of PUFA at various stages of life. He has also briefly highlighted the changing trends in dietary habits and touches upon Nutrition policies. He further emphasized that governing bodies and policy makers should create awareness and also identify dietary vehicles to deliver PUFA to consumers. Bruce Holub has discussed the regulatory aspects of ω3 fatty acid labelling of food products as practised in Canada. Peter Howe has addressed the concerns of regulatory agencies in Australia in promoting ω3 fatty acid enriched foods for therapeutic benefits. He urged that it was the responsibility of experts in the area to encourage the regulatory authorities to dessiminate the information in such a way that consumers get reliable information about the ever expanding sources and benefits of ω3 fatty acids. Simopoulos has discussed the need for redefining the nutritional concepts with a rethinking on the dietary recommendation by taking into consideration, the genetic variations and concepts of food safety to include nutrient structure and food consumption patterns.

The proceedings also contains 12 poster abstracts echoing the sentiments and views expressed in the main articles.

The overall views expressed in the deliberations can be summarised as follows :

- There is a change in the dietary pattern over a period of time pushing ω3 fatty acids into the sideline.
- Increasing awareness in recent years about the essentiality of ω3 fatty acids has created a rethinking process in our dietary habits.
- The benefits of ω3 fatty acids in promoting health and prevention/ alleviation of chronic diseases are receiving greater attention.
- Efforts are being made to increase the availability of ω3 fatty acids in food chains. In this effort, modifications of land-based animal food products can make substantial contributions without altering the current food habits.
- The first step in the return of ω3 fatty acids into the food supply from beef, pork and poultry products has been taken and hopefully, this will introduce substantial amounts of ω3 fatty acids into our food chain.

The book is useful for libraries and research workers as a reference material and the subject matters covered in the conference can generate further interest in creating alternate sources of ω3 fatty acids through land-based animal products.

B.R. LOKESH
CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE, MYSORE - 570 013

EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS, Forty-ninth report of Joint FAO/FAO Expert Committee on Food Additives, WHO Technical Report Series No. 884, World Health Organization, 1211, Geneva 27, Switzerland, 1999, pp 96, Price SW. fr. 20/-.

This report is published under the World Health Organization Technical Report Series, which

makes available the findings of various international groups of experts with the latest scientific and technical advice on a broad range of medical and public health subjects. This report presents the conclusion of Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives and contaminants, with a view to recommend acceptable daily intakes for humans, and to formulate specifications for the identity and purity of food additives.

This report is divided into 7 chapters, which include Introduction, General considerations, Specific food additives and Food ingredients, Substances evaluated using the procedure for the safety evaluation of flavouring agents, Contaminants-aflatoxins, Revision of certain specifications and Recommendations.

Chapter 1 deals with Introduction, which highlights the meeting of Joint FAO/WHO Expert Committee, which met in Rome from 17 to 26 June 1997. The committee has recommended that scientific committees that advise the Codex Alimentarious Commission must have adequate expertise and experience to enable them to perform accurate and scientifically sound evaluations.

Chapter 2, which deals with general consideration, lists the task before the committee to elaborate further principles for evaluation of food additives and contaminants, toxicological evaluation, review and preparation of specification for selected food additives and flavouring agents. In making recommendations on the safety of food additives, food ingredients, flavouring agents and contaminants, the committee took into consideration the principles established and present in Environmental Health criteria No. 70. Apart from this, procedure for the safety evaluation of flavouring agents, revision of specifications, limits for arsenic, lead and other heavy metals, analytical methods have been thoroughly discussed.

Chapter 3 outlines specific food additives and food ingredients. Tert - butylhydro-quinone (TBHQ), which was previously evaluated by the committee, reviewed the results of the long-term toxicity studies in mice and rats. In addition, metabolism of TBHQ and its short term reproductive toxicity in rodent was also discussed. Elaborate feeding studies have been reported. In view of the conflicting results of the genotoxicity assays reviewed at previous meetings, many of the TBHQ studies were reevaluated. Animal studies conducted on microcrystalline cellulose, which is considered as an emulsifier. In some studies in humans, there have been reports of alterations in gastro-intestinal function following ingestion of microcrystalline cellulose. The committee has concluded that the toxicological data from humans and animals provided no evidence that the ingestion of microcrystalline cellulose can cause toxic effects in humans when used in foods. Regarding sucrose esters of fatty acids and sucroglycerides, the committee noted that no systematic studies were made in well conducted long term toxicity trials in rats upto the highest dose tested - 1970 mg/kg of body weight per day. The committee has noted that well documented non-pathogenic and non-toxicogenic strains of microorganisms had been used in the genetic modification process.

