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

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (INDIA)

Vol.34, No.4 July-August 1997

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 head-quarters at Mysore.
- The chapters of the Association are located at Bangalore, Bhopal, Calcutta, Chennai, Delhi, Hisar, Hyderabad, Jabalpur, Jaipur, Jammu, Kanpur, Karnal, Kharagpur, Ludhiana, Manipur, Mumbai, Nagpur, Pantnagar, Parbhani, Pune, 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)

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

EDITORIAL BOARD

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*

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Volume 34 Number 4 July/August 1997

CONTENTS

PRINTS INDIA
Booksellers & Publishers
11, Darya Ganj,
New Delhi-110002, INDIA
Telex : 031-61087 Cable : Indology

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.

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.

Studies on the Influence of Water Activity (a_w) on the Stability of Foods - A Critical Appraisal

H.M. JAYAPRAKASHA*[1], K. JAYARAJ RAO[2] AND W.A. LOKESH KUMAR[1]

[1] Department of Dairy Technology, University of Agricultural Sciences, Hebbal, Bangalore-560 024,

[2] National Dairy Research Institute, Southern Regional Station, Adugodi, Bangalore-560 030, India.

Water activity is one of the most significant parameters for the shelf stability of any food system. A good combination of humectants, which are compatible with a given foodstuff can contribute significantly to its stability. Each microorganism has its critical water activity level for its growth, sporulation, toxin and metabolites production etc. Understanding of water activity range of various foods and spoilage organisms is of utmost important for processing and formulation of various food products. Water activity in combination with other factors such as pH, temperature of heating, preservative added etc., in a right proportion can effectively keep in check the chemical, microbiological and enzymatic spoilage with minimum effect on rheological and sensory characteristics of food system and thus contribute to the stability of foods. The present review attempts to appraise the role of water activity on the stability of foods.

Keywords: Food stability, Hurdle technology, Humectants, Non-enzymatic and enzymatic chemical changes, Rheology of foods, Water activity.

Requirement of water for the survival of human race is universally recognised. It has been reported that foodstuffs of high moisture content are readily susceptible to deterioration by microorganisms (Scott 1957). Not all the water present in a food is available for microbial growth. Some of the water is tightly bound to the product and hence cannot be utilized by microbes. The bound water increases, when some solutes (humectants) are added to a product (Sperber 1983). It is the state of water (free or bound) rather than the total content that is important as far as microbial proliferation is concerned. It has been observed that the state of water is related to the vapour pressure of a food. Greater the proportion of free water present, greater is the vapour pressure and *vice-versa*. For any food, if bound water increases, the vapour pressure decreases (Scott 1957). Thus, if a product is kept in a closed container and allowed to equilibrate, the humidity inside the container will be a measure of free state of water inside the product. The distilled water shows a humidity of 100% and all foods show a humidity of <100%. Generally, foods when kept at different humidities absorb (or) desorb moisture, depending on their water activity (a_w). When a_w is less than the surrounding humidity, the product will absorb and when reverse is the case, it will desorb moisture.

The concept of preservation of foods by control of water content happens to be one of the oldest methods. By the process of sun-drying, salting and/or sugaring foods knowingly or unknowingly, the water activity used to be reduced in foodstuffs to extend shelf-life. However, the physical and chemical basis of this was not understood, until the late 1950s. Later on, the concept of a_w of a food was introduced and defined as the ratio of vapour pressure of water in food (p_f) to vapour pressure of pure water (p_o) at the same temperature (Scott 1957).

$$a_w = \frac{P_f}{P_o}$$

$$= \% \frac{ERH}{100}$$

where, ERH=Relative humidity at which food neither gains nor loses moisture to the atmosphere.

In the subsequent years, the concept of a_w gained momentum and considerable amount of work has been done on chemical kinetics of reactions, effect of type of humectant used, effect of a_w in relation to other parameters such as pH, temperature, preservatives etc. (Duckworth 1975; Rockland and Stewart 1981; Simatos and Multon 1985; Rockland and Beuchat 1987). Several traditional as well as recent methods of food preservation (drying, curing, salting, freezing, intermediate moisture foods) are atleast partially based on the reduction of a_w (Leistner and Roedel 1975; Troller 1979; Plitman et al. 1973; Vigo et al. 1981). By this reason, various methods have been developed for measuring the a_w of foods and extensive information is available on a_w values of

* Corresponding Author

raw/processed low moisture and intermediate moisture foods (Labuza et al. 1976; Troller and Christian 1978; Chirife 1978; Stoloff 1978; Jordan and Cogan 1992; Fox 1993; Wojciechowski and Matylla 1984). The concept of a_w is considered to be of paramount importance in food stability. Based on this concept, various mathematical models have been developed to predict shelf-life of foodstuffs. Each foodstuff has its critical a_w level. Water activity in combination with other factors such as pH and temperature of heating has a key role to play in the shelf-stability of foodstuffs, as it controls the microbial (proteolysis, lypolysis), chemical (browning, oxidation) and physical (rheological characteristics) spoilage. This concept can be used to a great advantage in developing ready-to-use convenience foods. At present, monitoring the food stability is possible by carefully controlling the a_w by selecting appropriate humectants, with a minimal effect on textural and organoleptic characteristics. Therefore, there is a need to have a clear understanding of this concept, to develop technology for preparing novel convenience food products with long shelf-life at different temperatures.

Influence of water activity on microorganisms in foods

Microorganisms exhibit varied responses to a_w in foods. Their growth, sporulation, germination, morphology and production of metabolites like toxins, acids etc., are controlled by a_w. The response of microorganisms varies widely, depending on their physiological make-up. Morphology of bacteria changes due to reduced a_w. But, this may not be true for all microorganisms. There are not many reports available in the literature on the effect of a_w on the motility due to reduced a_w. As for example, when present in an environment of low a_w, the bacteria lose water as well as their morphological characteristics (Burdon and Williams 1968).

Microbial growth

Reduction of a_w has a variable effect on different genera of bacteria. As a_w is reduced, some bacteria stop growing at high values, while others are able to grow at much lower values. A decrease in a_w causes physiological problems within the microorganisms due to the higher concentration of solutes in the external environment. The intracellular a_w of bacterial cells is slightly lower than that of external medium and the cells are able to maintain turgour pressure. When a_w of the external medium is reduced, cells are subjected to osmotic shock and rapidly lose water (plasmolysis) (Sperber 1983). Koujima et al (1978) have shown that *S. aureus* loses about 50% of its intracellular water, when switched from a medium of a_w 0.995 to one of a_w 0.950. Under similar circumstances, Gibson (1973) has shown that the cell volume of *Salmonella typhimurium* decreases by 44%. To grow, the cell must reduce its intracellular a_w to regain its turgour. The resistance offered by some microorganisms to such osmotically hostile environment is basically due to intracellular accumulation of some osmotically active compounds called 'Compatible solutes' (Table 1).

TABLE 1. COMPATIBLE SOLUTES ACCUMULATED WITHIN THE CELL TO COUNTERACT OSMOTICALLY HOSTILE ENVIRONMENT

Microorganism	Compatible solute
Halophilic bacteria	Potassium ion
Osmophilic bacteria	Protein
Moderately tolerant bacteria	γ-amino butyric acid
Least osmotolerant bacteria	Glutamic acid
Yeast	Polyols

At reduced a_w, a general decrease in the growth occurs. An outright cell death rarely occurs. Each organism has its own characteristic optimum a_w, at which growth will occur most rapidly. The stages at which, growth suppression occurs are shown by arrows in Fig. 1 (it occurs in lag, logarithmic and maximum stationary phases of growth). Lag time of *L. lactis* var. *lactis* was not observed at a_w 0.988 (0 h), at a_w 0.975, lag time was at 2-7 h after incubation and at a_w 0.967, lag time was from

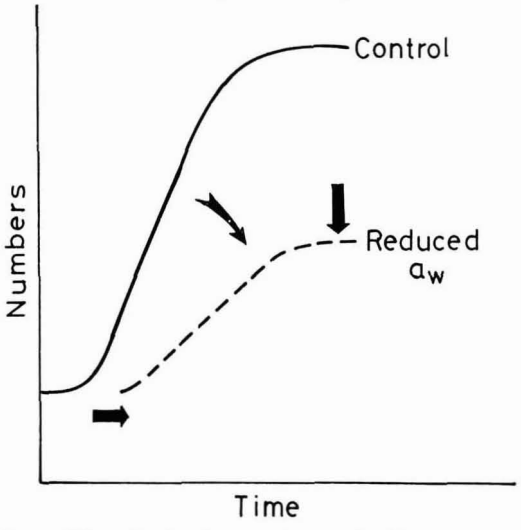

Fig. 1. Effect of reduced a_w on the growth of bacteria

2-8 h. Also, at a_w 0.967, the growth rate was retarded approximately 300 times (Ismail 1990). When a_w was reduced from 0.979 to 0.955, the lag time of *P. fluorescens* increased 3 times (0.3 to 1.5 h) (Ken-Yuon L i and Antonio Torres 1993). The same effects can be observed in moulds and yeasts also. Lag time for yeast and mould growth increased three times by reducing a_w from 0.97 to 0.91 in yoghurt (Lacroix and Lachance 1990). The minimum a_w for growth of yeasts depended on a_w controlling solutes as well as yeast species and strain. Most species showed the highest minimum a_w for growth in NaCl medium and about half of the species showed the lowest minimum a_w for growth in sucrose medium. One strain of *Zygosaccharomyces rouxii* had minimum a_w for growth as low as 0.67 in fructose medium. Generation times of *S. salivarius* sub sp. *thermophilus* and *L. lactis* increased, as the a_w was decreased by the addition of NaCl from 0.994 to 0.962 (Valik et al. 1995). Microorganisms may also get adapted to reduced a_w by continuous exposure to adverse a_w conditions. For example, pre-incubation in the presence of high concentration of glucose or fructose decreased minimum a_w for growth of yeast in glucose, or fructose medium (Tokuoka and Tshitoni 1991). Minimum a_w level for growth of *Listeria monocytogenes* occurred at 0.90. Its cells survived the longest in glycerol medium and died earliest in propylene glycol medium (Miller 1992). In milk medium, maximum acid production by *Streptococcus thermophilus* was at a_w 0.99 and by *Lactobacillus bulgaricus*, it was at a_w 0.983 (Larsen and Anon 1990). Humectants may also have protective effect on bacteria, as was observed with *S. thermophilus* and *L. bulgaricus* during the storage of yoghurt. Among bacteria, some of the halophilics can grow at low a_w. *S. aureus* grows at a_w of as low as 0.80 (Davies et al. 1976). Minimum a_w for growth depends on the type of solute employed. *S. cremoris* had minimum a_w of 0.95 for growth, when glycerol was used and it was 0.97, when sucrose was used. The corresponding values for *S. diacetylactis* and *S. lactis* were 0.95 and 0.95, 0.93 and 0.97, respectively (Troller and Stinson 1981). Some foods are listed in Table 2 with their water content, water activity and spoilage organisms. Table 3 shows the minimum a_w required for some of the microorganisms. Water activity of some of the common foods are listed in Table 4. Minimal a_w for acid production, spore grermination and growth is affected by the types of humectant used for adjusting a_w. Humectants can be mineral salts (NaCl in cheese), organic acids (lactic acid), mono-, di- and oligo saccharides (sucrose and lactose), alcohols and polyols (glycerol and sorbitol in pastry), proteins and protein derivatives (amino acids in cheese) and lipids and lipid derivatives (fatty acids, phospholipids, emulsifiers and emulsions) (Guilbert 1992). Glucose has an additional inhibitory effect to that caused by decreasing the a_w (Lacroix and Lachance 1990). Among glucose, NaCl, glycerol, glycine, proline, alanine, sodium glutamate and lysine hydrochloride as humectants, glycine was found to be the most

TABLE 2. WATER ACTIVITY, SPOILAGE ORGANISMS AND WATER CONTENT OF SOME FOODS*

Range of a_w	Organism inhibited by the lowest value of this range	Examples of food with such a water activity
1.00-0.95	Gram -ve rods; bacterial spore; some yeasts	Many cooked sausages; bread and foods containing 40% (w/w) sucrose or 7% (w/w) salts
0.95-0.91	Most cocci; lacto-bacilli; vegetative cells of Bacillacae; some moulds	Salami; old cheese; foods containing 55% (w/w) sucrose (i.e., saturated); foods with 15% NaCl
0.91-0.87	Most yeasts	Flour, rice, pulses, etc., containing 15-17% water; fruit cake, sweetened condensed milk
0.80-0.75	Most moulds *S. aureus*	Foods with 26% NaCl (i.e., saturated) old genuine Hungarian salami; margipan, containing 15-17% water; jam and marmalade
0.75-0.65	Xerophilic moulds	Rolled oats, containing 10% water
0.65-0.60	Osmophilic yeasts	Dried fruits containing 15-20% water, toffees and caramels containing 8% water
0.5		Noodles etc., containing 12% water, spices containing 10% water
0.4	Area of a_w which will not allow any microbial proliferation	Whole egg powder containing 5% water
0.3		Biscuits, rusks, bread crusts, etc., containing 3-5% water
0.2		Whole milk powder, containing 2-3% water, dried vegetables containing approx. 5% water, corn flakes, containing approx. 5% water

* Adapted from Davies et al (1976)

TABLE 3. MINIMUM WATER ACTIVITY REQUIRED FOR THE GROWTH OF SOME MICROORGANISMS*

Name of microorganism	a_w	Name of microorganism	a_w
Escherichia coli	0.960	*Bacillus subtilis*	0.950
Enterobacter aerogenes	0.945	*Staphylococcus aureus*	0.860
Clostridium botulinum	0.930	*Streptococcus diacetylactis*	0.950
Lactobacillus helveticus	0.970	*Listeria monocytogenes*	0.940
Zygosaccharomyces rouxii	0.670	*Bacillus thermophacta*	0.934
Pseudomonas fluorescenes	0.945	*Enterobacter faecalis*	0.941
Moraxella/Acinetobacter sp.	0.990	*Clostridium perfringens*	0.970
Vibrio parahaemolyticus	0.920	*Bacillus cereus*	0.920
Clostridium sporogenes	0.945	*Streptococcus thermophilus*	0.985
Streptococcus lactis	0.965	*Pseudomonas aeruginosa*	0.970
Lactobacillus plantarum	0.945	*Bacillus megaterium*	0.945
Serratia marcescens	0.943	*Klebsiella aerogenes*	0.940
Micrococcus lysodeikticus	0.930	*Sarcina lutes*	0.920

* aw values collected from different sources in literature

effective and glycerol was the least effective in retarding the growth of lactic acid bacteria (Streit et al. 1979).

Sporulation and spore germination

The effect of a_w on sporulation of various microorganisms has indicated that the water activity (a_w) and the solute, which is used to reduce the a_w, influence the rate of spore formation. During *B. cereus* sporulation, it was observed that glucose, sorbitol and NaCl limited sporulation at 0.95 a_w, whereas sporulation in the presence of glycerol occurred at an a_w as low as 0.91 (Jakobsen and Murrell 1977). Generally, the mineral a_w levels are appreciably below those permitting growth (Leistner and Roedel 1978), but it depends on the method of spore harvesting (Jakobsen and Murrell 1977). Chyr et al (1977) found that *Clostridium sporogenes* could not grow at a_w 0.95, but germination of its spores was only slightly retarded at a_w 0.95 in comparison to 0.99.

Production of metabolites

Optimal a_w for maximum growth and maximum metabolite production may not be the same. For example, maximum growth rates of *S. thermophilus*

TABLE 4. WATER ACTIVITY OF SOME FOODS

Name of food	a_w	Name of food	a_w
Mayonnaise	0.910	*Sandesh, Karapak*	0.810
Margarine	0.950	*Sandesh, Kachgolla*	0.950
Textured soy protein	0.530	Cream (40% fat)	0.979
Plum marmalade	0.910	Whole milk	0.994-0.995
Processed cheese spread	0.965	Milk (1.5% fat)	0.995
Dried yeast	0.176	Apples	0.980
Yeast extract	0.592	Apricot	0.985
Caseinate	0.509	Bananas	0.964-0.987
Dried whole milk	0.254	Cherries	0.960-0.986
Dried skim milk	0.259	Currants	0.990
Processed cheese	0.965	Dates	0.974
Kashkaval cheese (10 month old)	0.930-0.965	Figs	0.974
Unsalted butter	0.990	Grapes	0.963-0.986
Salted butter	0.920	Lemon	0.982-0.984
Khoa	0.960	Mangoes	0.979-0.987
Raisins	0.582	Papaya	0.990
Plain yoghurt	0.988	Pineapple	0.985-0.988
Sweetened yoghurt	0.980	Plums	0.969-0.982
Cheddar cheese	0.948	Strawberry	0.986-0.991
Edam cheese	0.951	Water melon	0.992
Parmesan cheese	0.760	Beans	0.990-0.996
Milk chocolate	0.600	Beets	0.979-0.985
Unripened fresh cheese	0.985	Cabbage	0.990-0.992
Curd cheese	0.995	Carrots	0.983-0.989
Whey cheese	0.997	Tomatoes	0.991-0.994
Brick cheese	0.965	Camembert cheese	0.967
Sweetened condensed milk	0.850-0.890	*Sandesh narampak*	0.920

* a_w values collected from different sources in literature

TABLE 5. EFFECT OF A_w ON GROWTH AND MYCOTOXIN FORMATION BY SEVERAL MOULD SPECIES

Mould	Toxin	Minimal a_w	
		For growth	For toxin production
Aspergillus flavus	Aflatoxin	0.78-0.80	0.83
Penicillium patulum	Patulin	0.81	0.85
Aspergillus ochraceus	Ocharatoxin	0.77	0.88
Aspergillus ochraceus	Penicillic acid	0.76	0.81
Stachybotyrs atra	Stachybotryn	0.94	0.94

and *L. bulgaricus* occurred at 0.992, while maximum acid production was observed upto an a_w of 0.983 (Larsen and Anon 1990). Diacetyl production of lactic cultures was found to increase by decreasing a_w (Troller and Stinson 1981). Minimum a_w for acid production by *S. thermophilus* and *L. bulgaricus* was always lower, when a_w of milk was adjusted with glucose or sucrose rather than with glycerol (Larsen and Anon 1989).

L. lactis and *S. salivarius* sub sp. *thermophilus*, which are lactic acid producers, tended to produce more of acetic acid as a_w of the medium was lowered (Valik et al. 1985). As the a_w reduced from 0.998 to 0.967, the amount of acid production by *L. lactis* var. *lactis* also reduced (Ismail 1990).

The mineral a_w so far observed for the production of mycotoxin has been reported as 0.81 (Davies et al. 1976). This is considerably higher than the corresponding minimal a_w for growth of many mycotoxigenic fungi. Table 5 shows the effect of a_w on growth and mycotoxin formation by several mould species. In general, it can be noticed that the minimal a_w for toxin production is higher than that for growth.

A study of bacterial toxins has revealed that minimum a_w for toxin formation in *C. botulinum* is 0.94 (Roberts et al. 1976). There were some variations in some strains. In *S. aureus*, the production of enterotoxin-B was more sensitive (production fell sharply, when a_w was reduced from 0.996 to 0.970) compared to enterotoxin-A (production caused at a_w 0.87 or 0.89) (Leistner and Roedel 1976).

Besides enterotoxins, the influence of a_w on the production of other extracellular metabolites by *S. aureus* has shown that the greatest activity of DNAase, triputyrinase, trioleinase, catalase, coagulase and acid phosphatase was present in spent media from enterotoxin-A and B-producing strains, grown at 0.996 a_w. Acid and alkaline protease activities, on the other hand, were greatest at 0.94 a_w. Enzyme production with the exception of protease activity was generally related to the extent of growth. Acid production during cold storage of yoghurt was completely inhibited by 4% added salt (a_w <0.96). Reduced a_w with sucrose (or) sorbitol did not inhibit the acid production (Lacroix and Lachance 1988a).

Interaction with other factors

Water activity, as discussed earlier, reacts with other growth factors. Usually, a_w acts as an inhibitor along with other growth factors, if they are away from their optimum.

Heat : A very low a_w level is protective as compared to heating at higher a_w level. By decreasing a_w from 0.98 to 0.83 in sucrose solution, the D 65.5°C value for *Salmonella typhimurium* increases by 100-fold, whereas 'Z' value increases from 6.5°C to 7.7°C. The heat resistance of *L. monocytogenes* was determined in sucrose solution by decreasing a_w from 0.98 to 0.90. There was nearly a 10-fold increase in D 65.6°C value, whereas 'Z' value increased from 7.6°C to 12.9°C (Summer et al. 1991). Sodium chloride and glycerol are also known to offer protection to microorganisms (Jakobsen and Murrell 1977). For instance, spores of *Bacillus subtilis* are killed in less than 10 min in steam at 120°C, but in anhydrous glycerol, 170°C for 30 min is required. Sodium chloride in low concentrations has a protective effect on some spores. Solutes differ in their effect on bacteria. Glucose, for example, protects *E. coli* and *P. fluorescens* against heat better than sodium chloride at a_w levels near the minimum for growth. On the other hand, glucose affords practically no protection, or is even harmful to *S. aureus*, whereas sodium chloride is very protective. The optimal concentration for protection varies with the organism. It is high for osmophilic organisms and low for others, high for spores and low for non-osmophilic cells. The protective effect of sugar may be related to a resulting decrease in a_w (Frazier and Westhoff 1984). The osmoregulation process involves synthesis of intracellular compatible solutes to balance the external osmolality. This osmoregulation process requires considerabe energy (Prior 1978) and since at low temperature, chemical reactions are slowed down, the combined effect of refrigeration temperature and low a_w lengthens the lag phase dramatically (Ken-Yuon Li and Antonio Torres 1993).