Six groups of flavouring agents have been evaluated using the procedure for safety evaluation of flavouring agents and the results are presented in Chapter 4. The committee noted that in applying the procedure, assigned the substances as follows:

Class I : Substances that have simple chemical structures and efficient mode of metabolism which would suggest low order of oral toxicity.

Class II : Substances that have structured features and are less inocuous than those of substances in class I, but are not suggestive of toxicity. Substances in this class may contain reactive functional groups.

Class III : Substances that have structured features, which permit no strong initial presumption of safety, or may even suggest significant toxicity.

Six exhaustive tables are given in this chapter depicting the results of safety evaluation of 38 saturated aliphatic acyclic branched-chain primary alcohols and aldehydes and acids; 35 aliphatic lactones; 32 esters of aliphetic acyclic primary alcohols with branched chain aliphatic acyclic acids; 67 esters of aliphatic primary alcohols with aliphatic linear saturated carboxylic acids and 26 esters derived from branched - chain terpenoid alcohols and aliphatic acyclic carboxylic acid compounds.

Chapter 5 is devoted on contaminants but solely deals with aflatoxins using the global monitoring system. Food Contamination Monitoring and Assessment Programme, as well as regional diets combined with data on levels of aflatoxins contamination are discussed. The committee was able to provide relative estimates of the mean dietry intake of aflotoxins for various regions.

Revision of certain specifications is considered in Chapter 6. A total of 40 substances were examined for specifications only and specifications for 30 were revised. The specifications for agar, carthamus yellow, microcrystalline wax, propylene glycol, propylene glycol alginate and gellan gum were revised with minor changes.

Recommendations are presented in chapter 7. One of the substances referred to the present meeting for evaluation is an example of materials that have been defined as "novel foods" by some countries and organizations. The committee recommended that FAO and WHO arrange at future meetings of the committee for the review of the procedure in Environmental Health Criteria No. 70 for safety evaluation of these types of food additives and contaminants in food.

Annexed to the report are tables summarizing the committee's recommendations for acceptable daily intake of the food additives and food ingredients, changes in the status of specifications for these substances for which further toxicological studies are required. The book is very useful for developing countries, where it can be procured at a subsidised rate of Sw. Fr. 14/-.

O.P. KAPUR
RETD. SCIENTIST, CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE, MYSORE - 570 013

We are PUBLISHING for a PURPOSE ...

A purpose of linking

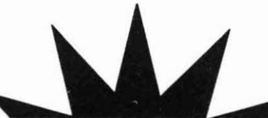
through the ....

NISSAT / DSIR

DIRECTORY OF
CURRENT RESEARCH PROJECTS IN
FOOD SCIENCE AND
TECHNOLOGY IN INDIA

National Information Centre for Food Science and Technology
CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE
MYSORE - 570 013. INDIA

Total Pages : 237 ; Price : Rs. 900/-
(DD in favour of Dirctor, CFTRI)
Extra Rs. 40/- for handling
& Forwarding charges.



An investment worth making.... for the information you need that you won't find elsewhere !

DIRECTORY OF CURRENT RESEARCH PROJECTS IN FOOD SCIENCE AND TECHNOLOGY IN INDIA

A Publication of the
National Information Centre for Food Science and Technology (NICFOS)

Which covers information on
477 projects pertaining to
25 subject areas in Food Science and Technology from
104 institutions.

Provides

- **Subject Index,**
- **Institution Index** and
- **Investigator Index**

for easy retrieval.