The concept of 'wet heat' and 'dry heat' may be used to describe these results. The heat-resistance is increased, when there is a decrease

in the unbound water. The transfer of heat takes place at a slower rate and hence higher temperature is required for destruction of the microorganisms. A general explanation is that at lower a_w, the removal of cell water has been considered to have a stabilizing effect on the heat-labile components of bacteria similar to that which occurs in the case of proteins. Comparisons of heat resistance studies and studies of the biophysical properties of microbial cell water indicate that maximum heat resistance probably occurs in the region of the 'water monolayer' zone of cell hydration or 'localized water region', where cell water is most firmly bound. Water content of maximum heat resistance presumably corresponds to the optimum water content of heat stability of the protein or other cell component(s), whose destruction causes thermal death (Stumbo 1973).

pH: As a rule of thumb, it can be stated that as a_w is lowered, the pH limits for growth will be narrowed. These have been studied for *C. perfringens* and S. *aureus* (Webster et al. 1985). Similar effects have been observed for yeasts and moulds.

Oxygen: The minimal a_w at which growth occurs is lower under aerobic than under anaerobic conditions for those organisms capable of growing facultatively. For aerobic organisms, reduced oxidation reduction potential of the medium enhances the lethal effect of either pH, or heat or both (Leistner 1991).

Chemicals: There are descriptions about a method of fruit processing by first removing tissue gases under vacuum and replacing these gases with a mixture, containing 30% sucrose plus small amounts of $NaHSO_4$ and potassium sorbate (Woodroof and Luh 1975). Similar processes in which sucrose and chemical preservatives are infused into fruits have been under development in Eastern European countries for several years. One of the most effective means of preventing mould growth in shelf-stable intermediate-moisture (IM) foods is the combined application of humectants, such as glycerol and a mycotal, such as potassium sorbate (Leistner 1992).

Sometimes, interaction effect of combination of additives/humectants may not be manifested. For example, the shelf-life of fish sausage was increased by reducing a_w to 0.92, but a_w did not show any interaction effect with additives such as water binders and nitrite (Nieto and Toledo 1989). Resistance of S. *thermophilus* to penicillin increased, when a_w of milk was reduced with glycerol from 0.990 to 0.975, but its susceptibility increased, when a_w was adjusted with glucose. Resistance to gentamycin decreased with reduction of a_w (Larsen and Anon 1989).

Hurdle concept and hurdle technology

Each factor responsible for retarding microbial growth is seen as "hurdle" for microorganisms (Leistner and Roedel 1975). According to hurdle concept, each preservation parameter is termed as "hurdle". Thus, all the parameters like a_w, pH redox potential, heat treatment etc., which are bacteriostatic or bactericidal, are "hurdle". These hurdles govern many preservation processes. Intense heat (F) preserves canned foods, low a_w prevents any microbial growth in dried products and low pH is the reason for prolonged shelf-life of fermented foods. More than one hurdle is often responsible for long shelf-life of certain food products (Leistner 1992).

Most processed foods have several inherent hurdles that accomplish the desired microbial stability of the product. The stability of the product depends on the intensity of hurdles present in it. For example, *khoa* has an a_w of 0.96, but has a conducive pH for growth of bacteria and moulds, which overcome the hurdle of a_w in 3-4 days and resume multiplying. That is why *khoa* has a keeping quality of 3-4 days at room temperature. If sugar is added, the intensity of hurdle a_w increases i.e., a_w value decreases. It can be decreased upto 0.78 by addition of 50% sugar (Prajapati et al. 1986). Hence, microorganisms need more time to overcome this hurdle. As the intensity of hurdle increases, microorganisms find it more difficult to overcome it and at certain stages can no longer "jump" it. According to hurdle concept, many foods cannot be preserved by a single hurdle alone without affecting their sensory and nutritional properties. For instance, *paneer* cannot be preserved by "lone" hurdle of heat treatment or dehydration (a_w) or pH, because its body and texture and nutritive value get affected. By using three or more hurdles together, not only the damage to sensory properties is kept to the minimum, but also their synergistic action is exploited. Thus, hurdle technology is a technology by which every preservation parameter is used at an optimum level in order to get a maximum lethality by a combination of two or more such parameters so that damage to the sensory properties of food is kept to the minimum. Interestingly, each hurdle may not be intense enough to cause destruction of microorganisms or

spores, but the sub-lethal damage caused by heat treatment is augmented by the adverse effects of other hurdles. Hurdle technology is used abroad in making shelf-stable meat products like different types of sausages (Leistner 1992). Preservation by combined effect of cheese whey (De Kanterwicz et al. 1985), yoghurt (Lacroix and Lachance 1988b), cheese spread (Kombila-Moundounga and Lacroix 1991), *paneer* (Rao et al. 1992) and canned liver-pate (Silveira et al. 1992) is also reported. Application of hurdle concept to preservation of foods includes studying the effect of hurdles on the physico-chemical properties, nutritional value and microflora of a food and then exploring the ways of achieving these hurdles in the food. A method was developed by Zwietering et al (1992) to combine qualitative and quantitative information to predict possible growth of microorganisms in foods. Water activity was taken as one of the parameters, which was coupled to the growth characteristics. A database of kinetic parameters of microorganisms was built. This helped in predicting the shelf-life as well as product development. Several workers have compiled the data and reviewed the scope of hurdle technology (Rao 1993; Incze 1994; Russo 1994; Spahr and Url 1994; Gould 1995).

Influence of aw on non-enzymatic chemical changes

Non-enzymic browning: Non-enzymic browning is one of the major spoilage factors in high heat-treated foods. Maillard reaction occurs due to reaction between reducing sugars and amino groups of amino acids and proteins, subsequently resulting in visible browning. Two parameters are important (i) intensity of heat treatment and (ii) temperatue of storage. Higher processing temperatures initiate and enhance the reaction between the amino groups and the reducing sugars. Higher storage temperatures accelerate the intermediate browning reactions, which lead to the formation of several compounds like reactive unsaturated polycarbonyl compounds. These reactive components polymerize and bind simultaneously to α-amino terminal, ε- and other amino groups of different polypeptide chains bringing about the formation of coloured, high molecular weight, highly cross-linked protein-carbohydrate polymers with low solubility, digestibility and nutritional value (Cheftel 1979; Burton 1984), thereby resulting in altered texture (Matz 1962), detrimental effect on mineral homeostasis, increased allergenicity of milk proteins and changes in functional properties of proteins (O'Brien 1995). Water activity plays a major role in controlling these intermediate browning reactions.

Complex relationship exists between a_w and non-ezymatic browning. Minimum browning is observed at low and high a_w. The decrease in reaction at high a_w is due to the dilution of reacting species. The decreased reaction rate at low a_w, when the amount of mobile water is greatly reduced, has been ascribed to an increasing diffusion resistance, which lowers the mobility of the reactants. For example, addition of 10% sorbitol to casein-glucose solution reduced the rate of browning. This was attributed to decreased water mobility caused by increased viscosity. Diffusion of solutes can take place at water contents above the monomolecular layer value on the sorption isotherm, corresponding to the maximum amount of very strongly bound water, although browning can develop even below this moisture level to some extent (Labuza 1980). Generally, maximum browning occurs in the range of 0.3-0.7 a_w, depending on the type of food. As shown in Fig. 2, maximum browning occurs at a_w 0.6-0.7. Extent of browning not only varies with a_w, but also with type of humectants, pH and temperature. Warmbier et al (1976) reported that use of glycerol in intermediate moisture foods might decrease browning rates at a given a_w. However, with glucose-lysine solutions, Petriella et al (1989) observed no such effect. The effect of a_w on non-enzymatic browning in a water-glycerol-sorbate-glycine model system at pH 4.0 was studied by Seow and Cheah (1985). The rate constant and activation energy decreased with increasing a_w. Glycerol was found to react with sorbate or glycine to form brown pigments at a_w 0.80 (Seow and Cheah 1985).

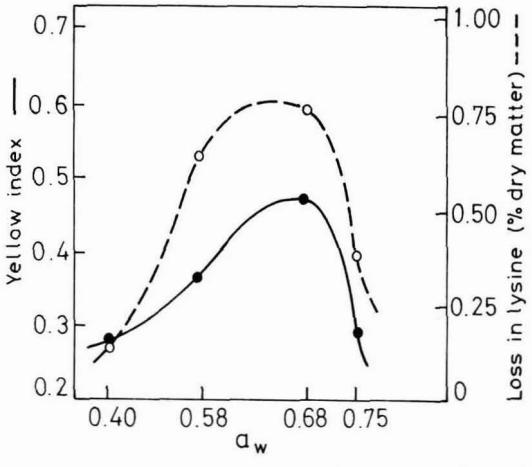

Fig. 2. Loss in lysine and browning in dried milk as influenced by a_w

The kinetic aspects of browning with regard to a_w have been discussed by Labuza (1980). Browning as measured by hydroxy-methyl-furfural (HMF) generally follows zero order reaction (Resnik and Chirife 1979; Ringe and Love 1988; Singh 1991). The activation energies for browning reactions may vary from 172 kJ/mol at zero moisture and 121 kJ/mol at 83% moisture (dry basis) in dehydrated apples (Resnik and Chirife 1979), 126-142 kJ/mol in sweet whey powders (a_w 0.33-0.65) (Labuza and Saltmarch 1981), 4.31-111.54 kJ/mol in UHT milk (Singh 1991) and 18.30 kJ/mol in *paneer* (a_w 0.95) (Rao 1993), depending on temperature of storage.

In general, Maillard reactions lead to losses of lysine. The losses may vary from 10 to 35% (Renner 1983) Fig. 2 indicates that loss of lysine in dried nuts is maximum between a_w 0.55 and 0.70. Wolf et al (1981) demonstrated that the losses of free lysine were highly dependent on a_w, protein and sugar. Reaction rates at 65°C decreased with increasing a_w (0.33-0.98). In the dried whey protein concentrate stored at 40°C, the loss of available lysine was maximum at a_w 0.41, the loss being 23% after three months (Lindemann-Schneider and Fennema 1989). In sweet whey powder, maximum loss was at a_w 0.44 (Saltmarch 1980).

Oxidation of ascorbic acid: The effect of oxygen and a_w on the destruction of ascorbic acid in orange juice crystals has a direct linear relationship of the loss rate with a decrease in moisture content down to the BET monolayer moisture value.

At low moisture contents, the rate of destruction of ascorbic acid in orange juice increased rapidly, as the moisture increased. This was due to the dilution of the aqueous phase which, in turn, decreased viscosity, thereby increasing reactant mobility. At high moisture contents, any further increase in the water content does not cause any further significant viscosity decrease. Therefore, the rate should remain constant (Labuza 1975).

Oxidation of food lipids: Water acts both as 'antioxidant' and 'prooxidant'. At a_w below the monolayer value, oxidation rate decreases with increasing a_w. The rate reaches a minimum around the monolayer value and increases with a further increase in a_w. The antioxidant effect of water at low a_w has been attributed to bonding of hydroperoxides and hydration of metal catalysts, whereas the prooxidant effect of water at higher a_w is due to increased mobility of reactions (Hiedelbaugh and Karel 1970). The major lipid oxidation mechanism is a free radical mechanism in which polyunsaturated methylene-interrupted double bonds react with oxygen, as controlled by moisture content, light and trace metal catalysts. Besides the production of objectionable odours and flavours, the free radicals and peroxides produced can react with pigments bleaching them, react with proteins, causing toughening and reducing digestibility, destroy vitamins and lead to the production of possibly toxic material (Eichner 1980).

As the monolayer region of water is exceeded, the solution properties of water affect the oxidation reaction in several ways, causing the rate to eventually increase. As a_w increases, the metal catalysts, although less effective, are more easily mobilized to reaction sites from their aqueous environment. Higher moisture system oxidises faster than lower moisture at a constant a_w. The difference increases with an increase in sorption hysteresis. At low metal content (10-50 ppm), as moisture increases, the rate of oxidation increases due to the decreased viscosity, increased mobility and the swelling, which exposes new catalytic sites. However, in systems containing high concentrations of trace metals, the rate at high a_w decreases, as a_w increases and is slower for the higher moisture systems. The reasons given are that of high metal concentration and metal catalysis as a predominant force in inducing formation of free radicals. Thus, any substantial decrease in the concentration depresses the rate steeply, as would be the case, when the water content is increased (Labuza 1975).

It was shown that in methyl linoleate used for oxidation studies, oxidation proceeded at a rapid rate at a 0.55 rather than in dry state (Kacyn et al. 1983). Oxygen uptake was shown to decrease in a model system containing browning intermediates as products, which reportedly have antioxidant properties (Eichner 1980). In some studies, it was observed that the rate of lipid oxidation was maximum at a_w of about 0.3-0.4. This was because of stabilization of the propagation rate (Kahl 1986). Oxidation stability of dried whole milk stored for one year at 30°C improved significantly, when a_w was increased in the range of 0.09-0.28. It is recommended that the dried milk should have an a_w of 0.21-0.24 for which the moisture content should be in the range of 2.5-3.0 to 3.4% (Wewala 1990).

Lipolysis: During food spoilage, a fraction of lipids is generally hydrolysed by lipases by spoilage microorganisms. The lipolytic activity of these enzyme is influenced by the water activity of the system.

Not many studies are available on the influence of a_w on lipolysis in foods. However, in studies on model system containing wheat germ and olive oil, it was observed that at a given a_w, the rate of fatty acid release increased quickly at first, followed by slow, continuous progression of the reaction, which is probably related to the mobility of the substrate. As a function of a_w, low linear reelationships were observed between fatty acids released in a given time and the quantity of water sorbed. The first corresponds to the water fraction that is very strongly absorbed and whose role is only that of substrate and the second corresponds to the linear portion of the sorption curve. Lipolysis measured as mg oleic acid formed after 72 h increased linearly with a_w, reflecting the role of water in deacylation of olive oil. The per cent olive oil in the system decreased rapidly, till a_w 0.4, but thereafter increased, showing maximum affinity of enzyme for the substrate at a_w 0.4 (Caillat and Drapron 1974).

Proteolysis: Influence of a_w on proteolysis is important in ripened cheese, though minor. The plasmin activity on β-casein (appearance of r-fractions) increased upto 0.972 (i.e., salt in moisture content of 2% and then decreased with decrease in a_w). Proteolysis of α-casein decreased with cheese a_w lowering the cohesiveness of the hard cheese. Secondary proteolysis as measured by the ratios of water soluble N : total N and phosphotungstic acid soluble N : total N varied little in relation to a_w values (Delacroix-Buchet and Trossat 1991).

Influence of a_w on rheology of foods

Water activity has a major effect on textural properties of foods. The effect of a_w on food texture is specific to the kind of food under consideration. Rockland (1969) listed food characteristics as a function of their localised moisture sorption isotherms : (a) local isotherm one (low moisture content) - dry, hard, crisp, shrunken, (b) local isotherm two (intermediate moisture content) - dry firm, flexible and (c) local isotherm three (high moisture content) - moist, soft, flaccid, swollen and sticky.

Hardness and brittleness increase and springiness and elasticity decrease with decreasing a_w (Ziegler et al. 1987). In freeze-dried beef, hardness and chewiness were found to increase at a_w 0.50 or 0.75 during storage for 4 months at 37°C (Heldman et al. 1973). Texture profile analysis of intermediate moisture meat as affected by a_w during storage was studied by Ledward et al (1981). Chordash and Potter (1972) showed that dehydration alone was not feasible in intermediate moisture technology. To achieve a_w sufficient to inhibit bacterial growth, the product has to be dried to such an extent that the texture becomes granular. Similar observation was made by Vishweshwaraiah (1987) with *paneer*, which, on dehydration, lost cohesive property and crumbled on rehydration. Generally, the *paneer* is fried and cooked before consumption. At a_w 0.90, the hardness of fried *paneer* was found to be about ten times more than a_w 0.88 (Rao 1993). It was observed that frying - which leads to lowering of a_w to approximately 0.97 - and cooking of *paneer* in 2.25% salt solution - which leads to restoration of original a_w - enhanced elasticity and chewiness of *paneer* and decreased the firmness and smoothness (Desai 1988). Deep-frying (175°C/4-5 min) causes compaction of the protein particles and cooking in salt water restores the overall structure (Kalab et al. 1988). Similar changes in freeze-dried beef were attributed to decrease in standard differential entropy change caused by sorption of water (Kapsalis et al. 1970). Sharp transition in textural properties are displayed near BET (Brunauer-Emmett-Teller) monolayer moisture content (Kapsalis 1975).

The texture is also affected by the type of solute used. In case of soyflour suspension used for soy beverages, sucrose, glucose and NaCl tended to decrease apparent viscosity and increase Newtonian behaviour (Urbanski et al. 1982). Admixture of glycerol with *khoa* at 2% level significantly improved hardness, cohesiveness, adhesiveness and chewiness of the product (Sawhney et al. 1991). Glycerol was also reported to improve the spreadability of cheese spread, when used at 5% concentration, but salt and lactose had the opposite effect. These humectants are reported to modify rheological characteristics by affecting the hydration of the cheese proteins. Type of humectant and the level to be used should be selected carefully, as otherwise the sensory properties of a product may be affected. In yoghurt, consistency decreased and the flowability increased with increasing level of humectants in the order : NaCl < sucrose = sorbitol. Synersis increased with humectant level (Lacroix and Lachance 1988b).

Conclusion

As indicated in Fig. 3, one can expect certain generalizations of foods with regard to quality and stability. Water activity is definitely an important

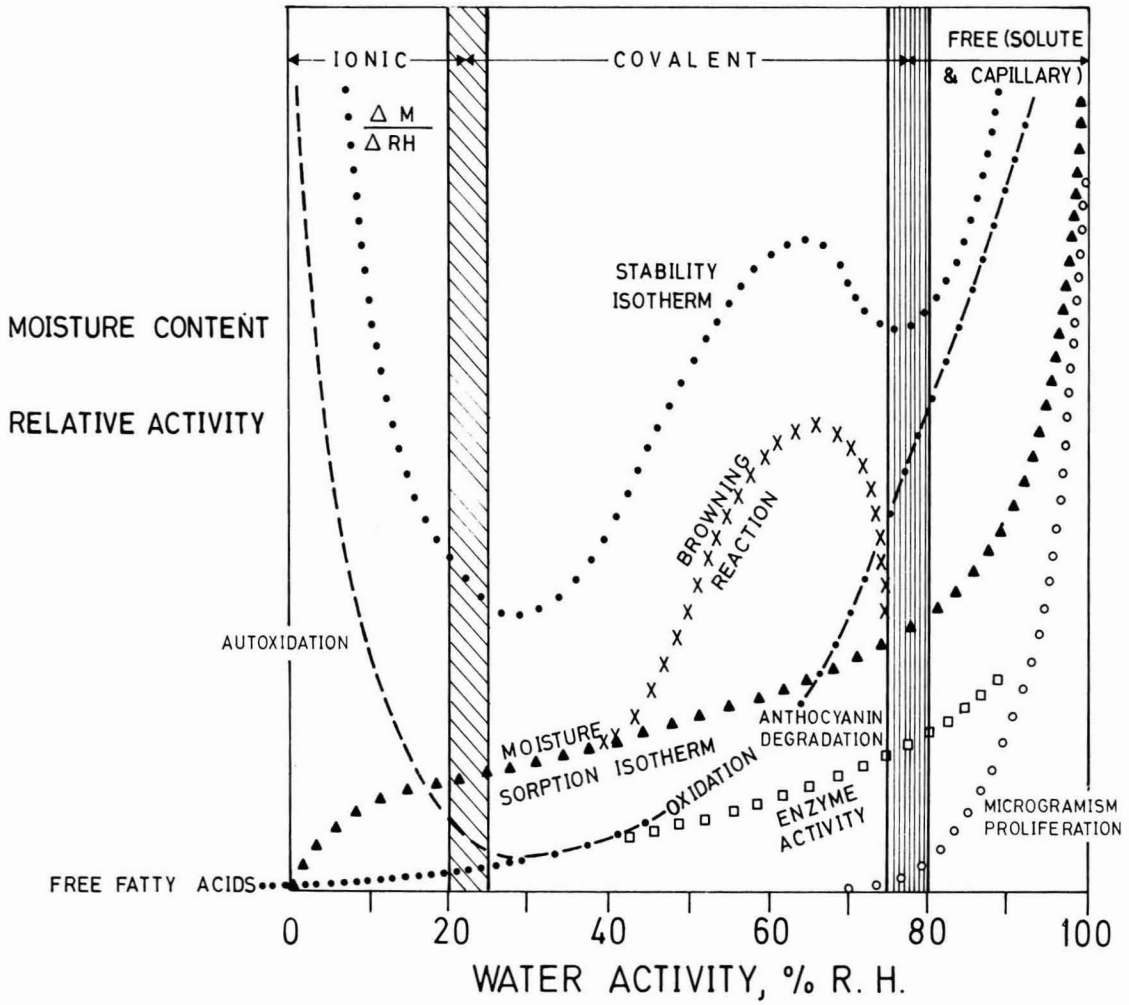

Fig. 3. Diagramatic representation of the influence of a_w on chemical enzymatic and microbiological changes and on overall stability and moisture sorption properties of food products

parameter in judging the quality and shelf-life of a product. Somewhere, certain loopholes could lead to perplexity over the universality of the concept. Russo (1994) pointed out the contradictions, resulting from the applications of a_w to foods and its limits. For example, when a solute such as sucrose is used, a_w is decreased, but the water mobility is increased. The result is dependent on the physical state of the solute. One aspect, which one should bear in mind is that though a_w is a very weighty parameter, it may not be complete in all respects and it needs to be supplemented with another parameter(s). The interactions of a_w with other growth factors (pH, temperature, preservatives, oxygen content) have been discussed in this review. Instead of using a_w alone to achieve stability, it would be worthwhile to combine it with some other parameter suitably. In fact, this has been in vogue and it is called 'hurdle technology'. Here, a_w is combined with pH, temperature, preservatives etc. By decreasing the a_w to a very low value, all the reactions could be controlled, but this would lead to textural problems, thus decreasing the general acceptability of the product. Therefore, a combination is preferred to control a_w alone, keeping in view the textural characteristics.