Please send your order to :

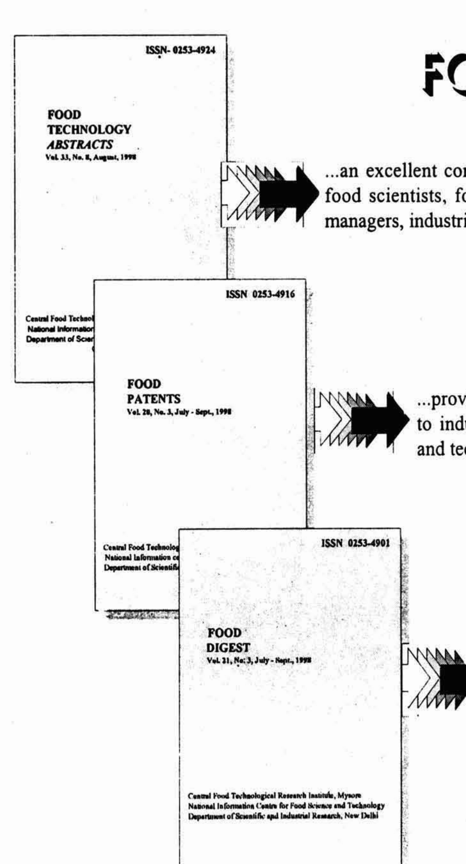
Head, FOSTIS, Central Food Technological Research Institute, Cheluvamba Mansion, Mysore - 570 013
Tel : 0821-515850 / 514760 Fax : 0821-517233 / 516308 Email : fostis@cscftri.ren.nic.in / fostis@nicfos.ernet.in

Design : Khadir M.M. Tel. : (0821) 516832

FOR COMPLETE

FOOD INFORMATION

SUBSCRIBE TO



...an excellent compilation of international information for food scientists, food engineers, technologists, researchers, managers, industrialists etc

...provide(s) up-to-date information and of immense help to industries, consultants etc, to refresh their industries and technology

....a unique source of valuable information on new raw materials, trade information, legislation, etc useful for industries, technologists, consultants, policy makers, managers etc.

For Details Contact:

Head

NICFOS

National Information Centre For Food Science and Technology
Central Food Technological Research Institute, Mysore - 570 013, India

Grams : FOODSEARCH, Mysore
Telex : 846 241 FTRI IN
Phone : 0821-515850
Fax : 0821 516308/517233
E-mail : fostis@nicfos.ernet.in
fostis@cscftri.ren.nic.in

ORDER FORM

Enter my subscription to (Tick box)

- ☐ Food Technology Abstracts (Monthly) at Rs 400/- per year
- ☐ Food Patents (Quarterly) at Rs. 300/- per year
- ☐ Food Digest (Quarterly) at Rs. 300/- per year

- ☐ I enclose a Demand Draft (in favour of Director, Central Food Technological Research Institute, Mysore) bearing No....................Dated................ for a sum of Rs.......................

- ☐ Please send me the proforma invoice.

Signature..

Name...

Address..

....

City/State...

International Conference on Processed Food For 21st Century January 14-16, 2000

The above conference is organised by the Department of Food Technology and Biochemical Engineering, Jadavpur University, Calcutta 700 032. India and sponsored by the AFST (I) Calcutta Chapter, Ministry of Food processing Industries, New Delhi, Department of Science and Technology, New Delhi, Council of Scientists and Industrial Research, New Delhi, University Grant Commission, New Delhi, Department of Biotechnology, New Delhi and a host of others.

Topics for Technical Session : Bio-technology; Food Processing and Engineering; Food Packaging; Health Foods; Nutrition and Food Safety and Quality; Food Laws; Global Marketing of Foods; Traditional Foods for Globalization; IPR and Patents; Human Resource Development; Policy Issues for Global Success. There will be poster session also under various areas.

For more detials, kindly contact :

Convenor, International Conference '99
Department of Food Technology &
Bio-Chemical Engineering, Jadavpur University, Calcutta - 700 032.
Phone : Office - 472-5822, 472-7635; Residence : 412-4838
Fax : 91-33-472-5822; 92-33-473-4266; E-mail : juftbe@cal2.vsnl.net.in

INDIAN FOOD INDUSTRY

A Publication of Association of Food Scientists and Technologists (India)

Contents of July-August 1999 Issue

FROM THE CHIEF EDITOR - A Word..... 203

INDUSTRY NEWS 204

US Patent for Vegetables/Herbs-based Drug Surprises Indian Manufacturers * Russia May Import 70 m kg Tea from India * India's Tea Output Likely to Fall During 1999 * Packaged Tea Market Drops 8.3 Per cent * Trade Agreement May Favour Lankan Tea Exporters to India * Belgium Company to Tie up with South Indian Firms * Organic Farming Scheme Launched in Britain * Indian Seafood Export in for Another EU Ban * Deep Sea Fishing Fund Mooted * Indian Seafood Sector Keen on Getting a Brand Name * Indian Shrimp Exports to US Likely to Boom * Atlantic Salmon to be Available in India Soon * Quality Processing Improves Seafood Export * Poultry Units Produce More Eggs * Marks and Spencer Faces Protests on Modified Foods * Spices Board to Evolve Codex Norm for Pesticide Levels * New Alternatives to Boost White Pepper Output * India May Fall Short to Meet Spices Export Target * Bumper 84 Lakh Tonnes Rabi Oilseeds Crop Envisaged * Oils, Fats Output up 2.1 % in 1999 * Packaged Edible Oil Soon * Vanaspati Firms in Favour of Tinplate Import Prices * Government to Create Buffer Storage for Onions * Government Brings Onions Under Essential Commodities Act * India to Test 400 Varieties of Super Rice Soon * Reducing Packaging Waste for Farmers * Fresh Regulations of Bottled Water * Consumers Demand Health and Convenience from Food * Supermarket Responds to Call for Low Salt * European Union to Lift Ban on British Beef Exports * SmithKline Beecham Re-launches 'Boost' * Transgenic Rice to be Introduced in India * Indian Food Grains Requirements to Reach 500 Million Tonnes by 2050 * India to Protect Traditional Agro Products * Biotechnology for Bharat *

FEATURE ARTICLES 217

Internet Applications in Food Industry
T. Kar and *A.K. Misra* 218

Applications of Image Processing Technique in Food Processing Industries
Abhijit Kar and *Sanjaya K. Dash* 223

Sorbitol and Other Sugar Alcohols in the Food Industry
Fausto F. Dias 229

Anti-Freeze Proteins : Prospects and Perspectives in Food Sector
V. Mishra and *P. Pattnaik* 238

DEPARTMENTS

AFST (I) News 216

Award 237

New Machinery 245

New Products 252

Research Round-up 255

Data Bank 259

Future Events 261

JFST Contents 262

Registered with the Registrar of Newspapers for India under No. 24918/64

Innovative Flavour Systems with high taste impact and functionality...

.... to meet the ever growing firce competition and demanding consumer....

Organoleptic preceptions - more particularly taste will continue to be the driving force in acceptance of a new food product in market place. High quality and high performance flavours promote innovative new food product ideas and satisfy unique process and product demands. In addition - provide to variety, consumer appeal and ultimately consumer acceptance into processed foods.

At Food Ingredient Specialities - We have achieved a quantum leap and professional expertise and are committed to providing flavours of high functionality and excellent taste attributes to processed foods. We are continuously capturing natures experience. This is a continuing commitment to work closely with user industry to bring about the best into processed food products, viz., - Soft drinks, Fruit and vegetable products, bakery products, confectionery, Dairy products including ice creams, snack foods and others. We are committed to consistant quality, responsive service and on-time delivery.

Experience the expertise ....

FOOD INGREDIENT SPECIALITIES PRIVATE LIMITED

Regd. Office : AI-111, ANNA NAGAR WEST, MADRAS - 600 040.

Phone : 0091-44-6250451/ 0091-44-6259536

Telegram : 'Foodingred' Fax : 0091-44-6264839

Published by the Secretary AFST (India) CFTRI, Mysore-570 013 and Printed at M/s. Jwalamukhi Job Press, 44/1, K.R. Road, Bangalore-560 004, India.