References

Burton M (1984) The bacteriological, chemical, biochemical and physical changes that occur in milk at temperatures of 100-180°C. J Dairy Res 51:341-363

Burdon KL, Williams RP (1968) Microbiology. The MacMillan Co, Collier-Macmillan Ltd., London, p 174

Caillat JM, Drapron R (1974) Wheat lipase : Characteristics of its action in aqueous and low moisture media. Ann Technol Agric 23:273–275

Cheftel JC (1979) Proteins and amino acids. In: Tannenbaum, SR (ed) Nutritional and Safety Aspects of Food Processing, Marcel Dekker Inc. New York, USA, pp 153-217

Chirife J (1978) Prediction of water activity in intermediate moisture foods. J Food Technol 13:417-419

Chordash RA, Potter NN (1972) Effect of dehydration through the intermediate moisture range on water activity, microbial growth and texture of selected foods. J Milk Food Technol 35:395-398

Chyr CYL, Walker HW, Hinz P (1977) Influence of pH, temperature curing agents and water activity on germination of PA 3679 *(Clostridium sporogenes)* spores. J Food Prot 40:369-372

Davies R, Birch GG, Parker KJ (1976) Intermediate moisture foods. Applied Science Publishers Ltd., London, UK

Dekanterwicz R, Chrife J, De Lagard EA (1985) Preservation of concentrated cheese whey by combined factors. J Food Sci 50:1629-1632

Delacroix-Buchet A, Trossat P (1991) Proteolysis and texture of scalded curd hard cheeses - I. Influence of water activity Lait (Lyon) 71:299-311

Desai HK (1988) Rheological properties of heat and acid coagulated Indian milk products. Ph.D. Thesis, Kurukshetra University, Kurukshetra

Duckworth RB (1980), In: Duckworth RB (ed.) Water Relations in Foods. Academic Press, New York, USA

Eichner K (1980) Antioxidative effect of Maillard reaction intermediates In: Simic MG, Karel M (eds), Autoxidation in Food and Biological Systems. Plenum Press, London, UK

Fox PF (1993) Salt in cheese: Physical chemical and biological aspects In: Guinee TP, Fox PF (eds) Cheese: Chemistry Physics and Microbiology Vol 1, General Aspects. 2nd edn., pp 257-302

Frazier WC, Westhoff DC (1984) Food Microbiology, Tata McGraw Hill Publishing Ltd, New Delhi pp 5-9

Gibson B (1973) The effect of high sugar concentrations on the heat resistance of vegetative microorganisms. J Appl Bacteriol 36:365-376

Gould GW (1995) New Methods of Food Preservation. Unilever Res. Labor, UK

Guilbert S (1992) Additives and agents reducing water activity. Dairy Sci Abstr 54:6827

Heldman DR, Reidy GA, Palanikkar MP (1973) Texture stability during storage of freeze-dried beef at low and intermediate moisture contents. J Food Sci 38:282-285

Hiedelbaugh N, Karel M (1970) Effects of water binding agents on oxidation of methyl linoleate. J Am Oil Chem Soc 47:539

Incze K (1994) Shelf-stable meat products (SSP) storable temperature. A-Hus (Hungary) 4:187-191

Ismail A (1990) Influence of water activity on the growth rate and acid production during growth of lactic acid bacteria in milk. Assiut J Agril Sci 21:111-131

Jakobsen M, Murrell WG (1977) The effect of water activity and water activity controlling solute on sporulation of *Bacillus cereus*. J Appl Bacteriol 43:239-245

Jordan KN, Cogan TM (1992) Cheese as a growth medium for bacteria. Proceedings of Third Cheese Symposium, Moorepark, pp 100-107

Kacyn LJ, Saguy I, Karel M (1983) Kinetics of oxidation of dehydrated food at low oxygen pressures. J Food Proc Preserv 7:161-165

Kahl J (1986) Effect of water and cobalt on methyl linoleate autoxidation. MS Thesis, Washington State University, Pullman, WA

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

Kapsalis JG (1975) The influence of water on textural parameters in foods and intermediate moisture levels. In: Duckworth RB (ed), Water Relations in Foods, Academic Press, New York, USA, pp 627-637

Kapsalis JG, Walker Jr JE, Wolf M (1970) A physico-chemical study of the mechanical properties of low and intermediate moisture foods. J Texture Studies 1:464-483

Ken-Yuon Li, Antonio Torres J (1993) Water activity relationship for selected mesophiles and psychrotrophs at refrigeration temperatures. J Food Prot 56:612-615

Kombila-Moundounga, Lacroix C (1991) Effect des combinaisons de chlooryre de sodium de lactose et de glycerol sur les characteristiques rheologiques et la couleur des fromages foundus a tartiner. Can Inst Food Sci Technol J 24:239-251

Koujima I, Hayshi H, Tomochika K, Oklase A, Kanemasa Y (1978) Adaptional change in proline and water content of *Staphylococcus aureus* after alteration of environmental salt concentration. Appl Environ Microbiol 35:467-470

Lubuza TP (1975) Oxidative changes in foods at low and intermediate moisture levels In: Duckworth RB (ed) Water Relations of Foods Academic Press, New York, USA

Labuza TP (1980) The effect of water activity on reaction kinetics of food deterioration. Food Technol 34:36-41, 59

Labuza TP, Acott KA, Tatini SR, Lee R, Flink J, McCall W (1976) Water activity determination: A comparative study of different methods. J Food Sci 41:910-917

Labuza TP, Saltmarch M (1981) Kinetics of browning and protein quality loss in whey powders during steady state and non-steady state storage conditions. J Food Sci 47:92-96

Lacroix C, Lachance O (1988a) Effect of water activity on the survival of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and on the development of acidity in yoghurt during cold storage. Can Inst Food Sci Technol J 21:501-510

Lacroix C, Lachance O (1988b) Effects of humectants on water activity and rheological properties of yoghurt. Can Inst Food Sci Technol J 21:511-519

Lacroix C, Lachance O (1990) Effects of various humectants and water activity on proteolysis yeast and mold growth and shelf-life during cold storage of yoghurt. Can Inst Food Sci Technol J 23:101-108

Larsen RF, Anon MC (1989) Interaction of antibiotics and water activity on *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. J Food Sci 54:922-924, 939

Larsen RF, Anon MC (1990) Effect of water activity of milk upon growth and acid production by mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. J Food Sci 55: 708-800

Ledward DA, Lymn SK, Mitchell JR (1981) Textural changes in intermediate moisture meat during storage at 38°C. J Texture Studies 12:173-184

Leistner L (1991) Food preservation by combined processes (Hurdle Technology). In : Summary of Minutes of FLAIR Concerted Action No. 7 Meeting of Subgroup B, April 10-12 1991. Federal Centre of Meat Research, Kulmbach Germany, pp 1-2

Leistner L (1992) Food preservation by combined methods. Food Res Int 25:151-158

Leistner L, Roedel W (1975) The significance of water activity for microorganisms in meats. In: Duckworth RS (ed), Water Relations of Foods. Academic Press,New York, USA, pp 309-324

Leistner L, Roedel W (1976) The stability of intermediate moisture foods with respect to microorganism. In: Davis R, Birch GG, Parker KJ (eds). Intermediate Moisture Foods, Applied Science Publishers, London, UK

Leistner L, Roedel W (1978) Microbiology of intermediate moisture foods In: Jarvis B, Christian JHB, Michener HD (eds). Proceedings of the International Meeting on Food Microbiology and Technology, Medicina Viva Servizio Congreei, Parma, Italy

Lindemann-Schneider U, Fennema O (1989) Stability of lysine, methionine and tryptophan in dried whey concentrate during storage. J Dairy Sci 72:1740-1747

Matz SA (1962) Food Texture. The AVI Publishing Co. Inc., Westport, Connecticut, USA

Miller AJ (1992) Combined water activity and solute effects on growth and survival of *Listeria monocytogenes*. J Food Prot 55:414-418

Nieto MB, Toledo RT (1989) Fate of *Clostridium sporogenes* PA 3679 in fish sausage formulations with adjusted water activity using food binders. J Food Sci 54:1129-1132, 1135

O'Brien J (1995) Heat induced changes in lactose: Isomerization degradation Maillard browning In: Fox PF (ed), Heat Induced Changes in Milk, Bruxelles, Belgium

Petriella C, Chirife J, Resnik SL, Lozano RD (1989) Solute effects at high water activity on non-enzymatic browning of a glucose-lysine solutions. J Food Sci 53:987-988

Plitman M, Park Y, Gomez R, Sinskey AJ (1973) Viability of *Staphylococcus aureus* in intermediate moisture meats. J Food Sci 38:1004

Prajapati JB, Ramachandran L, Dave JM (1986) Effect of added sugar on water activity and shelf-life of *khoa*. Asian J Dairy Res 5:25-29

Prior BA (1978) The effect of water activity on the growth and respiration of *Pseudomonas fluorescens*. Appl Bacteriol 44: 97-106

Rao KJ, Dresel J, Leistner L (1992) Application of hurdle technology in developing countries considering *paneer* as an example. Mitteilingsblatt-der-Bundesanstalt fur. Fleischforschung Kutmach, Germany 31:117, 293-297

Rao KJ (1993) Application of hurdle technology in the development of long-life *paneer*-based convenience food. Ph.D. Thesis, National Dairy Research Institute, Karnal

Renner E (1983) Content of available lysine in heat treated milk products. In: Proceedings of Symposium on Role of Milk Proteins in Human Nutrition, Kiel, Germany

Resnik S, Chirife J (1979) Effect of moisture Cintent and temperature on some aspects of non-enzymatic browning in dehydrated apple. J Food Sci 44:601-605

Ringe ML, Love MH (1988) Kinetics of protein quality change in extruded cowpea - corn flour blend under varied steady-state storage conditions. J Food Sci 53:584-588

Roberts TA, Jarvis B, Rhodes AC (1976) Inhibition of growth of *Clostridium botulinum* by curing salts in pasteurised pork slurry. J Food Technol 11:25-40

Rockland LB (1969) Water activity and storage stability. Food Technol 23:1241-1246, 1248, 1251

Rockland LB, Beuchat LR (1987) Water activity Theory and Applications in Food. Marcel Dekker Inc, New York, USA

Rockland LB, Stewart GF (1981) Water activity: Influences on Food Quality. Academic Press Inc, New York, USA

Russo C (1994) Water activity of foods. How Credible is its measure today? Industrie-Alimentari (Italy) 33:505-521

Saltmarch M (1980) The influence of temperature, water activity and physico-chemical state of lactose on the kinetics of the Maillard reaction in spray-dried sweet whey powder stored under steady state and non-steady state storage conditions. Diss Abstr Inter B 41:879

Sawhney IK, Patil GR, Bikram Kumar, Jayaraj Rao K (1991) Evaluation of glycerol as a water activity modifier in *Khoa*. In: Proceedings of 27th Convention of ISAE, ICAR, New Delhi, Oct 9-11

Scott WJ (1957) Water relations of food spoilage microorganisms. Adv Food Res 7:83-127

Seow CC, Cheah PB (1985) Reactivity of sorbic acid and glycerol in non-enzymatic browning in liquid intermediate moisture model systems. Food Chem 18:71-80

Silveira ETF, Tardim DCP, Silveira NFA, Barbieri MK, Arima HK, Shirose I (1992) Hurdle technology applied to the production of canned poultry liver pate. In: Proceedings of 38th International Congress Meat Sci Technol, Clermont-Ferrand, France 4: 751-754

Simatos D, Multon JL (1985) Properties of Water in Foods. Martinus Nijhoff Publishers, Dordrecht, The Netherlands

Singh RRB (1991) Kinetic expression of certain deteriorative reactions in stored UHT milk. Ph.D. Thesis, National Dairy Research Institute, Karnal

Spahr U, Url B (1994) Behaviour of pathogenic bacteria in cheese - A synopsis of experimental data. Bull Inter Dairy Fed No 298:2-16

Sperber WH (1983) Influence of water activity on foodborne bacteria - A review. J Food Prot 46:142-150

Stoloff L (1978) Calibration of water activity measuring instruments and devices: Collaborative study. J Assoc Off Anal Chem 61:1166-1178

Streit K, Ruegg M, Blanc B (1979) The influence of water activity on the growth of lactic acid-propionic acid bacteria and the dependence on the solute added to the culture media. Milchwissenschaft 34:459-462

Stumbo CR (1973) Thermobacteriology in Food Processing. Academic Press, London

Summer SS, Sanders TM, Harmon MC, Scott VN, Bernard DT (1991) Heat resistance of *Salmonella typhimurium* and *Listeria monocytogenes* in sucrose solutions of various water activities. J Food Sci 56:1741-1743

Tokuoka K, Tshitoni T (1991) Minimum water activities for the growth of yeasts isolated from high sugar foods. J Gen Appl Microbiol 37:111-119

Troller JA (1979) Food spoilage by microorganisms tolerating low water activity environments. Food Technol 39:72-75

Troller JA, Christian JHB (1978) Water activity and Food. Academic Press, New York

Troller JA, Stinson JV (1981) Moisture requirement for growth and metabolite production of lactic acid bacteria. Appl Environ Microbiol 42:682-687

Urbanski GE, Wei LS, Nelson AI, Steinberg MD (1982) Effect of solutes on rheology of soy flour and its components. J Food Sci 47:792-795

Valik L, Gorner F, Zemanovic J (1995) The effect of water activity on the growth and metabolism of lactic-culturing cheese starter bacteria. Potravinarske Vedy 13:355-367

Vigo MS, Chirife J, Scorza OC, Catanco P, Bertoni MH, Sarrailh P (1981) Estudio sobre alimentos tradicipnales de humidad intermedia elaborados en la Argentina I Determinacion de actividad acuosa (a_w) pH humedad Y solidos solubles. Rev Agrog Y Technol de Alimentos (Spain) 21:91

Vishweshwaraiah L (1987) Studies on dehydration and deep freezing of *paneer*. J Food Sci Technol 24:95-96

Warmbier HC, Schnickels RA, Labuza TP (1976) Effect of glycerol on non-enzymatic browning in a solid intermediate moisture model food system. J Food Sci 41:528-531

Webster SN, Fowler DR, Cooke RD (1985) Control of a range of food related microorganisms by a multi-parameter preservation system. J Food Technol 20:311-318

Wewala AR (1990) Manipulation of water activity: An important aspect of extending the shelf-life of whole milk powder. In: Report 1990, New Zealand Dairy Research Inst, Palmerston North, New Zealand

Wojciechowski J, Matylla P (1994) Water activity in selected dairy products. Przeglad-Mleczarski (Poland) 2:38-40

Wolf JC, Thompson DR, Warthesen JJ, Reineccius GA (1981) Relative importance of food composition in free lysine and methionine losses during elevated temperature processing. J Food Sci 46:1074-1078

Woodroof JG, Luh BS (1975) Commercial Fruit Processing. AVI Publishing Co. Inc, Westport, Connecticut, USA

Ziegler GR, Rizvi SSH, Acton JC (1987) Relationship of water content to textural characteristics water activity and thermal conductivity of some commercial sausages. J Food Sci 52:901-905

Zwietering MH, Wijtzes T, De Wit JC, Van't Riet K (1992) A decision support system for prediction of the microbial spoilage in foods. J Food Prot 55:973-979

Received 11 April 1996; revised 5 March 1997; accepted 3 April 1997

Evaluation of New Grape Hybrids for Dehydration

I.N. DOREYAPPA GOWDA*, R. SINGH AND B.N.S. MURTHY
Indian Institute of Horticultural Research, Bangalore - 560 089, India.

Seventeen new grape hybrids, consisting of seven yellow seedless, five black seedless or soft seeded and five seeded grapes, were compared with 'Thompson Seedless' and 'Arkavati' to test their suitability for dehydration. All the seedless hybrids had thin skin, except 'E-31/4', 'E-30/4', 'E-24/2' and 'Thompson Seedless' varieties. The total soluble solids were higher in all the new seedless hybrids, except 'E-31/4' and 'E-30/4', than those in 'Thompson Seedless' variety. All the hybrids had distinctly lower acidity than 'Arkavati' and 'Thompson Seedless', except 'E-31/4', 'E-24/2' and it was lower than 0.5% in 10 hybrids. The yield of dry product was better than 'Thompson Seedless' in all hybrids except 'E-30/4' and 'E-26/8. The sensory evaluation of the dry product revealed that hybrids 'E-29/5', 'E-12/3' and 'E-12/7' produced raisins superior to 'Thompson Seedless' and were comparable to 'Arkavati' with a distinctly lower acidity. 'E-31/5' was better than 'Thompson Seedless' only. Among currants, 'E-25/11' and in lexiors, 'E-26/8' and 'E-6/10' were better than the others. Storage studies also confirmed the above results.

Keywords : Grape hybrids, Physico-chemical characters, Dehydration, 'Thompson Seedless', Sensory quality.

A great breakthrough has been achieved in grape cultivation in India during the last 2-3 decades (Arve 1992). India is now fast emerging as a leading grape producing country and has already achieved a great success by producing world's finest grapes with productivity level, surpassing any other country (Arve 1992). The present needs of grape industry in India induce a compelling attempt to develop grape varieties exclusive for uses like table purpose, raisin, juice and wine making. Not much impact has been noticed due to several introductions made from abroad and their subsequent screening along with the indigenous ones, especially with regard to usage for dehydration (Singh et al. 1993). Research work carried out for the past two decades at the Indian Institute of Horticultural Research, Bangalore, resulted in the release of 'Arkavati' hybrid, which is very well suited for raisin making (Amba Dan et al. 1987). During the last few years, further hybridization work involving various inter-and intra specific crosses led to some more promising hybrids for dehydration purposes (Singh et al. 1993). In this study, 17 hybrid seedlings, both seedless and seeded, including black coloured hybrids were raised and evaluated to identify the suitable ones for dehydration. Similarly, comparison between certain clones of 'Thompson Seedless' (Peter and Marry 1994), 'Arkavati' (Amba Dan et al. 1987) and grapes grown under dry temperate conditions (Bhutani et al. 1980) has been reported.

Materials and Methods

Raw materials : Seventeen new grape hybrids consisting of seven yellow/green seedless, five black seedless or soft seeded and five seeded grapes were compared with 'Thompson seedless' and 'Arkavati'. Five kg grapes of each hybrid/variety, harvested during the main seasons of 1991-1993, from the vineyard of the Institute at the stage of commercial harvesting maturity, beyond which there was no further improvement in quality in terms of increases in TSS, were used for the study.

Physico-chemical parameters of grapes : Few bunches were selected randomly from each hybrid/variety for noting down colour of the berries, size, shape, skin thickness, flavour and presence or absence of seeds. Further, total soluble solids (TSS) in the juice of the berries were determined by a hand refractometer (Erma, 0-32 °Brix) at room temperature and necessary temperature corrections were applied. Acidity of juice was determined by AOAC (1984) method and expressed as tartaric acid.

Dehydration of grapes : The grape bunches were prepared for drying by removing diseased, damaged, immature or green berries and made into 2.5 kg. lots. Bunches of black/blue hybrids were dried as such in a tray drier (60-65°C),whereas each lot was tied in a cloth and dipped in a boiling NaOH solution of 0.3% for 3 sec, followed by cold water washing in case of all other varieties. Treated bunches were spread on perforated trays, kept in an air tight suphur box, fumigated by burning sulphur at the rate of 3 g/kg of fresh grape for 3h and dehydrated to optimum moisture in a tray drier (60-65°C). The dry grapes were separated from rachis, washed in lukewarm water, air-dried and kept for moisture equalization for a period of one month in an airtight container. The yields of raisins (dry seedless grapes), currants (dry black seedless grapes) and lexior (dry seeded grapes) were recorded.

* Corresponding Author

TABLE 1. DESCRIPTION AND SPECIAL CHARACTERISTICS OF NEW GRAPE HYBRIDS

Hybrid/variety	Parents	Yield/special characters
Raisins		
'E-29/5'	'Black Champa' X 'Thompson Seedless'	High yielder, vigorous vines, clusters medium and well-filled small to medium-sized seedless berries of golden yellow colour with pink blush
'E-31/5'	'Black Champa' X 'Thompson Seedless'	Very vigorous vines and good cropper, clusters medium sized, well-filled, golden yellow colour bold berries. Good cropper
'E-31/4'	'Black Champa' X 'Thompson Seedless'	Vigorous vines and very heavy cropper, large bunches, slightly compact, seedless to soft seeded berries of greenish yellow colour
'E-29/7'	'Black Champa' X 'Thompson Seedless'	Vigorous vines and prolific bearer, large bunches, well-filled, greenish yellow seeded to soft-seeded, slightly elongated berries
'E-11/29'	'Anab-e-shahi' X 'Thompson Seedless'	Moderately vigorous vines and prolific bearer, medium-sized bunches, cylindrical and well-filled, greenish yellow coloured seedless berries
'E-12/7'	'Anab-e-shahi' X 'Thompson Seedless'	Vigorous vines, medium to good cropper, large, winged bunches, golden yellow seedless berries
'E-12/3'	'Anab-e-shahi' X 'Thompson Seedless'	Moderately vigorous, good yielder, less compact bunches, medium sized, seedless berries with crispy pulp
Thompson Seedless'	Multipurpose grape variety also known as 'Sultana' in Australia.	Moderately vigorous, moderate yielder, well-filled, medium size elongated seedless berries of greenish yellow colour
'Arkavati'	Black Champa X Thompson Seedless	Clusters medium in size, yellowish green, ellipsoidal to spherical sweet seedless berries
Currants		
'E-25/11'	'Bangalore Blue' X 'Thompson Seedless'	Medium cropper, bunches small to medium, deep-red, round, seedless berries
'E-29/3'	'Black Champa' X 'Thompson Seedless'	Moderately vigorous vines, very heavy yielder, suitable for double cropping, deep red, seedless to soft-seeded berries
'E-32/8'	'Black Champa' X 'Thompson Seedless	Moderately vigorous, medium cropper, clusters small to medium -sized, well-filled, berries medium-sized seedless, dark tan coloured
'E-30/4'	'Black Champa' X 'Thompson Seedless'	Moderately vigorous vines, prolific bearer, long and cylindrical bunches, deep red, seedless berries
'E-24/2'	'Bangalore Blue' X 'Thompson Seedless'	Moderately vigorous vines, moderate yielder, clusters are medium-sized seedless and well-filled berries
Lexior		
'E-26/8'	'Angur Kalan' X 'Black Champa'	Moderately vigorous vines, prolific bearer, large-sized well-filled bunches, bold, greenish yellow, round berries
'E-7/12'	'Anab-e-shahi' X 'Convert Large Black'	Moderately vigorous vines, medium to high yielder, greenish yellow, slightly elongated berries
'E-6/10'	'Anab-e-shahi' X 'Black Champa'	Moderately vigorous, medium cropper, medium, well-filled bunches, elongated, golden yellow coloured berries with crispy, pulp and distinct muscat flavour
'E-18/10'	'Bangalore Blue' X 'Beauty Seedless'	Moderately vigorous vines, high yielder, clusters are medium and well-filled, black-coloured seeded berries
'E-2/7'	'Angur Kalan' X 'Convert Large Black'	Vigorous vines, good yielder, clusters are medium-sized, seeded black coloured berries with distinct flavour

Physico-chemical composition of dry product : The dried samples of raisins, currants and lexiors were analysed for moisture, acidity, reducing and total sugars according to AOAC (1984). Sulphur dioxide (SO_2) was determined according to the method described by Monier and Shipton (1960), whereas non-enzymatic browning (NEB) was estimated by the method of Handel et al (1950).

Storage studies : The dried samples were packed in 400 gauge polyethylene bags, stored at refrigerated temperature (3-5°C) in duplicate for a period of one year and analysed again for the above mentioned parameters.

Sensory quality : Fresh and stored raisins were assessed by a panel of 10 judges using Hedonic scale having 30, 30 and 40 marks for colour, texture and flavour, respectively. All the data were analysed statistically, using randomised block design (RBD) with two replications and means were compared either at a probability level of 1% or 5% (Sundararaj et al. 1972).

Results and Discussion

Physical characters : A brief description such as parents, yield and special characters of new hybrids and varieties is given in Table 1. The physical characteristics such as colour, berry size,

TABLE 2. PHYSICO-CHEMICAL CHARACTERISTICS OF DIFFERENT GRAPE HYBRIDS

Hybrid/variety	Colour	Berry size	Shape	Seeds	Skin	Flavour	TSS, °Brix	Acidity, %	Raisin yield, %
Raisins									
'E-29/5'	Y-G	S	R-O	A	Thin	G	23.6	0.45	24.0
'E-31/5'	Y-G	B	O	A	Thin	G	22.6	0.48	23.3
'E-31/4'	Y	M	O	A	Thick	G	20.0	0.71	19.7
'E-29/7'	Y	M	O	A	Thin	G	22.3	0.62	22.5
'E-11/29'	Y	B	O	A	Thin	G	21.2	0.46	21.0
'E-12/7'	Y	S	O	A	Thin	G	23.3	0.52	23.7
'E-12/3'	Y	M-S	O-R	A	Thin	G	23.0	0.48	23.0
'Thompson Seedless'	Y-G	B	O	A	Thick	G	20.7	0.67	19.7
'Arkavati'	Y-G	S	O-R	A	Thin	G	23.0	0.72	23.0
Currants									
'E-25/11'	B	M-B	R-O	A-SS	Thin	G	23.4	0.56	22.7
'E-29/3'	B	S	O-R	A	Thin	G	22.5	0.50	21.5
'E-32/8'	B	S	O-R	SS-A	Thin	M	21.2	0.48	20.8
'E-30/4'	B	S	R-O	A	Thin-Thick	G	20.0	0.39	19.5
'E-24/2'	B	S	R-O	A	Thick	VG	22.5	0.70	22.2
Lexiors									
'E-26/8'	Y	B	O	P	Thin	VG	18.9	0.39	19.5
'E-7/12'	Y	B	O	P	Thick	M	20.0	0.42	20.5
'E-6/10'	Y	B	O	P	Thick	G	20.9	0.37	20.2
'E-18/10'	B	S	R	P	Thick	M	22.0	0.40	22.2
'E-2/7'	B	B	O	P	Thick	G	20.0	0.61	20.3
SEM ±							0.62	0.03	0.89
CD at 5%							1.77	0.09	2.55
CD at 1%							2.38	0.12	3.75

Note: Y-Yellow, G-Green, B-Black, S-Small, B-Big, M-Medium, R-Round, O-Oval, A-Absent, SS-Soft seeded, VG-Very good, P-Present, M-Moderate

TSS-Total Soluble Solids

shape, presence or absence of seeds and flavour of grape berries are presented in Table 2. Out of 17 new hybrids and two controls, 9 were yellow to green seedless, 3 were black seedless, 2 were black and soft seeded, 3 were yellow seeded and 2 were black seeded. Among seedless, all had small to medium sized berries, except 'Thompson Seed-less' and 'E-31/5'. Most of the hybrids had an oval-shaped berries except 'E-18/10', 'E-24/2', 'E-30/4' and 'E-25/11'. The berry skin was thin in all seedless hybrids, except 'E-31/4','Thompson Seedless' and 'E-24/2, while it was moderate in 'E-30/4'. The flavour of fresh berries was judged to be very good in case of 'E-24/2' and 'E-26/8', moderate in 'E-32/8','E-7/12' and 'E-18/10'. In the rest of the hybrids/varieties, the flavour was good.

Chemical composition of berries : The TSS was higher than 'Thompson Seedless' in all the seedless grapes except 'E-31/4' and 'E-30/4' (Table 2). All the hybrids had distinctly lower acidity than 'Arkavati' and 'Thompson Seedless' except 'E-29/7', 'E-24/2', 'E-31/4' and 'E-2/7', while it was lower than 0.5% in 'E-29/5', 'E-31/5', 'E-11/29', 'E-12/3', 'E-32/8', 'E-30/4', 'E-26/8', 'E-7/12', 'E-6/10' and 'E-18/10'. Among the seeded hybrids, only 'E-18/10' had higher TSS.

Yield of dehydrated product : The raisins yield was better than 'Thompson Seedless' in all the hybyrids except 'E-31/4' and 'E-11/29' (Table 2). The yields of 'E-29/5', 'E-12/7', 'E-31/5' and 'E-12/3' were comparable with those of 'Arkavati'. 'E-25/11' and 'E-24/2' (currants) and 'E-2/7'. 'E-18/10' lexiors also gave better yield. The higher yield in these hybrids was attributed to higher TSS of fresh berries.

Physico-chemical composition of dry products: Significant differences were observed in acidity, non-enzymatic browning, moisture and total sugars (Table 3). The moisture contents of dehydrated products varied from 14 to 15% and significantly different only at a probability level of 5%. The acidity values of all the raisin samples were lower than those of 'Thompson Seedless' and 'Arkavati', except 'E-31/4'. Among currants, lower acidity was

TABLE 3. PHYSICO-CHEMICAL COMPOSITION AND SENSORY QUALITY OF RAISINS, CURRANTS AND LEXIORS OF DIFFERENT GRAPE HYBRIDS BEFORE STORAGE

Hybrid/variety	Moisture, %	Acidity, %	Reducing sugars, %	Total sugars, %	SO_2, ppm	NEB, OD at 420 nm	Sensory quality Colour 30	Texture 30	Flavour 40	Overall Total, 100
Raisins										
'E-29/5'	14.3	2.25	72.8	73.8	410	0.07	22	25	25	72
'E-31/5'	14.1	2.12	71.3	71.4	320	0.28	21	23	24	68
'E-31/4'	14.6	2.53	67.8	68.4	285	0.35	19	19	20	58
'E-29/7'	14.3	2.40	68.7	70.6	290	0.32	20	21	22	63
'E-11/29'	14.7	2.31	70.4	70.6	360	0.09	21	20	21	62
'E-12/7'	13.9	2.05	70.6	72.9	405	0.08	22	24	24	70
'E-12/3'	14.9	2.33	70.4	72.5	400	0.07	22	24	25	71
Thompson Seedless'	14.8	2.40	68.0	68.6	280	0.28	21	21	21	63
'Arkavati'	14.9	2.80	71.5	71.8	425	0.07	23	25	25	73
Currants										
'E-25/11'	14.3	2.05	71.7	72.8	-	0.38	20	23	21	64
'E-29/3'	14.2	2.00	71.0	71.2	-	1.02	21	20	21	62
'E-32/8'	14.1	2.20	68.0	68.7	-	1.07	22	18	20	60
'E-30/4'	14.0	1.92	67.5	69.4	-	0.48	19	21	21	61
'E-24/2'	15.0	2.62	71.0	71.3	-	0.59	18	22	19	59
Lexiors										
'E-26/8'	14.5	2.07	67.4	68.8	250	0.09	21	21	23	65
'E-7/12'	15.0	2.24	70.5	70.7	200	0.26	16	20	21	57
'E-6/10'	14.8	1.81	71.4	71.9	260	0.12	21	20	21	63
'E-18/10'	14.2	2.57	72.0	72.5	-	1.17	19	18	22	59
'E-2/7'	15.0	1.92	70.7	70.8	-	1.00	17	21	20	58
SEM ±	0.30	0.07	1.73	1.48	-	0.07	0.67	0.59	0.96	1.76
CD at 5%	0.86	0.21	NS	4.29	-	0.21	1.91	1.68	2.88	5.05
CD at 1%	NS	0.28	-	NS	-	0.27	2.56	2.26	3.84	6.78

observed in 'E-29/3', 'E-30/3' and 'E-32/8'. All lexiors possessed lower acidity, except 'E-18/10'. The lower acidity of dehydrated products of these hybrids can be attributed to the lower acidity of fresh grapes. The total sugar content was only marginally higher in raisins of hybrids 'E-29/5', 'E-31/5', 'E-12/7', 'E-12/3', currants 'E-25/11' and lexior 'E-18/10'. Though marked differences were observed in TSS of fresh grapes, non-occurrence of significant differences in reducing sugars and a very narrow differences in total sugar contents can be attributed mainly to removal of water by dehydration to uniform level of moisture, irrespective of sugar contents of fresh berries. The non-enzymatic browning of raisins was significantly lower than 'Thompson Seedless' in case of 'E-29/5', 'E-12/3', 'E-12/7' and 'E-11/29' and it was comparable to 'Arkavati'. The lower non-enzymatic browning of these hybrids was attributed mainly to thinner skin leading to better checking and infiltration of sulphur dioxide during lye treatments and sulphur fumigation, respectively. Though all the currants were made of thick or moderately thick- skinned berries, higher browning existed due to black colour of the berries. In lexior, low non-enzymatic browning was observed in 'E-26/8' and 'E-6/10'.

Sensory quality : The sensory evaluation of the products indicated that the raisins of 'E-12/3', 'E-29/5' and 'E-12/7' were superior to 'Thompson Seedless' and were comparable with 'Arkavati' (Table 3). The superiority of these hybrids was attributed to their better score for colour due to lower non-enzymatic browning, soft texture due to thin skin and good taste due to higher TSS and lower acidiy. Similarly, superiority of 'Arkavati' over 'Thompson Seedless' has been reported by Amba Dan et al (1987). Though 'E-31/5', had very good colour and flavour, it got slightly lower score for overall acceptability due to moderate texture and hence, was judged to be superior to 'Thompson Seedless' only. Among currants, 'E-25/11' and 'E-26/8' in addition to 'E-6/10' lexiors were superior to other currants and lexiors, respectively.

Storage studies : Storage studies conducted upto one year also confirmed the above results

TABLE 4. PHYSICO-CHEMICAL COMPOSITION AND SENSORY QUALITY OF RAISINS, CURRANTS AND LEXIORS OF DIFFERENT GRAPE HYBRIDS AFTER STORAGE

Hybrid/variety	Moisture, %	Acidity, %	Redusing sugars, %	Total sugars, %	SO_2, ppm	NEB, OD at 420 nm	Sensory quality: Colour 30	Texture 30	Flavour 40	Over all Total, 100
Raisins										
'E-29/5'	14.2	2.20	70.0	70.8	260	0.20	21	25	23	69
'E-31/5'	13.9	2.30	70.7	71.7	200	0.32	21	22	23	66
'E-31/4'	14.1	2.50	70.0	70.3	120	0.36	15	18	19	52
'E-29/7'	14.2	2.50	70.0	70.3	130	0.42	19	20	22	61
'E-11/29'	14.3	2.30	69.0	70.0	200	0.26	20	20	20	60
'E-12/7'	13.9	2.00	70.1	70.3	255	0.23	21	23	23	67
'E-12/3'	14.2	2.07	69.3	69.8	260	0.30	21	24	22	67
'Thompson Seedless'	14.3	2.30	69.0	69.5	150	0.46	20	20	20	60
'Arkavati'	13.9	2.76	70.7	70.8	250	0.28	23	23	24	70
Currants										
'E-25/11'	14.2	2.27	69.0	70.0	-	0.49	21	22	28	62
'E-29/3'	13.9	2.13	68.9	69.3	-	1.10	19	20	21	61
'E-32/8'	13.9	2.17	66.0	66.7	-	1.09	20	19	20	58
'E-30/4'	14.2	2.13	68.0	68.4	-	0.20	20	20	23	63
'E-24/2'	14.9	2.24	69.2	69.6	-	0.86	14	23	17	55
Lexiors										
'E-26/8'	14.5	2.23	68.0	68.4	160	0.20	20	20	23	63
'E-7/12'	14.7	2.17	69.7	70.0	110	0.38	15	20	21	55
'E-6/10'	14.2	2.00	70.0	70.2	150	0.24	20	21	21	60
'E-18/10'	14.0	2.58	70.0	70.7	-	1.40	14	25	19	58
'E-2/7'	14.8	1.96	70.1	70.3	-	1.23	13	22	19	58
SEM ±	0.43	0.06	1.25	1.28	-	0.08	0.86	1.04	1.08	1.75
CD at 5%	1.25	0.17	NS	NS	-	0.23	2.48	2.98	3.10	5.01
CD at 1%	NS	0.23	-	-	-	0.35	3.32	3.95	4.16	6.72

(Table 4). Though there was slight reduction in overall acceptability score at the end of storage period, it was attributed mainly to lower colour scores, due to increase in non-enzymatic browning and change in flavour due to variations in taste during storage. However, new hybrids 'E-29/5', 'E-12/3' and 'E-12/7', were found to be superior for raising making.

Acknowledgements

The authors are grateful to Dr. I.S. Yadav, Director, I.I.H.R., Bangalore for encouragement. Thanks are due to Mr. K. Reddeppa and Mrs. Sarojini Jalali for technical assistance.

References

Amba Dan, Adsule PG, Negi SS (1987) Evaluation of new grape cultivars for processing. J Food Sci Technol 24(4):194-196

AOAC (1984). Official Methods of Analysis, 14th edn. Association of Official Analytical Chemists, Washington, DC

Arve VR (1992) Grape industry in India: Export strategy and constraints. In: Souvenir, International Symposium on Recent Advances in Viticulture and Oenology. 14-17 Feb. Hyderabad, India, Andhra Pradesh Grape Growers Association, Hyderabad, pp 45-48

Bhutani VP, Negi CD, Chadha TR (1980) Studies on raisin making of grapes grown under dry temperate conditions. Punjab Hort J 20:203-207

Handel CE, Bailey GE, Taylor (1950) Measurement of non-enzymatic browning in dehydrated vegetables during storage. Food Technol 4:344-346

Monier W, Shipton D (1960) Estimation of sulphur dioxide. Laboratory Techniques in Food Analysis. Butterworth, London, pp 79-81

Peter CL, Marry LB (1994) Comparison of Thompson Seedless' clones for raisin production. Am J Vitic 45(2): 150-154

Singh R, Murthy BNS, Doreyappa Gowda IN (1993) Development of grape hybrids for dehydration. Paper presented at the Golden Jubilee Symposium on Horticultural Research, Changing Scenario, 24-28 May, Horticultural Society of India, Bangalore, pp 96-96

Sundararaj N, Nagaraja S, Venkata Ramu MN, Jagannath MK (1972) Design and Analysis of Field Experiments. University of Agricultural Sciences, Bangalore, India

Received 26 December 1995; revised 25 February 1997; accepted 27 February 1997

J. Food Sci. Technol., 1997, Vol 34, No. 4. 291-295

Comparative Properties of Rice Flakes Prepared Using Edge Runner and Roller Flaker

S. EKANAYAKA[1] AND H.V. NARASIMHA*

Department of Grain Science and Technology,
Central Food Technological Research Institute, Mysore -570 013, India.

Properties of rice flakes with varying physical properties (flake thickness and surface area) prepared by the traditional (edge runner) process and the newly developed continuous (roller flaking) process were studied. Moistness, tenderness and lumping nature were nearly similar for the flakes of similar physical properties of these two types of flakes. However, minor differences existed in lumping and stickiness of the reconstituted flakes of varying physical properties. Roller flakes were judged to be more moist, tender with a greater tendency for lumping, compared to the edge-runner flakes, which were rather more chewy. Expansion on deep-fat-frying was found to be more for roller flakes. Normal differences observed by the consumers between the two types of flakes could be attributed to the differences in the physical properties of the samples.

Keywords : Rice flakes, Properties, Edge runner, Roller flaker.

Beaten rice or flaked rice (also called *Poha, Aval, Avalakki*) is a popular whole rice product widely produced at cottage/small scale in India. About 5% of the paddy produced is converted to flakes in the country. Essentially, the process consists of soaking paddy in warm/hot water, draining, roasting with sand (in batches of about 2 kg lots) in a shallow iron pan (*Bhatti*) over a strong fire followed by flattening in an edge runner for 40-60 sec to obtain the desired thickness (Ananthachar et al. 1982).

A continuous process for flaking of rice has been developed at this Institute, where roasting of the soaked paddy is carried out in a continuous grain roaster (Shanmugam Pillai 1972), which was modified for the purpose (Narasimha et al. 1982). The roasted paddy is dehusked, polished and then flattened instantaneously, to the desired thickness in a roller flaker. Here, flattening of the grain is achieved instantaneously, whereas in the edge runner process, it is gradual and occurs over a period of 40 to 60 sec. The properties of flakes by the two systems were investigated and the results are reported in this paper.

Materials and Methods

Coarse paddy (Variety: 'Jaya'), which is commonly used for producing flakes, was purchased from the local market, stored in cold room and drawn as and when needed.

Preparation of flakes : Cleaned paddy was dumped into hot water at 70°C, left overnight (about 18-20 h) and drained. Soaked paddy was roasted in a *bhatti* in a commercial establishment (Ashoka Industries, Mysore) at an initial sand temperature of 220-230°C for 30-35 sec with a sand:paddy ratio of 4:1 (w/w) with continuous stirring in a roasting pan. Roasted paddy was sieved out and then held in open baskets for 3 min (tempering). Sand temperature at the end of roasting was 115-120°C. Half of the roasted paddy was flaked at the factory itself in the commercial edge runner to obtain flakes of 0.82, 1.03 and 1.29 mm thickness (E_1, E_2, E_3, respectively) by suitably controlling the flaking time. Other half of the roasted paddy was dehusked, polished and then flaked to 0.84, 0.93 and 1.22 mm thickness (R_1, R_2, R_3, respectively) in the roller flaker by adjusting clearance between the rolls. Flakes were also prepared by the commercial edge runner process (E) and in the continuous flaking process (R) as described by Narasimha et al (1982).

All samples were dried in the shade for 48 h and packed in polythene bags and stored in sealed tins in cold room (around 10°C) until further analysis. Length (l) and breadth (b) of flakes were determined as described by Bhattacharya et al (1972). Surface area of flakes was computed using the formula, π lb/4, assuming the flakes to be of elliptical shape. Thickness of 50 flakes was measured, using a Dial type thickness gauge (make: Mitutoyo, Japan) and values averaged.

Water uptake of flakes was determined by soaking the flakes in water at room temperature. Samples were drawn after soaking for 0, 2, 5, 8, 10, 15, 20, 25, 30 min. They were gently pressed between layers of filter paper and moisture was

* *Corresponding Author: Present address : [1]Division of Food Technology, P.O. Box 11 CARI, Gannoruwa, Peredenia, Sri Lanka*

determined at 105°C for 18 h in an oven (Indudhara Swamy et al. 1971). Flake samples were soaked in water for 24 h and the moisture was similarly determined and designated as equilibrium moisture (EMC-S).

Bulk density of flake samples was measured in an one litre volume bulk density apparatus (OHAUS, New York, PVI, USA,). Texture of such soaked flakes (at a moisture of 70% wb) was determined in a Chopin-INRA viscoelastograph with 250g load by placing three flakes on the anvil of the instrument in a triangular form and original thickness (a) and compressed thickness (b) were noted with the instrument settings of 08–35–04–20 sec for the plunger movement. Minimum of six replicates were done for each sample. Firmness of the sample was calculated as per Liagnelet and Feillet (1978).

Expansion of flakes after frying in oil : Twenty grams of flakes were fried in refined groundnut oil at about 170°C for 10 to 15 sec. Initial and final volumes were determined, using a graduated cylinder and their ratio computed as expansion ratio.

Damaged starch : Flakes were ground to semolina (–25+60 BSS) in a hammer mill and damaged starch in it was determined as per standard procedure (AACC 1969).

Organoleptic evaluation of rice flakes : Each sample was soaked exactly for a moisture content of 69-71%, as noted from the hydration curves, water drained out and flakes were transferred to white porcelain bowls for organoleptic evaluation for tenderness, lumping, moistness and chewing count (eating quality). Thinner flakes of edge-runner (E_1) and roller flaker (R_1) processes as well as commercial flake samples (E and R, which were not graded by thickness and surface area) were soaked in water at room temperature for 10 min. after which excess water was drained out and evaluated separately for stickiness and lumpiness alone by 10 trained panel members. The score card used is given in Table 1.

TABLE 1. SCORE CARD* FOR ORGANOLEPTIC EVALUATION OF RICE FLAKES

Score	Tenderness	Stickiness	Lumping	Moistness
1	Hard	Not sticky	Well separated	Very dry
3	Firm	Slightly sticky	Fairly separated (very few grains sticking together)	Rather dry
5	Moderate	Rather sticky	Partially separated (small lumps, occasional)	Slightly moist
7	Soft	Very sticky	Large lumps visible, individual grains not seen	Moist
9	Mashy	Like paste	Grains not seen (fully lumpy)	Very moist

* specially devised for the purpose

Results and Discussion

Flakes from edge-runner were long and narrow, while those from roller flaker were short but broad and occasionally circular (Fig. 1). These were perhaps due to differences in the orientation of grains in the two machines prior to compression. Grains tend to get gradually compressed randomly (both along length and breadth) in the edge-runner, while in the roller flaker, they tended to fall on to the rollers vertically and got compressed instantaneously. The edge-runner flakes appeared to have more damaged (frayed/cracked) edges compared to roller flakes. Materials from edges of flakes appeared to be lost in the edge-runner process, due to the gradual thinning, where drying and powdering of the grain occurred (Shankara et al. 1984).

Many properties of rice such as water uptake and its cooking quality depend on its physical properties (Indudhara Swamy et al. 1971). This may be true for flakes also. For instance, thickness and surface area of flakes may influence the rate and quantity of water absorbed by them. In order to study the basic differences between them, flakes of almost similar physical properties (Table 2) were prepared in the two systems. As could be seen from the data, the edge runner flakes (E_1, E_2 and E_3) were nearly similar to roller flakes (R_1, R_2 and R_3, respectively) in their physical properties like flake thickness and surface area for the corresponding samples. However, there were larger differences in the level of damaged starch. Roller-flaked samples always had higher levels of damaged starch.

Data on the progressive water uptake by the two types of flakes are depicted in Fig 2. It is seen that there was a steady rise of moisture upto 30% (wb) in the first few minutes for all flakes, irrespective of flake thickness. However, hydration rate dropped sharply at this stage (5 min and beyond) for thicker flakes. Thin flakes (E_1 and R_1) attained 70% moisture in less than 10 min. Thicker flakes (E_2, E_3 and R_2, R_3) needed much longer time (>20 min) for hydration and always had lower moisture at any given hydration time. Interestingly, some differences were observed in the water uptake behaviour of even similar samples of the two types (e.g., E_1 and R_1), though both types of flakes attained a moisture content of 70% within 10 min.

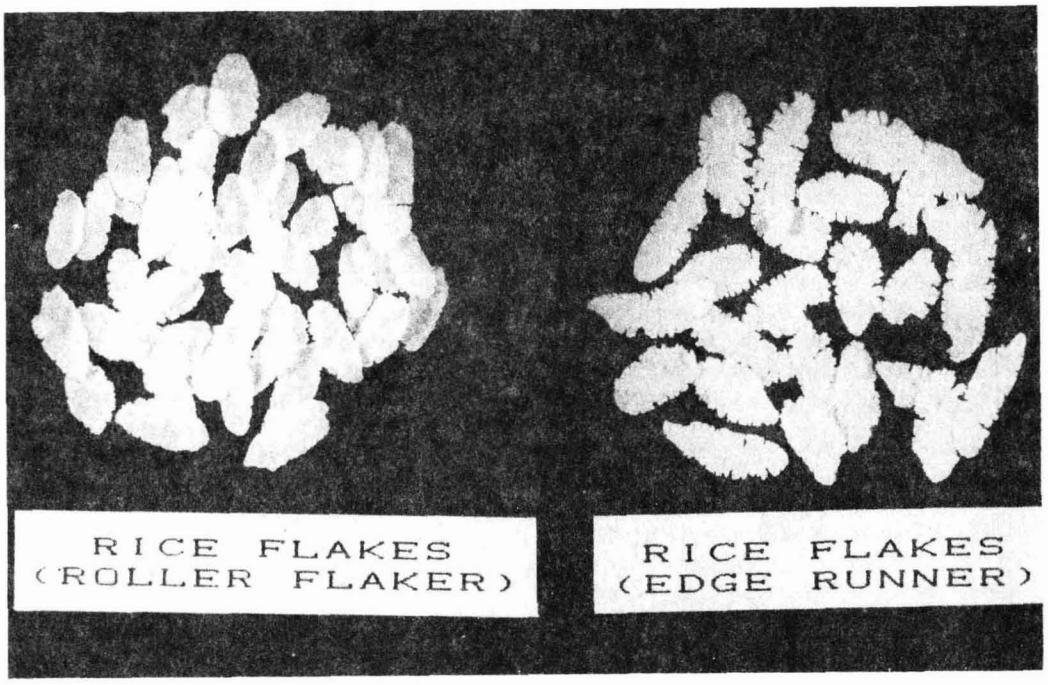

Fig. 1. Appearance of rice flakes from roller flaker and edge runner

These differences narrowed down, as the flake thickness increased, perhaps due to larger differences in the thinner flakes.

Data on the sensory qualities and expansion during deep-fat-frying are presented in Table 2. All flake samples, when rehydrated, tended to be more tender as thickness reduced. Thinner type edge runner samples (E_1) were much more tender or soft compared to the roller flakes of similar dimensions (R_1), as judged in terms of mouthfeel by the panel members. However, this was reversed in the firmness data obtained from the viscoelastograph. Tenderness score was inversely related to firmness value. Differences in the moistness values and tendency to lump formation were marginal, when the two types of flakes with similar physical properties were compared, though some panel members judged roller flakes as more lumpy. Chewing counts were almost identical for the two types of flakes, when samples of similar physical properties were compared. A plot of various properties against surface area presented in Fig 3,

TABLE 2. PROPERTIES OF FLAKES FROM EDGE RUNNER (E) AND ROLLER FLAKER (R)

Sample code	Length, mm	Breadth, mm	Thickness, mm	Surface area*, mm	Bulk density, g/L	Damaged starch, %	Equilibrium moisture content-soaking	Tenderness	Lumping	Moistness	Chewing count	%Firmness viscoelastogram	Expansion ratio	Stickiness
E_1	10.53	4.80	0.82	41.86	311	55.5	83.8	6.45	3.54	6.4	17	58.9	3.0	2.90
E_2	8.23	3.47	1.03	22.42	442	45.9	82.5	5.72	2.09	5.8	19	76.5	2.8	ND
E_3	6.73	3.07	1.19	16.23	638	29.5	79.4	2.60	1.36	2.6	30	85.3	2.2	ND
E	10.50	4.35	0.84 ±0.05	36.11	341	53.9	82.9	5.50	1.45	5.4	19	62.2	3.1	1.14
R_1	9.87	5.40	0.84	41.87	301	62.3	82.7	5.55	3.82	6.0	18	62.9	3.3	3.00
R_2	7.83	3.53	0.93	21.71	470	60.3	82.5	5.72	2.81	6.0	19	72.8	3.0	ND
R_3	7.40	3.43	1.22	19.93	652	35.7	80.5	3.36	1.81	4.0	29	83.2	2.1	ND
R	8.90	5.80	0.50 ± 0.10	41.43	256	72.4	76.1	8.10	6.64	7.8	14	57.1	3.6	6.54

* Surface area calculated as π lb/4 for an elliptical surface

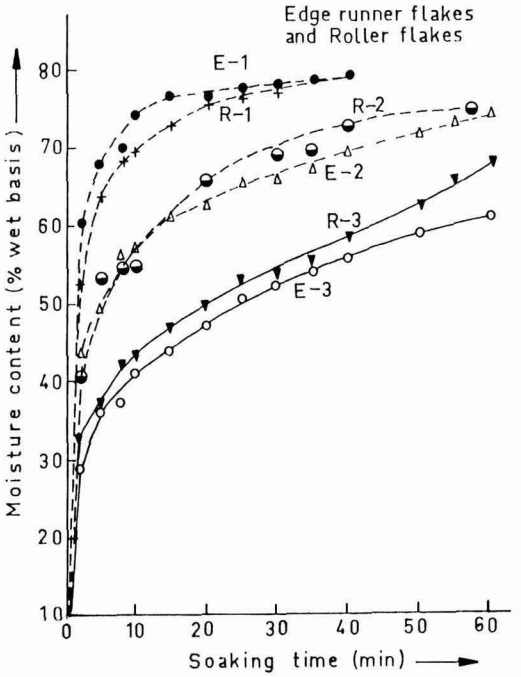

Fig. 2. Progressive water uptake during soaking of rice flakes
E_1. Flakes from edge runner (thin flakes)
E_2. Flakes from edge runner (thick flakes)
E_3. Flakes from roller flaker (thin flakes)
R_1. Flakes from roller flaker (thick flakes)
R_2. Flakes from roller flaker (medium flakes)
R_3. Flakes from roller flaker (thick flakes)

shows that paired samples like E_1-R_1; E_2-R_2; and E_3-R_3 behaved very similarly except for some marginal differences between E_3 and R_3, specially in moistness values, which may perhaps be due to larger differences between them in thickness and surface area. Among all the properties studied, moistness and lumping appeared to have closer relation to surface area. This brought out the observed differences between the two types of flakes. Roller flakes always had higher values for moistness and lumping, as compared to edge runner flakes. There appeared to be a direct relationship between these properties and surface area of flakes. Another property related to flake thickness and surface area was expansion on deep-fat-frying. Data presented in Table 2 indicate that the expansion was in the range of 2 to 3.3 times for different flake samples and was inversely related to the thickness of flakes. Roller flakes had somewhat higher volume expansion.

These results clearly showed that many

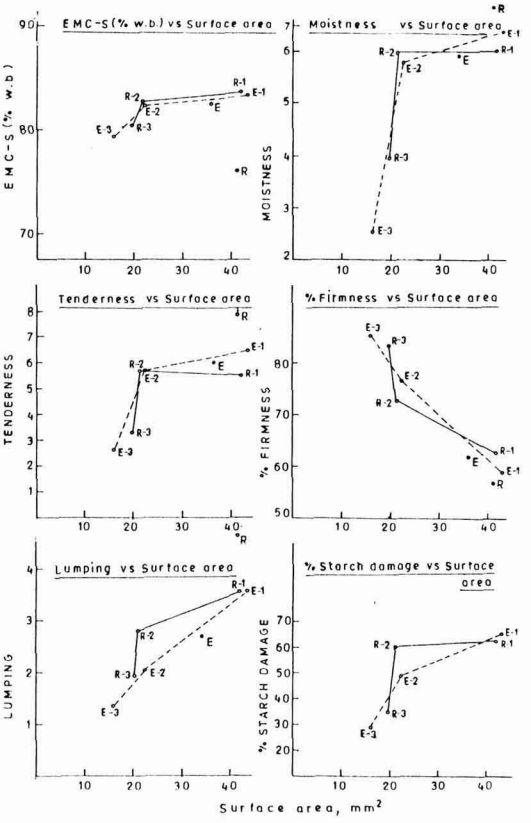

Fig. 3. Relationship of textural properties of the flakes to their surface area.
E-Flakes from commercial edge runner; R- Flakes from CFTRI continuous roller flaking process other symbols as in Fig 2

properties studied were very nearly similar for the two types of flakes prepared under carefully controlled conditions. Nevertheless, in practice, the roller flakes have been generally described by consumers to have a greater tendency for lumping and swell to a lesser extent than edge-runner flakes. To closely examine this point, flakes of the two types were prepared and tested without size gradation (E and R). Data on their physical and textural properties are also presented in Table 2. It is seen from these data that roller flakes (R) were thinner than edge-runner flakes (E) and variation was much smaller in the commercial edge runner sample (E), as compared to the roller flakes (R). The mean thickness value of E was around 0.84 ± 0.05 mm, while that of R was found to be about 0.50 ± 0.10 mm. Consequently, the surface area of the two flakes also varied, the values being 36.11 and 41.43 mm^2 for E and R, respectively (Table 2).

The equilibrium moisture content EMC of edge-runner flakes (both E and E_1) was about 83% as against around 82 and 76% for roller flakes R_1 and R, respectively (Table 2). It is rather unusual that commercial type roller flakes (R) had lower EMC, though these were thinner and had larger surface areas. This may be either due to lower severity of heat treatment in the roller flaking process (since sand roasting is carried out at initial temperatures of about 175°C and 230°C, respectively in the two processes) or due to variations in other processing steps. In the commercial edge runner process, flaking of paddy is done soon after roasting (after a holding time of just 3 to 4 min.), while there is a time gap of a few hours (about 4 to 6 h) between roasting and flaking in the roller flaking process. There may be some retrogradation of starch during this holding time, which could influence the water absorption capacity of the flakes (Ali and Bhattacharya 1976). Another significant observation on the reconstituted flakes was that roller flakes did not appreciably swell along their thickness, while the edge-runner flakes tended to regain their original grain shape. This could be due to their greater compactness or collapse of cell structure or even due to higher starch damage in the roller flakes (compare data for samples E, E_1 and R, R_1).

Organoleptic evaluation of the two types of flakes (E and R) indicated that rehydrated roller flakes (R) tended to be more moist and lumpy compared to the edge-runner (E) flakes (Table 2). Differences between them were quite appreciable. This was also reflected in their eating quality (chewing count) and instrumental firmness values. Both these parameters were lower for roller flakes compared to the edge runner flakes, indicating roller flakes (R) had smooth mouthfeel. Roller flakes also had higher expansion ratio on deep-frying compared to edge runner flakes.

It is concluded that the observed differences in properties of commercial roller flakes in various properties were pronounced more due to non-uniformity of the sample. This appeared to enlarge also due to the differences in the method of their production. This was clearly brought out, when samples E-R and E_1-R_1 were compared. If the properties (thickness and surface area) of the two types of flakes are kept similar (as in the sets of E_1-R_1, E_2-R_2 and E_3-R_3), they tend to behave more closely. However, in practice, it is difficult to achieve this (E and R). Other methods of reducing moistness, pastiness/lumping need to be worked out to popularize roller flakes. The roller flakes, however, had improved volume expansion on deep-fat-frying compared to edge runner flakes and had better organoleptic acceptability in the fried form.

References

Ali SZ, Bhattacharya KR (1976) Comparative properties of beaten rice and parboiled rice. Lebensm-Wiss. u. Technol. 9:11-13

AACC (1969) American Association of Cereal Chemists, Cereal Laboratory Methods, St. Paul Minnesota, USA 76:30A;1-2

Ananthachar TK, Narasimha HV, Shankara R, Gopal MS, Desikachar HSR (1982) Improvement of traditional process for rice flakes. J Food Sci Technol 19:47-51

Bhattacharya KR, Sowbhagya CM, Indhudhara Swamy YM (1972) Some physical properties of paddy and rice and their interrelations. J Sci Food Agri 23:171-175

Indhudhara Swamy YM, Ali SZ, Bhattacharya KR (1971) Hydration of raw and parboiled rice and paddy at room temperature. J Food Sci Technol 8(1):20-24

Liagnelet B, Feillet (1978) Use of viscoelastograph for measuring the texture of cooked rice; In: Chemical Aspects of Rice Grain Quality, International Rice Research Institute, Los Banos, Lagunna, Philippines, pp 355-361

Narasimha HV, Ananthachar TK, Shankara R, Gopal MS, Desikachar HSR (1982) Development of a continuous process for making rice flakes. J Food Sci Technol 19:233-236

Shankara R, Ananthachar TK, Narasimha HV, Krishnamurthy H, Desikachar HSR (1984) Improvements to the traditional edge runner process for rice flake production. J Food Sci Technol 21:121-123

Shanmugam Pillai (1972) Gram roasting machine, Indian patent No. 67546-1963 and 77726-1963, Patents Office, Government of India, Calcutta, India

Received 6 June 1996; revised 17 March 1997; accepted 19 March 1997

J. Food Sci. Technol.,1997, Vol 34, No 4. 296-302

In vitro Enzymic Oxidation of Apple Phenols

K.L. BAJAJ[1]*, C.A. DIEZ DE BETHENCOURT[2], B. JUNQUERA[2] AND M.L. GONZALEZ-SAN JOSE[2]

[1]Department of Biochemistry, Punjab Agricultural University, Ludhiana -141 004, India.
[2]Instituto de Fermentaciones Industriales C.S.I.C. Juan de la Cierva, 3, 28006 - Madrid, Spain.

4-methylcatechol and (-) epicatechin have been found to be the most important phenols, contributing towards browning due to their synergic effect in enzymic oxidation of the other phenols of apples. Initially, chlorogenic acid and caffeic acid play a role towards browning, but in later stages, (-) epicatechin contributes towards browning significantly. These catalyse the enzymic oxidation of procyanidin B_2, which is a non-substrate for polyphenol oxidase and also catalyse the hydroxylation of phloretin, to form hydroxyphloretin, which is further enzymatically oxidised. 2,4-dihydroxybenzoic acid has been found to be an effective inhibitor of polyphenol oxidase-mediated oxidation of (-) epicatechin, whereas p-coumaric acid inhibits oxidation of (-) epicatechin, 4-methylcatechol and chlorogenic acid.

Keywords : Apple, Polyphenol oxidase, Mushroom tyrosinase, Phenolic compounds, *In vitro* oxidation, Browning reaction.

The principal phenolic constituents of apple are known to be pyrocatechol, D (+) catechin, procyanidin B_2, (-) epicatechin, 4-methylcatechol, phloridzin, chlorogenic acid, naringin and rutin, the latter two being mainly concentrated in peel (Coseteng and Lee 1987). Of these phenolic compounds, the major ones are chlorogenic acid, phloridzin, (-) epicatechin and procyanidin B_2 (Robertson 1983). Apple fruit and their juice are susceptible to enzymic browning and this is a major problem encountered during processing. Both the concentration of phenolic compounds and activity of the enzyme polyphenol oxidase are considered to contribute towards browning of apple fruit and juice (Prabha and Patwardhan 1985, a,b). The aim of the present study was to investigate the interaction of different phenols during their enzymic oxidation for their contribution towards browning reaction in view of different reports available in literature (Coseteng and Lee 1987; Harel et al. 1966; Weurman and Swain 1955). This has become more important in view of recent reports of synergic effect of (-) epicatechin in polyphenol oxidase; mediated oxidation of other phenolic compounds (Bajaj et al. 1987; Robertson 1983).

Materials and Methods

Enzymic oxidation of 14 phenolic compounds, viz., D (+) catechin, (-) epicatechin, procyanidin B_2, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, naringin, pyrocatechol, phloretin, (-) epigallocatechin, (-) epigallocatechin, (-) epigallocatechin gallate and (-) epicatechin gallate was studied. The concentration of the compounds studied was chosen as present in apple (variety: 'Golden Delicious') juice, assuming 66% juice recovery and complete extraction of phenols (Cosetang and Lee 1987; Mosel and Herrman 1974) (Table 1). Enzymic oxidation was carried out both with tyrosinase (Sigma Chemical Co. St. Louis, Mo.) and polyphenol oxidase isolated from apples by the procedure as described by Goodenough et al (1983) with the modification that enzyme after ammonium sulphate precipitation was desalted, using G-25 Sephadex^R column, freeze-dried and dissolved, in citrate-phosphate buffer (pH 4.0) and used as such without further purification.

The enzyme activity was determined as described by Sciancalepore and Longone (1984). For enzymic oxidation, 0.1 ml of enzyme extract, containing 2.8×10^4 units, as defined by Coseteng and Lee (1987), was added to 2.4 ml of the reaction mixture, containing the substrate and 2.35ml 0.1m of citrate phosphate buffer (pH 4.0). In case of two substrates, the quantity of the buffer was accordingly adjusted. Incubation was done for different time intervals at 30°C. The development of the browning of the extract was measured by the increase in absorbance at 420 nm in Beckman DU-70 U.V. visible spectrophotometer.

Regression analysis : The model chosen to explain the contribution towards browning of different phenolic compounds, was a polynomial regression model with an independent variable. The hypothesis of this model is:

$$Y_1 = B_0 + B_1T + B_2T^2 + B_3T^3 + ... + B_pT^p + u$$

where Y_1 are the individual phenolic compounds; T is time, independent variable; B_1 are the regression coefficients and u are the residuals.

* *Corresponding Author*

TABLE 1. EFFECT OF ADDITION OF (-) EPICATECHIN/CHLOROGENIC ACID TO DIFFERENT PHENOLS OF ENZYMIC BROWNING

Phenol	Conc, mM	Mushroom tyrosine: % change in absorbance by addition of (-) epicatechin	Mushroom tyrosine: % change in absorbance by addition of chlorogenic acid	Apple polyphenol oxidase: % change in absorbance by addition of (-) epicatechin	Apple polyphenol oxidase: % change in absorbance by addition of chlorogenic acid
Phloridzin	0.03	+14.1	+14.3	+10.7	+1.5
(-) Epigallocatechin	0.01	+21.9	-4.5	-15.3	-20.0
(-) Epigallocatechin gallate	0.02	+10.6	-6.8	-33.8	-17.4
(-) Epicatechin gallate	0.01	-	-6.7	-17.4	+1.5
Phloretin	0.03	+30.1	+4.3	+36.6	+14.5
Pyrocatechol	0.04	-	-35.3	+10.6	-14.1
(+) Catechin	0.03	-13.5	-28.8	-5.9	-20.6
Naringin	0.01	-	-12.2	-	-13.7
Chlorogenic acid/ Epicatechin	0.33/0.18	+22.2	+23.3	+37.3	+36.3
Caffeic acid	0.85	-7.8	-22.5	-5.8	-28.0
4-Methylcatechol	0.21	+33.7	-12.5	+55.2	-15.2
p-Coumaric acid	0.17	-38.5	-37.5	-54.0	-52.7
Ferulic acid	0.04	-10.1	-18.6	-12.7	-17.6

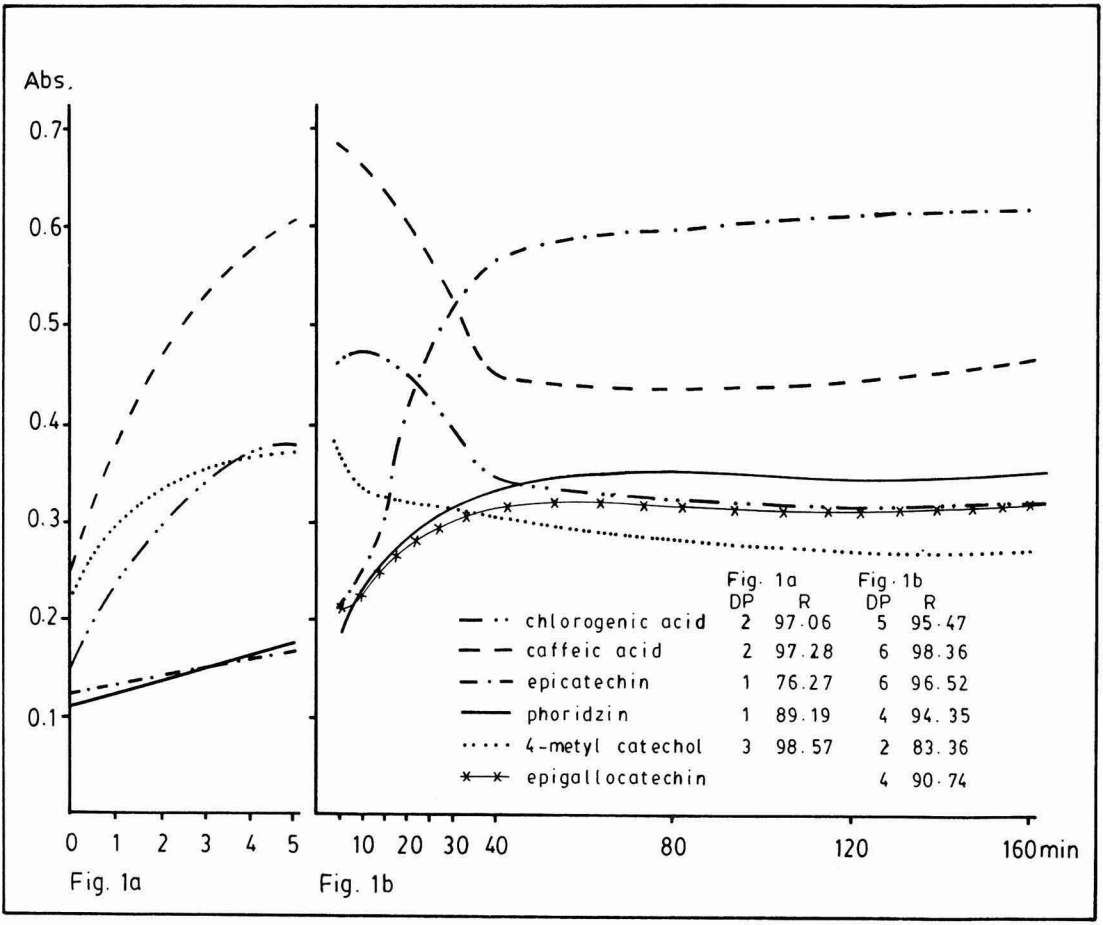

Fig. 1. Mushroom tyrosinase catalysed oxidation of major phenols of apple in initial stage of reaction (Fig. 1a) and in later stages of reaction (Fig. 1b). DP = polynomial degree, R= determination coefficient

Programme 5R of the BMDP statistic pack (Jennich and Mundie 1987) was used for the estimation of the regression coefficients B1 and the analysis of the residuals, with a CYBER 855 CDC computer. The polynomial degree (DP) was effected with a goodness-of-fit test by calculating statistic F, quotient between the variance explained by each DP and the variance of the residuals. A high value for F indicated a poor fit and the need to successively increase a DP, until an acceptable value was reached, corresponding to a tail probability of 0.05.

Results and Discussion

Enzymic studies with mushroom tyrosinase : Oxidation studies with individual phenolic compounds showed that p-coumaric acid, ferulic acid, phloridzin, naringin and procyanidin B_2 were not substrates for tyrosinase and there was a little contribution towards browning by (–) epigallocatechin and its gallate. However, there was some contribution towards browning by D (+) catechin and pyrocatechol.

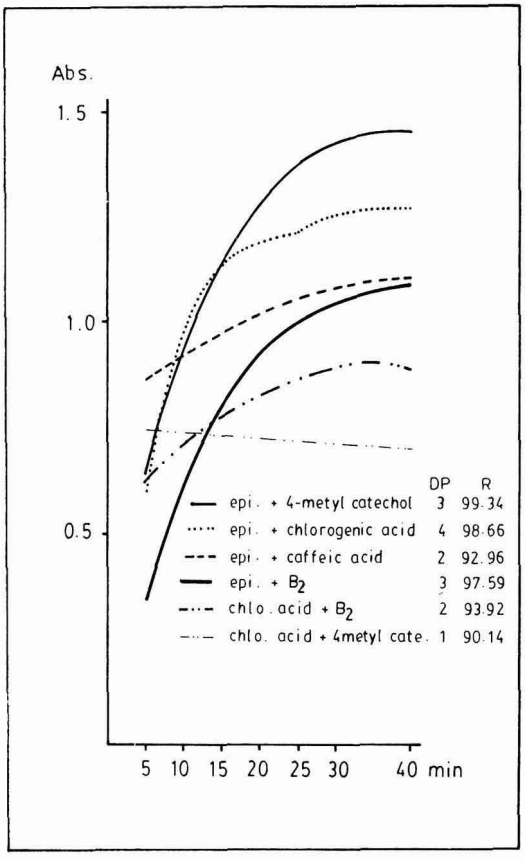

Fig. 2. Interaction of different phenols of apple for their contribution towards browning catalyzed by mushroom tyrosinase. DP= polynomial degree, R=determination coefficient, epi = (–) epicatechin, chlo= chlorogenic acid

Fig 1a and 1b refer to two separate experiments. The experiment as detailed in Fig 1b was carried out first, as the reaction was fast at 5 min. During the first 5 min of enzymic reaction, the contribution towards browning by caffeic acid, 4-methylcatechol and chlorogenic acid increased remarkably, whereas there was a little increase in browning by (–) epicatechin and phloretin (Fig. 1).

It is of interest to note that during the first 40 min of their oxidation, the contribution towards browning by oxidation of caffeic acid, chlorogenic acid and 4-methylcatehol decreased with time, whereas those of (–) epicatechin and phloretin progressively increased. There was practically no variation in absorbance during the period of 40 to 160 min. The contribution towards browning, was mainly by the oxidation of (–) epicatechin, caffeic acid, phloretin, chlorogenic acid, (–) epicatechin gallate and 4-methylcatechol.

Enzymic oxidation of phenolic compounds in combination with (–) epicatechin/chlorogenic acid : Data given in Table 1 show the effect of addition of (–) epicatechin and chlorogenic acid to different phenolic compounds and their contribution towards enzymic browning. The results show that by addition of (–) epicatechin, the contribution towards browning was increased in case of (–) epigallocatechin gallate, phloridzin, (–) epigallocatechin, chlorogenic acid and 4-methylcatechol in the increasing order, whereas the browning was inhibited partially in case of D (+) catechin, ferulic acid and p-coumaric acid. In case of addition of chlorogenic acid, in most cases, there was decrease in browning except in case of phloridzin, phloretin and (–) epicatechin. The maximum inhibition of browning was observed in case of p-coumaric acid, followed by pyrocatechol, D (+) catechin, caffeic acid, ferulic acid and 4-methylcatechol.

Time-course studies on the interaction of different phenolic compounds during enzymic oxidation: The results (Fig. 2) show that (–) epicatechin is the most efficient in increasing the browning compared to chlorogenic acid, particularly in case of their interaction with 4-methylcatechol.

After 10 min of reaction, (–) epicatechin had higher synergic effect in oxidation of chlorogenic acid than in case of caffeic acid. (–) epicatechin also catalysed the oxidation of procyanidin B_2 more than chlorogenic acid.

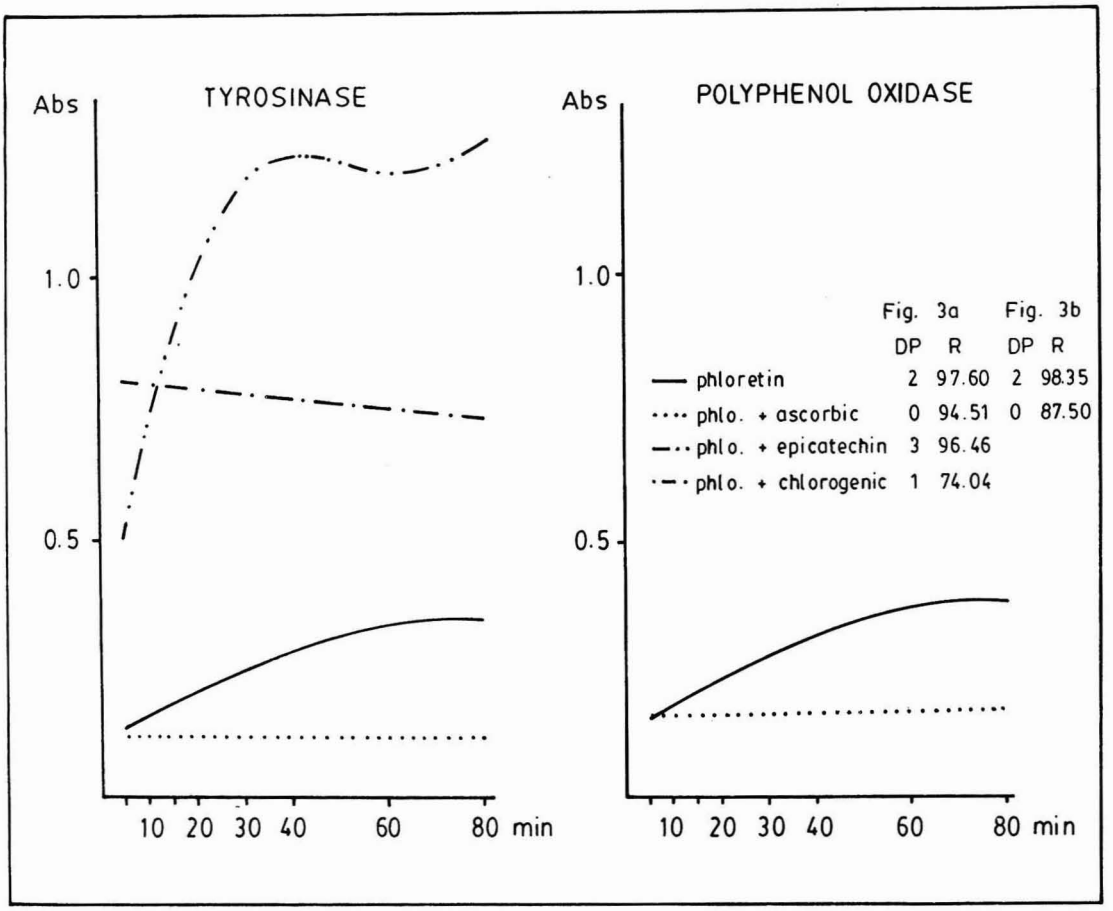

Fig. 3. Mushroom tyrosinase and apple polyphenol oxidase, catalysed hydroxylation and oxidation of phloretin (phlo). DP=polynomial degree, R=determination coefficient

Since phloretin is a monohydroxyphenolic compound, it is very likely that phloretin is first hydroxylated to 3-hydroxy phloretin, then oxidised to its quinone (Goodenough et al. 1983). Furthermore, in the presence of ascorbic acid, no browning reaction took place, thus further confirming that phloretin is oxidised via 3-hydroxy phloretin. In the present study, no lag phase in the oxidation of phloretin was observed, while Khan and Pomerantz (1980) reported lag phase in oxidation of phloretin in hydroxylation of monophenols in case of avocado polyphenol oxidase. Fig.3 shows that (–) epicatechin is more efficient than chlorogenic acid in increasing the browning in association with phloretin.

Studies with apple polyphenol oxidase : Apple polyphenol oxidase did not show any activity towards phloridzin, naringin, p-coumaric acid, ferulic acid and procyanidin B_2 and a very low activity towards (–) epigallocatechin and its gallate. However, Goodenough et al (1983) observed the enzymic oxidation of phloridzin. High activity was observed

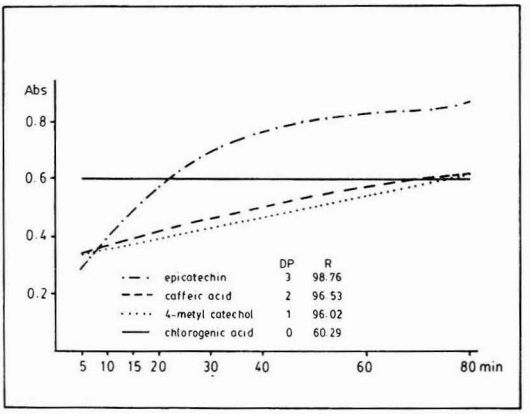

Fig. 4. Apple polyphenol oxidase, catalysed oxidation of major phenols of apples. DP=polynomial degree, R=determination coefficient

towards 4-methylcatechol, caffeic acid, chlorogenic acid and (–) epicatechin in increasing order after 40 min of incubation (Fig.4). Apple polyphenol oxidase showed high activity towards (–) epicatechin even in the initial stages of reaction (Fig. 1a), whereas mushroom tyrosinase showed poor activity towards it. After 5 min of incubation, the extent of browning by different substrates was as under: (–) epicatechin > 4-methylcatechol > caffeic acid > chlorogenic acid.

Initially, in case of apple polyphenol oxidase, the activity was remarkably high towards chlorogenic acid, as compared with other phenolic compounds. There was no change in the extent of browning with time in case of oxidation of 4-methylcatechol, caffeic acid and chlorogenic acid as observed in case of mushroom tyrosinase (Fig. 1b).

Apple polyphenol oxidase was also capable of oxidising phloretin, as observed in case of mushroom tyrosinase (Fig. 3). However, a lag period of 10 min was observed. Addition of ascorbic acid did not result in the formation of quinone.

Enzymic oxidation of phenolic compounds in combination with (–) epicatechin/chlorogenic acid: Data given in Table 1 show the effect of addition of (–) epicatechin on the enzymic oxidation of different phenols. The contribution towards enzymic browning was increased due to the addition of (–) epicatechin in case of pyrocatechol, phloridzin, phloretin, chlorogenic acid and 4-methylcatechol in the increasing order, whereas the browning was inhibited partially in case of ferulic acid, (–) epigallocatechin, (–) epicatechin gallate and p-coumaric acid. In case of addition of chlorogenic acid, in most cases, there was decrease in browning

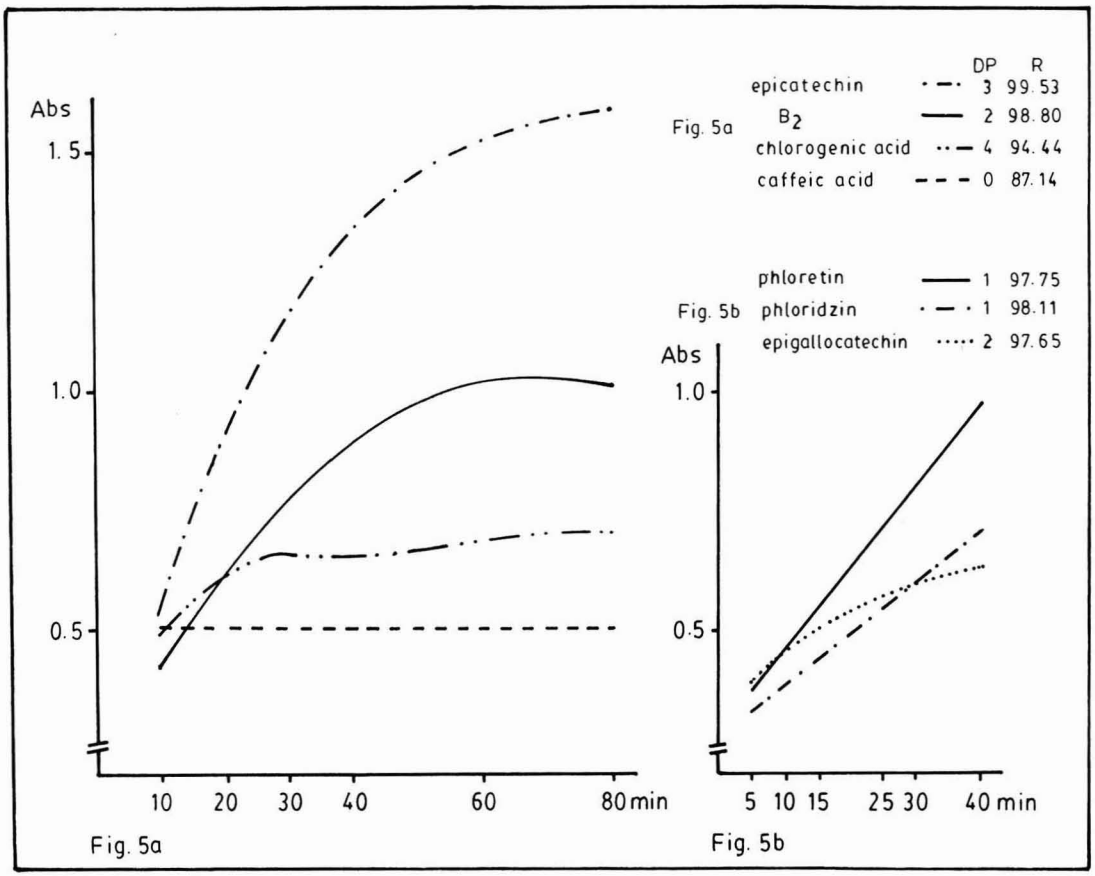

Fig. 5. Interaction fo different phenols of apple for their contribution towards browning catalysed by apple polyphenol oxidase (Fig. 5a and 5b). DP=polynomial degree, R=determination coefficient

except in case of phloretin and (-) epicatechin. The maximum inhibition was observed in case of p-coumaric acid, followed by caffeic acid, D (+) catechin, (-) epigallocatechin and its gallate ester.

Time-course studies on the interaction of different phenolic compounds during enzymic oxidation :

The results depicted in Fig.5a show that as compared to chlorogenic acid, addition of (-) epicatechin to 4-methylcatechol markedly increased the intensity of browning. Addition of 4-methylcatechol to procyanidin B_2 also markedly increased the intensity of browning. However, no marked increase was observed, when 4-methylcatechol was added to caffeic acid. Addition of 4-methylcatechol also had synergic effect on oxidation of (-) epicatechin gallate, phloridzin and phloretin in increasing order (Fig. 5b). Since 4-methylcatechol and (-) epicatechin are having low redox potential, these are rapidly oxidised to quinones, which act as electron acceptors to oxidise other phenolic compounds of higher redox potential. In these compounds, the simple substitution of a catechol with any electron supplying group lowers the oxidation-reduction potential and increase their susceptibility towards oxidation.

The present investigations, suggest that 4-methylcatechol and (-) epicatechin are highly effective in producing synergic effect as compared to chlorogenic acid in polyphenol oxidase-mediated oxidation of other phenols, as well as in catalysing the hydroxylation of monophenols to O-dihydroxy phenols, which are further oxidised to the corresponding quinones.

Procyanidin B_2, which is not a substrate of polyphenol oxidase, was more effectively oxidised in association with 4-methylcatechol/(-) epicatechin. This appears to be the first report of its kind.

Lea (1982) has reported that chlorogenic acid ≷ chlrogenoquinone redox shuttle, which is continuously regenerated, oxidises procyanidins. Keeping these facts in view, further experiments were conducted to inhibit the oxidation of 4-methylcatechol, (-) epicatechin, chlorogenic acid and caffeic acid.

Inhibition studies : Of various compounds tested viz., phloroglucinol carboxylic acid, 2,6-dihydroxy-benzoic acid, 2,4-dimethoxybenzaldehyde and 2,4-dihydroxybenzoic acid, the latter was found to inhibit effectively the enzymic oxidation of (-) epicatechin by apple polyphenol oxidase. Inhibition achieved with 4 mM concentration of 2,4-dihydroxy-benzoic acid was 70%, whereas 0.1 mM concentration of p-coumaric acid inhibited its oxidation by 74% (Table 2). None of the structural analogues of 4-methylcatechol tested viz., resorcinl, phenyl-ethyl, p-hydroxybenzyl alcohol, 2-isopropyl phenol could inhibit its oxidation.

TABLE 2. EFFECT OF DIFFERENT INHIBITORS ON OXIDATION OF MAJOR PHENOLS BY APPLE POLYPHENOL OXIDASE

	p-coumaric acid % inhibition			p-coumaric acid % inhibition			2,4-dihydroxy benzoic acid % inhibition
	(-) epicatechin	4-methyl-catechol		Chloro-genic acid	Caffeic acid		(-) epicatechin
mM			mM			mM	
0.1	74.0	47.0	1	66.3	36.0	1	50.1
0.2	74.0	64.3	2	66.3	51.6	2	62.0
0.3	74.0	62.3	3	66.0	51.3	3	66.3
0.4	74.0	62.3	4	66.3	55.0	4	69.6
0.5	74.0	62.3	5	66.3	61.0	5	70.0

However, p-coumaric acid (0.2 mM) was responsible to effect 62% inhibition (Table 2). p-coumaric acid (1 mM) inhibited the oxidation of chlorogenic acid by 67%, whereas only 36% inhibition of caffeic acid was observed at 1mM concentration of p-coumaric acid and 61% inhibition was observed at 5 mM concentration (Table 2). Walker and McCallion (1980) also reported that 0.1 mM concentration of p-coumaric acid could inhibit the O-diphenol oxidation.

It may be concluded that 4-methylcatechol, (-) epicatechin, chlorogenic acid and caffeic acid contribute significantly towards browning reaction in apples catalysed by polyphenol oxidase. 2,4-dihydroxybenzoic acid is an effective inhibitor of polyphenol oxidase-mediated oxidation of (-) epicatechin, whereas p-coumaric acid inhibits oxidation of (-) epicatechin, 4-methylcatechol and chlorogenic acid.

Acknowledgement

The authors thank Dr. C.Y Lea, USA for the gift of procyanidin B_2 and Dr. K. Ikegays, Japan for the gift of (-) epicatechin gallate and (-) epigallocatechin gallate used in the present investigation.

References

Bajaj KL, Anan T, Tushida T, Ikegays K (1987) Effects of (-) epicatechin on oxidation of the flavins by polyphenol oxidase from tea leaves. Agri Biol Chem 51:1767-1772

Coseteng MY, Lee, CY (1987) Changes in apple polyphenol oxidase and polyphenol concentrations in relation to degree of browning. J Food Sci 52:985-989

Goodenough PW, Kessell S, Lea AGH, Loeffler T (1983) Mono- and diphenolase activity from fruit of *Malus pumila*. Phytochem 22:359-363

Harel E, Mayer AM, Shain Y (1986) Catechol oxidases, endogenous substrates and browning in developing apples. J Sci Food Agric 17:389-391

Jennich R, Mundie J (1987) Polynomial Regression in Statistical Software. University California Press, Los Angeles

Kahn V, Pomerantz SH (1980) Monophenolase activity of avocado polyphenol oxidase. Phytochem 19:379-385

Lea AGH (1982) Reversed-phase high performance liquid chromatography of procyanidins and other phenolics in fresh and oxidising apple juices using pH shift technique. J Chromatogr 238:253-257

Mosel H, Hermann K (1974) Changes in catechins and hydroxycinamic acid derivatives during development of apples and pears. J Sci Food Agric 25:251-256

Prabha TN, Patwardhan MV (1985a) A comparison of the polyphenolic patterns in some Indian varieties of apples and their endogenous oxidation. I. J Food Sci Technol 22:404-407

Prabha TN, Patwardhan MV (1985b) A comparison of the browning potential of some Indian cultivars of ripening apples. II. J Food Sci Technol 22:431-433

Robertson A (1983) Effects of physical and chemical conditions on the *in vitro* oxidation of tea leaf catechins. Phytochem 22:889-896

Sciancalepore V, Longone V (1984) Polyphenol oxidase activity and browning in green olives. J Agric Food Chem 32:320-321

Walker JRL, McCallion RF (1980) The selective inhibition of ortho-and para-diphenol oxidases. Phytochem 19:373-377

Weurman C, Swain T (1955) Changes in the enzymic browning of Bramley's seedling apples during their development. J Sci Food Agric 6:186-192

Received 5 January 1996; revised 10 March 1997; accepted 19 March 1997

J. Food Sci. Technol.,1997, Vol 34, No 4. 303-310

Effect of Long Term Feeding of High Fat Diets on Growth, Plasma and Tissue Lipids in Rats

K. NARASIMHAMURTHY, P.L. RAINA*, AND K. HARIHARAN

Department of Biochemistry and Nutrition
Central Food Technological Research Institute Mysore - 570 013, India.

The influences of long term feeding of peanut oil (PNO) sesame oil (SO) and coconut oil (CNO) in synthetic diets at 5 and 20% levels on growth, plasma and tissue lipids were studied in rats. The growth rates of 20% fed animals were higher than the 5% fed animals. No significant differences were observed in the total plasma cholesterol levels in any of the groups, but significantly low levels of HDL-C and high levels of LDL-C + VLDL-C were observed in CNO groups. Liver cholesterol and phospholipid levels were significantly lower in CNO and SO groups. Plasma fatty acid analysis indicated higher levels of capric (10:0), palmitic (16:0) and stearic acid (18:0) in CNO groups compared to others. Analysis of erythrocyte fatty acids indicated high levels of arachidonic acid (20:4, n-6) in CNO groups, despite low levels of linoleic acid (18:2, n-6) in CNO. The arachidonic acid levels were higher in erythrocytes and were independent of the supply of linoleate. Adipose tissue fatty acid analysis indicated low levels of linoleate and high levels of saturated fatty acids in CNO group as compared to other groups. The ratios such as oleic/linoleic, oleic/stearic and linoleic/arachidonic acid were normal, indicating the type of fat consumed. Thus, the present study has shown that feeding higher levels of CNO can lead to an increase in the saturated fatty acids, LDL and VLDL, as compared to SO and PNO fed rats.

Keywords : High fat diets, Plasma lipids, Lipoproteins, Erythrocyte fatty acids, Plasma and adipose tissue fatty acids.

Earlier studies have consistently demonstrated that addition of adequate amounts of polyunsaturated fatty acids (PUFA) in the diets of humans and those of animals lowers plasma cholesterol levels (Kris-Etherton et al. 1988; Vega et al. 1982). However, the effect of polyunsaturated fatty acids (PUFAs) on cholesterol levels in rats is not clear, as various workers have reported an increase (Beynen 1987; Meijer et al. 1987), decrease (Kritchevsky et al. 1983; Zhang et al. 1990) and no effect (O' Brien et al. 1977; Alfin Slater 1967) and thus, the results are conflicting. On the other hand, many studies in a number of species including man have demonstrated that coconut oil (CNO) is more likely to raise blood cholesterol level, leading to atherogenicity (Kritchevsky et al. 1977; Mendes et al. 1989; Malmros and Wigard 1978). Sesame oil (SO) contains more of monounsaturated fatty acids such as oleic acid (18:1, n-9) and diunsaturated fatty acids such as linoleic (18:2 n-6) in equal proportions. Recent studies indicate that monounsaturated fatty acids are equally effective in lowering HDL-C, although less frequently than linoleate (Koh 1987). Also, sesame oil is widely consumed in India and still there is a lack of evidence on its effect upon various lipid parameters. On the other hand, coconut oil (CNO) has an unequal distribution of saturated and unsaturated fatty acids. Although some studies have been done on CNO and SO and its effect on cholestremia (Mattson and Grundy 1985; Mendis et al. 1989), still there is a paucity of data, regarding the effect of feeding at higher levels and also feeding for a longer duration, since these fats differ in their unsaturation levels. In the present study, the effect of long term feeding of SO and CNO in a synthetic diet as compared to widely consumed PNO as control is reported. It is expected that such a study would provide a clue to the mechanism, underlying the differential aspects of saturated (SAFA) and monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) on the cholesterol and lipid metabolism as well as the species dependent traits in rats.

Materials and Methods

Materials : Sesame oil (SO), peanut oil (PNO) and coconut oil (CNO) were purchased from the local market and were of edible grade. Casein was obtained from Kaira District Milk Producers Cooperative Union, Anand, Gujarat, India. Starch was purchased from Anil Starch Products Ltd., Ahmedabad, India. Methyl esters of fatty acids, cholesterol, phospholipids, triolein were all products of Sigma Chemical Co., St. Louis, Missouri, USA. Higher fatty acid methyl esters were all from Nu Chek Prep., Elysian, Minnesota, Mn, USA. Solvents and chemicals were of the highest purity obtained from Glaxo and were of AnalaR grade.

Experimental design and diets : CFT-Wistar strain male albino rats (8 numbers in each group weighing each around 57±5g) were used in these studies. They were randomly divided into 6 groups.

* *Corresponding Author*

The animals were kept in individual stainless steel cages and maintained in a room at 22±2°C at a relative humidity of about 60% and to a 12h light (07.0-19.00 h) and 12 h dark cycle (19.00-7.00 h). The diets were identical in all respects except the oil source and amount of starch. The diets consisted of casein 18%, sucrose 10%, salt mixture 2% (Hubbel et al. 1937), vitamin mixture 1.0% (Chapman et al. 1959). In addition to this, vitaminised oil containing vitamin A (20 IU/day/rat), vitamin D (10 IU/day/rat) and vitamin E (1 IU/day/rat) was added to these diets. The 5% groups, in addition, contained 5% of PNO or SO or CNO and 64% starch, whereas in the 20% oil groups, the fat was 20% with PNO or SO or CNO and 49% starch, respectively. Groups I and II were fed with 5% and 20% PNO diets, groups III and IV with 5 and 20% SO, and groups V and VI were fed with 5 and 20% CNO diets, respectively. The fatty acid composition of the various diets are given in Table 1. The animals were fed with these diets for a period of 18 weeks. These diets provided 1800 Joules (430 calories) and 2219 Joules (506 calories), respectively for 5 and 20% oil diets. Food and water were provided *ad libitum* and the animals were weighed once a week. At the end of 18 weeks, the animals were fasted overnight and sacrificed under mild diethyl ether anaesthesia. Blood was collected by cardiac puncture over heparin and plasma was separated and stored at -20°C, until use. Erythrocytes were washed and the lipids were extracted by the method of Rose and Ocklander (1965) and the total fatty acids were determined by the method of Clark et al (1992). The tissues such as liver, kidney, adipose tissue, brain and heart were removed, cleaned of adhering tissue impurities, weighed and stored at-20°C, until analysis.

TABLE 1. FATTY ACID COMPOSITION OF THE EXPERIMENTAL DIETS[a]

Fatty acids, %[b]	Group I and II (PNO diet)	Group III and IV (SO diet)	Group V and VI (CNO diet)
8:0	-	-	7.0
10:0	-	-	6.0
12:0	0.06	0.36	45.75
14:0	0.16	0.52	19.03
16:0	15.96	11.20	10.08
16:1	0.08	0.04	-
18:0	5.55	4.03	2.15
18:1	45.80	42.00	7.50
18:2	32.40	41.00	2.65
18:3	-	0.10	-
SAFA	21.73	16.11	90.00
MUFA	45.88	42.00	7.50
PUFA	32.40	41.10	2.65
Unsaturation index[c]	110.60	123.95	12.87
P/S ratio	1.48	2.54	0.14
Ratio 18:1/18:2	1.41	1.02	2.85

[a] The composition of the diet is given in the text

[b] Values expressed as % total fatty acids. Average of three values obtained by gas liquid chromatography

[c] Unsaturation index was calculated by summing up the percentage of individual fatty acids multiplied by the corresponding number of double bonds

Methods : The lipids were extracted from the plasma, adipose tissue and also from the diets by the method of Folch et al (1957). Plasma cholesterol was determined according to the method of Searcy and Bergquist (1960). Plasma and liver triglycerides were determined by the method of Fletcher (1968) and phospholipids by the method of Stewart (1980). Lipoproteins were determined by the method of Burnstein and Samaille (1960). Liver cholesterol was determined after homogenising the liver in 0.74% KCl and then processed for cholesterol and phospholipid determination. Plasma and erythrocyte lipids were methylated according to the method of Kates (1972). Methyl esters were analysed in a Packard gas chromatograph fitted with FID detector, 10% DEGS column (2m x 0.2 cm I.D.) attached to Shimadzu 2A integrator. Fatty acids were identified using authentic methyl ester standards (Ackman 1969). Adipose tissues were processed essentially as described for liver and the extracted lipids were methylated as described earlier. Results are expressed as the means and the standard error of the mean (SEM). Statistical analysis of the data was performed by using ANOVA and Students 't' test (Snedecor and Cochran 1967). The results were considered significant ($P<0.05$).

Results and Discussion

Fatty acid composition : The fatty acid composition of the different diets used in these experiments is given in Table 1. The analysis of fatty acid composition was the same as in 5 and 20% PNO groups. SO and CNO groups were also carried out similarly. PNO and SO had high levels of oleic acid (18:1, n-9), when compared to CNO. Linoleic acid was highest in SO, followed by PNO and lowest, in CNO. CNO provided the highest amount of SAFAs (90%) and lowest was in SO. PUFA was high in SO (41.0%) and lowest in CNO (2.65%), indicating that CNO is a saturated fat.

Body weight : Mean initial body weights of animals in all the groups ranged from 57±2.7 to 57±5.6g. 20%. Animal fed gained more weight (388-400g), compared to 5% fed Animals (359- 367g).

Thus, the weights of these animals were higher due to the higher calories provided by the fat, as compared to 5% groups (Table 2). There was no significant difference in the weight gain in the group of animals fed similar amount of dietary fat. The P/S ratio was highest in SO, followed by PNO and CNO (Table 1). The results indicated that the P/S ratio had no significant effect on growth parameters. A high P/S ratio beyond 3.0 is known to increase the weight of rats (Beynen and Kritchevsky 1986).

Plasma cholesterol : Plasma cholesterol levels in all the groups are given in Table 2. There was no significant difference observed in PNO, SO and CNO groups, both at 5 and 20% levels, although there was a tendency to be lower in 5% group of SO and PNO, but not significantly.

Plasma triglycerides : The mean plasma triglycerides as shown in Table 2 indicate that there was no significant change, followed by an increase in the lipid content of the diet. However, SO groups showed the highest level of triglycerides, followed by CNO and PNO. The 5% SO group showed high triglyceride level, compared to 20% group, indicating the effect of PUFAs. In general, the circulating triglycerides in 20% groups are higher in the PNO

TABLE 2. GROWTH RATE, LIPIDS AND LIPOPROTEINS OF RATS FED VARIOUS DIETS FOR 18 WEEKS*

Parameter	Peanut oil diet		Sesame oil diet		Coconut oil diet	
	5% I	20% II	5% III	20% IV	5% V	20% VI
Body weight, g						
Initial	57.00 ±3.80	57.00 ±4.50	57.00 ±2.90	57.00 ±5.60	57.00 ±4.90	57.00 ±2.70
Final	358.60 ±10.23	400.40 ±2.80	366.10 ±13.61	388.00 ±11.74	366.80 ±9.62	394.00 ±10.42
Increase in weight	301.60 ±6.40	343.40 ±8.40	309.30[a] ±8.90	326.70[a] ±9.40	309.80[a] ±7.80	337.00[a] ±8.90
Plasma cholesterol, mg/100ml (final)	79.33 ±3.46	75.27 ±2.77	77.69 ±2.71[a]	80.54 ±4.37[b]	79.69 ±0.30[a]	81.78 ±5.08[b]
Phospholipids, mg/100ml	106.65 ±9.84	112.84 ±7.91	113.30 ±8.59[a]	109.90 ±7.64	116.62 4.26[a]	136.60 10.08[a]
Triglycerides, mg/100 ml	43.75 ±2.14	47.99 ±4.96	60.36 ±7.17	51.22 ±5.35[a]	47.86 ±1.87	55.77 ±7.56[d]
HDL-C, %	46.57 ±6.31	48.72 ±3.89	43.18 ±3.26[b]	44.75 ±3.68[c]	30.33 ±2.95[c]	35.78 ±4.42[c]
LDL-C + VLDC-C, %	30.76 ±2.64	23.75 ±3.89	32.58 ±3.26[a]	38.64 ±3.68[c]	46.44 ±2.95[c]	43.84 ±4.42[c]
HDL-C / TC 100	58.70	64.73	55.68	55.56[c]	38.06[c]	43.75[c]
HDL-C / (LDL-C±VLDL-C) 100	151.39	205.14	132.82	115.80	65.30	81.60
C/P ratio (plasma)	0.74	0.66	0.68	0.73	0.68	0.60
Liver weight, g	13.24 ±0.6	14.78 ±0.58	14.97 ±0.68[d]	13.72 ±0.77[e]	13.03 ±0.47[e]	13.82 ±0.43[e]
Relative liver weight	3.69 ±0.15	3.69 ±0.16	4.08 ±0.11[f]	3.54 ±0.18[a]	3.56 ±0.16[a]	3.51 ±0.08[a]
Liver cholesterol, mg/g	3.97 ±0.39	3.93 ±0.24	2.96 ±0.27[g]	2.05 ±0.21[c]	1.82 ±0.23[c]	1.63 ±0.23[c]
Total liver cholesterol,	52.56 ±3.80	58.08 ±4.90	30.83 ±2.90	26.69 ±3.20	23.71 ±4.20	22.52 ±4.50
Liver phospholipids,	4.14 ±0.45	3.20 ±0.34	1.93 ±0.22[c]	1.75 ±0.21[c]	2.31 ±0.3[c]	2.27 ±0.34[c]

* All values ± SEM for 8 rats. Superscripts refer to the data on that particular line only

a Not significant between I and III, I and V and II and VI

b Not significant between I and III, II and IV, II and IV and II and VI

c P<0.001 Highly significant between II and IV and between I and V and II and VI

d P<0.05 between I and III and V and I (decrease)

e P<0.001 Highly significant decrease between II and IV and II and VI

f P<0.02 between I and III

g P<0.01 between I and III

and CNO groups. Although PUFAs are known to reduce serum glycerides, several workers have reached conflicting conclusions (Reiser et al. 1985; Becker et al. 1983). It is apparent that several factors seem to play in maintaining the triglyceride levels, such as the levels of n-6 fatty acids, n-3 fatty acids and also the higher homologues of fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and the derivatives of arachidonic acids.

Plasma phospholipids : Animals fed CNO showed the highest phospholipid level. In general, there was a significant increase in the phospholipid level, followed by an increase in the dietary fat content. However, in the PNO and SO groups, the results were significant, even when the fat content of the diet increased to 20%.

Liver cholesterol : The liver cholesterol levels are shown in Table 2. In the SO and CNO groups, there was a significant decrease in the liver cholesterol with the increase in dietary fats to 20% (Table 2), particularly when compared to PNO ($P<0.001$). It is known that cholesterol concentration in the liver can be modified by the type of fat. In general, PUFAs are more hepatocholesterolic than SAFA (Kritchevsky et al. 1988). Moreover, linoleic acid (18:2, n-6) at higher concentration has a stimulatory action on cholesterogenesis (Dupont et al. 1972). On the other hand, an increased concentration of cholesterol in the SO group is due to oleic acid (18:1, n-9), which has also been shown to be more hepatocholesterolic, than linoleate (Beynen 1989). In the case of CNO, the liver cholesterol is lowest ($P<0.001$) because of the SAFAs, which are known to inhibit the cholesterol accumulation in liver (Kritchevsky et al. 1988). It has also been reported that in the case of CNO, the plasma cholesterol is not parallel to liver cholesterol, indicating apparently that the type of fat may influence the cholesterol distribution between plasma and liver (Zhang et al. 1990) and in some cases, the SAFA has been shown to decrease serum cholesterol (Meijer and Beynen 1988), due to the interrelated effects of fat and type of carbohydrate (Beynen and Katan 1984).

Liver phospholipids : Liver phospholipids were reduced significantly in SO and CNO groups (Table 2), when compared to PNO groups. Also, there was a significant decrease in the liver phospholipid level with the increase in fat content of the diet for the PNO and SO groups. But no such change was observed for the CNO group.

Cholesterol/phospholipid ratio (C/P) : The C/P ratio of the plasma ranged from 0.60–0.74 in all the groups. It has been demonstrated that in healthy conditions, the C/P ratio in the plasma is less than one, while in hypercholestremia, it is greater than one (Mahley and Holcome 1977). In the present study, the ratio is less than one, indicating that the diets are not hypercholestremic.

Organ weights and organ to body weight ratios : Absolute organ weights and organ to body weight ratios of liver, kidney, brain and heart did not show any statistically significant differences between groups for these rats (Data not shown).

Lipoproteins : HDL-C was found to be significantly lower in the CNO fed animals. There appeared to be a significant change in the level of HDL-C with the change in the fat content of the diet (Table 2). The lower level of HDL-C was also reflected for the CNO group, when the level of HDL-C to serum cholesterol was tabulated (Table 2). The HDL-C/LDL-C +VLDL-C ratio indicates that highly saturated fats increase β-lipoprotein and reduce α-lipoproteins, as reported by various workers (Lewis 1977; Baudet et al. 1980). The lower HDL-C/LDL-C + VLDL-C ratio is indicative of the presence of saturated fats in the CNO diet, whereas in unsaturated fats in PNO and SO diets, it is higher. The ratio is indicative of the atherogenic power of the fat (Miller and Miller 1976).

Fatty acid composition of plasma, erythrocyte and adipose tissue : The results of analysis of plasma, erythrocytes and perirenal adipose tissue fatty acid composition of rats fed the various diets are shown in Table 3, 4 and 5. In the control PNO group, palmitic (16:0) acid decreased, when the level of PNO changed from 5 to 20%. There was no other significant change in the PNO group. In the CNO fed groups, there was a higher concentration of 10:0 (capric), palmitic (16:0), stearic (18:0), and arachidonic acids (20:4, n-6). The arachidonic acid levels were almost equal to SO and PNO, in spite of low intake of linoleate, indicating probably the endogenous synthesis from linoleate. Similarly, in the SO group, there was a reduction of 16:0, when the fat content of diet increased from 5 to 20%, whereas in the CNO group, the reduction was comparatively, low. There was no other significant difference with respect to other fatty acids. The SAFA levels were higher in the CNO group in both 5 and 20%, when compared to the other PNO and SO groups, whereas the PUFA levels were higher in PNO and SO groups due to

TABLE 3. PLASMA FATTY ACID PATTERN OF RATS FED VARIOUS DIETS BASED ON PEANUT, SESAME AND COCONUT OILS*

Fatty acid %	5% PNO	20% PNO	5% SO	20% SO	5% CNO	20% CNO
C10:0	0.40 ±0.17	0.56 ±0.23	0.80 ±0.17	1.42 ±0.28	1.43 ±0.33**	3.80 ±0.71**
C12:0	1.31 ±0.29	1.56 ±0.32	0.44 ±0.09	0.82 ±0.18	0.86 ±0.26	0.58 ±0.15
C14:0	0.56 ±0.29	0.61 ±0.40	0.77 ±0.09	0.74 ±0.06	0.46 ±0.06	0.84 ±0.17
C16:0	7.25 ±0.37	4.27 ±0.33	8.27 ±9.69	4.63 ±0.35	13.46 ±0.17**	8.92 ±0.63**
C18:0	6.56 ±0.54	6.05 ±0.91	6.93 ±0.59	9.35 ±0.57	9.15 ±0.31**	12.70 ±0.14**
C18:1,n-9	32.06 ±1.12	36.86 ±2.27	32.38 ±0.85	30.23 ±1.58	32.73 ±1.23	34.95 ±0.60
C18:2,n-6	18.24 ±0.86	19.21 ±1.51	18.05 ±0.84	20.10 ±0.66	6.61 ±0.30[a]	7.01 ±0.17[a]
C18:3,n-6	0.60 ±0.08	0.69 ±0.07	0.60 ±0.20	0.62 ±0.09	0.67 ±0.04	0.57 ±0.10
C20:0	1.05 ±0.21	1.42 ±0.40	0.13 ±0.01	0.72 ±0.22	0.59 ±0.33	0.81 ±0.48
C 20:4 n-6	8.12 ±0.23	9.34 ±0.31	10.01 ±0.31	12.03 ±1.15	10.63 ±0.91[b]	11.15 ±0.22[b]
SAFA	32.06	29.02	29.92	31.65	35.44	46.5[b]
MUFA	40.93	41.73	41.42	35.60	46.65	34.71
PUFA	26.96	29.24	28.66	32.75	17.91	18.73
P/S ratio	0.84	1.0	0.95	1.03	0.50	0.40
Ratio of						
18:1/18:2	1.76	1.82	1.79	1.50	4.95	3.55
18:1/18:0	4.89	6.05	4.67	3.23	4.57	1.96
18:2/20:4	2.24	2.05	1.80	1.67	0.62	0.63
20:4/18:2	0.97	0.49	0.55	0.59	1.60	1.59

* All values ± SEM for 8 rats. ** P<0.001 highly significant when compared to PNO and SO

[a] Significant increase when compared to the intake of linoleate which was very low.

[b] Arachidonic acid levels (20:4, n-6) are similar to PNO and SO

the higher intake provided by the diet. The lowest P/S ratio was with the plasma of CNO fed animals which resembled the dietary ratio, which was also low. In the erythrocytes, the CNO fed animals had higher levels of 10:0, 12:0, and 14:0, when compared to other fatty acids in both 5 and 20% groups, indicating that the increase was proportional to the higher level of fat. The level of 16:0 decreased by 11% in PNO, 20% in SO, and only 12% decrease in CNO groups, indicating probably a higher utili-sation. The linoleic acid levels uniformly increased in all the 20% groups, indicating the relationship to the intake. Arachidonic levels also increased in 20% group of PNO, SO and CNO groups by 5, 16 and 18%, respectively. The erythrocyte membranes are known to be rich in arachidonate, which confers fluidity to the membranes (Roth and Kirchgessner 1992; Farquhar and Ahrens 1963). The increases in levels even in CNO groups, indicates a higher activity of Δ^6 desaturase (Huang et al. 1986). Linoleic acid is an essential fatty acid for these rats and the present results indicate that higher intakes of these fatty acids in SO and PNO groups lead to higher levels in the adipose tissue, when compared to the CNO groups. As seen in Table 1, the unsaturation indices of PNO and SO are higher, when compared

TABLE 4. FATTY ACIDS OF TOTAL ERYTHROCYTE LIPIDS OF RATS FED VARIOUS DIETS BASED ON PEANUT, SESAME AND COCONUT OILS*

Diet	5% PNO	20% PNO	5% SO	20% SO	5% CNO	20% CNO
C10:0	1.07 ±0.22	0.70 ±0.19	0.50 ±0.25	0.33 ±0.09	1.52 ±0.37[a]	1.45 ±0.42[a]
C12:0	0.65 ±0.03	0.52 ±0.03	0.23 ±0.05	0.20 ±0.04	5.67 ±0.22**	7.32 ±1.42**
C14:0	1.61 ±1.22	1.34 ±0.10	2.15 ±0.23	2.09 ±0.27	8.55 ±0.26**	7.36 ±1.26**
C16:0	33.61 ±0.50	29.90 ±0.53	35.32 ±1.17	28.20 ±0.83	28.92 ±1.48	25.44 ±1.30
C16:1	0.16 ±0.01	0.22 ±0.02	0.09 ±0.001	0.10 ±0.01	0.10 ±0.01	0.12 ±0.01
C18:0	13.08 ±0.23	15.17 ±0.35	10.78 ±0.54	16.54 ±0.27	10.33 ±0.44	12.61 ±0.69
C18:1,n-9	17.30 ±0.34	15.86 ±0.29	20.12 ±0.60	16.29 ±0.40	18.65 ±1.12	14.71 ±0.05
C18:2,n-6	7.85 ±0.25	10.06 ±0.47	9.76 ±0.53	10.82 ±0.26	5.80 ±0.43[a]	6.73 ±0.41[a]
C18:3,n-6	0.21 ±0.02	0.24 ±0.02	0.18 ±0.03	0.06 ±0.02	0.13 ±0.05	0.28 ±0.05
C20:0	0.73 ±0.03	0.82 ±0.02	0.48 ±0.04	0.92 ±0.04	0.31 ±0.08	0.39 ±0.09
C20:1	0.34 ±0.08	0.51 ±0.11	0.39 ±0.15	0.42 ±0.11	0.64 ±0.12	0.86 ±0.23
C20:2	0.32 ±0.07	0.48 ±0.02	0.24 ±0.02	0.40 ±0.14	1.01 ±0.10	0.67 ±0.06
C20:3,n-6	0.36 ±0.03	0.53 ±0.08	0.39 ±0.02	1.00 ±0.45	0.44 ±0.08	0.70 ±0.03
C20:4,n-6	22.48 ±0.75	23.52 ±0.53	19.14 ±1.19	22.32 ±0.44	17.72[b] ±0.35[b]	20.96[b] ±0.90[b]
SAFA	50.80	48.45	49.46	48.28	55.30	55.07
MUFA	17.90	16.72	20.60	17.12	19.60	16.09
PUFA	31.22	34.83	29.71	34.60	25.10	29.39
Ratio of						
18:1/18:2	2.20	1.57	2.06	1.50	3.22	1.16
18:1/18:0	1.32	1.05	1.87	0.98	1.81	1.17
18:2/20:4	0.35	0.43	0.51	0.48	0.33	0.33
20:4/18:2	2.86	2.34	1.96	2.06	3.05	3.12
P/S ratio	0.62	0.72	0.60	0.72	0.45	0.53

* All values ± SEM for 8 rats

** P<0.001 Highly significant when compared to PNO and SO

[a] Linoleic acid is higher when compared to the intake of coconut oil by these rats where the values are low

[b] Arachidonic acid levels (20:4, n-6) are high in CNO fed rats in spite of low levels of feeding linoleate

to CNO groups and the higher unsaturation index is contributed mostly by oleic and linoleic acids. The palmitic acid levels were lower in 20% fed animals by 38, 34 and 12%, respectively in the PNO, SO and CNO groups. Apart from utilisation for energy purposes, the excess palmitic acid might have been deposited in adipose tissue. Feeding higher levels of PNO, SO and CNO increased the level of oleic acid by 6, 7 and 10%, respectively. The results are in agreement with those of Fordyce et al (1983). It has been demonstrated in rodents that palmitic acid can be converted into oleate by elongation and desaturation of the 9th carbon atom of oleic acid (Elovson 1965). Studies in mice have demonstrated that increased intake of SAFAs induces desaturase activity in the liver, leading to the concentration of oleate in adipose tissue (Tove and Smith 1959). Thus, adipose tissue fatty acid composition is a valid indicator of the dietary fat intake and useful in monitoring the nature of the diet (Herodek and Csakvary 1972). (A high oleic/linoleic/18:1/18:2) ratio is an indicator of essential fatty acid deficiency, as there is a competition between oleic and linoleic for the same desaturase and elongase enzymes (Dhopheshwarkar and Mead 1967). Besides, it also controls the endogenous synthesis of MUFA with exogenous supply of linoleate (Jeffcoat and James 1978). Since the availability of linoleate is high in PNO and SO groups, the ratio is reduced, whereas in the CNO, it is high, indicating low levels of linoleate, but not absent. Due to prolonged feeding, the ratio becomes less and less, as the level of linoleate increases. The ratio of 18:1/18:0 is highest in plasma followed by erythrocytes, indicating high Δ^9 desaturase activity (Leikin and Brenner 1987). The ratio of 18:2/20.4 is very high in adipose due to the absence of arachidonate. In plasma, the ratio is high due to the large availability of linoleate. In the erythrocytes, due to the large amount of arachidonate contributed by the membranes and the converted arachidonate from linoleate, the ratio is lowered from 0.33 to 0.51. Similar observations are also made in the ratio of 20:4/18:2, which is highest in plasma and erythrocytes and lowest in adipose tissue. In the adipose tissue, it is low because of the absence of 20:4, n-6 in the storage form. Thus, all these ratios indicate the activity of elongase and desaturase enzymes in an indirect way in the tissues.

TABLE 5. FATTY ACID COMPOSITION OF PERIRENAL ADIPOSE TISSUE OF RATS FED PEANUT, SESAME AND COCONUT OILS*

Diet	5% PNO	20% PNO	5% SO	20% SO	5% CNO	20% CNO
C10:0	ND	ND	ND	ND	5.21 ±0.23**	6.02 ±0.65**
C12:0	0.19 ±0.06	0.39 ±0.05	0.50 ±0.12	0.40 ±0.07	28.06 ±1.25**	25.48 ±1.87**
C14:0	1.33 ±0.03	0.20 ±0.07	1.17 ±0.08	0.73 ±0.09	12.13 ±0.21	11.03 ±0.18
C16:0	24.92 ±1.13	15.54 ±0.02[b]	18.80 ±0.63	12.41 ±0.12[b]	18.47 ±0.31	16.32 ±0.19[b]
C18:0	1.73 ±0.07	2.10 ±0.42	3.58 ±0.12	4.21 ±0.35	8.45 ±0.59**	10.62 ±0.64**
C18:1,n-9	48.70 ±0.69	51.52 ±0.73	48.92 ±0.78	49.10 ±0.21	22.64 ±0.80[b]	25.13 ±0.66[b]
C18:2,n-6	19.10 ±0.37	28.52 ±0.90	26.98 ±0.94	29.30 ±0.58	5.32 ±0.13[a]	6.14 ±0.17[a]
C18:3,n-6	0.66 ±0.03	0.30 ±0.16	0.37 ±0.09	0.58 ±0.30	0.52 ±0.03	0.51 ±0.03
SAFA	26.64	16.13	24.05	17.75	72.32	69.47
MUFA	48.70	51.52	48.92	49.10	22.64	25.13
PUFA	19.76	28.20	27.05	29.88	5.34	6.15
Ratio of 18:1/18:2	2.54	1.81	1.81	1.68	4.25	4.09
P/S ratio	0.74	1.78	1.12	1.68	0.07	0.09

* All values ± SEM for 8 rats

** P<0.001 highly significant when compared to PNO and SO

[a] P<0.001 significantly low because of low intake

[b] At 20% level 16:0 is reduced uniformly in all groups

ND Not detected

The present study has shown that SO is also effective as PNO, since the composition of the fatty acids differs marginally in reducing the lipid concentrations in rats. In the case of CNO, several factors viz., the species, type of diet, starch, cellulose and composition of fat, seem to play a role in inducing atherogenicity, leading to cardiovascular diseases. Recent studies indicate that oleate alone can reduce lipid levels more than linoleate (Heyden 1994). However, in the present study, it is not clear as both PNO and SO are similar in composition.

Acknowledgement

The authors are grateful to the Director, Central Food Technological Research Institute, Mysore, for keen interest and Dr. S.G. Bhat, Head, Biochemistry and Nutrition Department for encouragement and support.

References

Ackman RG (1969) Gas liquid chromatography of fatty acids and esters. Meth Enzymol 14:330-391

Alfin Slater RB (1967) Carbohydrate-lipid effects on cholesterol metabolism. J Dairy Sci 50:781-786

Baudet MF, Estera O, Delplanque B, Winchenne N, Jacotot B (1980) Effect of three dietary fats on plasma lipids and lipoproteins in fasting and post-prandial humans after a short term diet. Lipids 15:216-223

Becker N, Illingsworth R, Alaupovic P, Connor WE, Sundberg EG (1983) Effect of saturated, monounsaturated and n6 polyunsaturated fatty acids on plasma lipids, lipoproteins and apoproteins in humans. Am J Clin Nutr 37:355-360

Beynen AC, Katan MB (1984) Relation between the response of serum cholesterol to dietary cholesterol and to the type of dietary fat, protein and fibre in hypo and hyper-responsive rats. Nut Rep Int 29:1369-1381

Beynen AC, Kritchevsky D (1986) Dietary fats and serum cholesterol. In: Beynen AC (ed) Nutritional Effect of Cholesterol Metabolism, Transmondial, Voorthuizen, Netherlands

Beynen AC (1987) Serum and liver cholesterol of rats fed cholesterol free or high cholesterol diets differing in type and amount of fat. Nut Rep Int 35:1327-1332

Beynen AC (1989) Olive oil and liver cholesterol. Am J Clin Nutr 49:392-393

Burnstein M, Samaille J (1960) Sun un dosage rapide du cholesterol lie aux -et aux β-lipoproteins du serum. Clin Chim Acta 5:609-619

Chapman DG, Castillo R, Campbell JA (1959) Evaluation of protein in foods: A method for the determination of protein efficiency ratios. Can J Biochem Physiol 37:673

Clark KJ, Makridas M, Newmann MA, Gribson RA (1992) Determination of optimal ratio of linoleic to a linolenic acid in infant formulas. J Pediatrics 120:5151-5158

Dhopheshwarkar GA, Mead JF (1967) Role of oleic acid in the metabolism of essential fatty acids. J Am Clin Soc 38:297-301

Dupont J, Mathias MM, Cabacungan NB (1972) Dietary lipid, fatty acid synthesis and cholesterol metabolism in ageing rats. Lipids 7:576-580

Elovson J (1965) Conversions of palmitic and stearic acid in the intact rat. Biochem Biophys Acta 106:291-303

Farquhar JW, Ahrens EH (1963) Effects of dietary fats on human erythrocyte fatty acid patterns. J Clin Invest 42:675-683

Fletcher MJ (1968) A calorimetric method for estimating serum triglycerides. Clin Chim Acta 22:303-307

Folch J, Lee M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-502

Fordyce MK, Cristakis G, Kafatos A, Duncan R, Cassidy J (1983) Adipose tissue fatty acid composition of adolescents in a US-Greece cross cultured study of coronary heart disease risk factors. J Chronic Disease 18:143-145

Herodek S, Csakvary G (1972) Effect of dietary fatty acids on the desaturation of stearic acid in rat liver. Acta Biochim Biophys Acad Sci (Hung) 7:207-213

Heyden S (1994) Polyunsaturated and monounsaturated fatty acids in the diet to prevent coronary heart diseases via cholesterol reduction. Ann Nutr Metabol 38:117-122

Hubbel RB, Mendel LB, Wakeman AJ (1937) A raw salt mixture for use in experimental diets. J Nutr 14:273-277

Huang YS, Cunnane SC, Horrobin DF (1986) Effect of different proteins on plasma and liver fatty acid composition in growing rats. Proc Soc Expl Biol Med 181:399-402

Jeffcoat R, James AT (1978) The control of stearoyl COA-desaturase by dietary linoleic acid. FEBS Lett 85:114-117

Kates M (1972) Techniques in lipidology. Isolation analysis and identification of lipids In: Laboratory Techniques in Biochemistry and Molecular Biology 3:279--597

Koh ET (1987) Comparison of hypolipidemic effect of corn oil, sesame oil and soybean oil in rats. Nut Rep Int 36:903-908

Kris Etherton PM, Krummel D, Dreon D, Mackey S, Borchers J, Wood PD (1988) The effect of diet on plasma lipids. lipoproteins and coronary heart disease. J Am Diet Assoc 88:1373-1400

Kritchevsky D, Tepper SA, Story JA (1977) Influence of dietary fats on lipids metabolism in rats fed a semi-purified diet. Nut Rep Int 15:65-70

Kritchevsky D, Tepper SA, Bises G, Klurfeld DM (1983) Influence of cocoa butter on cholesterol metabolism in rats: Comparison with corn oil coconut oil and palm kernel oil. Nut Res 3:222-236

Kritchevsky D, Tepper SA, Lloyd LM, Davidson LM, Klurfeld DM (1988) Sesame and liver lipids of rats fed cocoa butter, corn oil, palm kernel oil and cholesterol. Nutr Res 8:287-294

Leikin AI, Brenner RR (1987) Cholesterol induced microsomal changes modulate desaturase activities. Biochim Biophys Acta 922:294-302

Lewis B (1977) Plasma lipoprotein interrelationships. Biochem Soc Trans 5:589-601

Mahley RW, Holcome KS (1977) Alterations in plasma lipoproteins and apoproteins following cholesterol feeding to the rat. J Lipid Res 18:312-317

Malmros H, Wigard G (1978) The effect of serum cholesterol diets containing different fats. Lancet 2:1-7

Mattson FH, Grundy SM (1985) Comparison of effects of dietary saturated, monounsturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J Lipid Res 26:194-202

Meijer GW Debruijne JJ, Beynen AC (1987) Dietary cholesterol fat type combinations and carbohydrate, lipid metabolism in rats and mice. Int J Vit Nut Res 57:310-326

Meijer GW, Beynen AC (1988) Interrelated effects of the type of dietary fat and carbohydrates on cholesterol metabolism in rats. Int J Vit Nut Res 58:241-245

Mendis S, Wissler RW, Bridenstone RT, Podielski FJ (1989) The effect of replacing coconut oil with corn oil on human serum lipid profiles and platelet derived factors active in atheorogenesis. Nut Rep Int 40:773-778

Miller GJ, Miller NE (1975) Plasma high density lipoprotein concentration and development of ischaemic heart disease. Lancet 1:16-19

O'Brien BC, Skutches CL, Henderson GR, Reiser R (1977) Interrelated effects of food lipids on steroid metabolism in rats. J Nutr 107:1444-1454

Reiser R, Probstfield JL, Silvers A, Scott LW, Shorny ML, Wood RD, O'Brien BC, Gotto AM, Insull Jr W (1985) Plasma lipid and lipoproteins response of humans to beef fat, coconut oil and sunflower oil. Am J Clin Nutr 42:190-197

Rose HG, Ocklander M (1985) Improved procedure for the extraction of lipids from human erythrocytes. J Lipid Res 8:428-433

Roth HP, Kirchgessner M (1992) Einflass verschiedener ungesattigter fettsavren auf die stabilitat der erythrozytenmembran bei der ratte. Ann Nut Metabol 36:113-120

Searcy RK, Bergquist LM (1960) A new color reaction for the quantification of serum cholesterol. Clin Chim Acta 5:192-199

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

Stewart JCM (1980) Colorimetric estimation of phospholipids with ammonium ferrothiocyanate. Anal Biochem 104:10-14

Tove SB, Smith FH (1959) Kinetics of the depletion of linoleic acid in mice. Arch Biochem Biophys 85:352-365

Vega GL, Groszeck E, Wolf R, Grundy SM (1982) Influence of polyunsaturated fats on composition of plasma lipoproteins and apoliproteins. 23:811-822

Zhang X, Meijer GW, Beynen AC (1990) Liver cholesterol concentration in rats fed diets containing various fats of plant origin. Int J Vit Nut Res 60:275-290

Received 23 July 1996; revised 24 March 1997; accepted 26 March 1997

J. Food Sci. Technol., 1997, Vol 34, No. 4. 311-315

The Effect of Flour-blending on the Physico-chemical and Sensory Qualities of Bread

CHINYERE I. IWUOHA*, ALEX C. ANYADIKE AND ONYEKWERE S. EKE

Department of Food Science and Technology,
Federal University of Technology, P.M.B. 1526, Owerri, Imo State, Nigeria.

Composite flour mixture (CFMs) were prepared by blending the flours of wheat (WT), cassava (CS) or other cereals [maize, (MZ) and sorghum, (SG)], and legumes [soybean, (SB) and cowpea, (CP)] in the proportions of 60:20:20 and 60:30:10 by weight. These were made into doughs and baked to generate 12 composite bread samples (CBSs). Both the CFMs and CBSs were compared with one another and with their all-WT counterpart. Results of proximate analyses of CFMs showed that those containing SB had relatively more proteins and fat and lesser carbohydrates, while those with CP had higher fibre content. The same trend was observed for the residual gluten (GC) and water retention capacity (WRC). Gluten content dropped by at least 36.41%, while WRC increased by more than 15%. Cowpea (CP)-containing CBSs exhibited higher specific volume (3.42ml/g) as against the all-WT (control loaves (2.79ml/g) and were statistically different (P=0.05). The WT-SG-SB (60:20:20) CFM had the highest after-bake proteins (15.13%), which was not significantly different from the WT-MZ-SB (600:20:20) CBS. Despite the superior nutrient status of soy flour-containing CBSs, panelists rated the cowpea flour-containing CBS the highest (7.40) in mean overall acceptance on a 9-point Hedonic scale, which was also significantly different from the other CBSs. Finally, results showed that age group and sex had significant influence on the sensory scoring pattern of the consumers.

Keywords : Flour-blending, Bread, Chemical, Physical, Sensory, Quality.

Bread has become increasingly popular in the diets of the less developed countries (LDCs) like Nigeria (Rychnovsky 1990). Wheat flour is the preferred raw material, as its proteins impart desirable qualities to the resultant bread (Van der Made 1977; Perlmann 1977; Sosulski 1977).

Wheat is a temperate crop not conveniently adapted to the tropical climate. Nigeria's wheat import bill as at 1973 was estimated at 40 million naira and was projected to run into billions of naira by the turn of 1987 (Akobundu et al. 1988). The dependence on imports for the baking flour would drain the foreign reserves. To ease this burden on the exchequer, the Nigerian government decided in 1986 to discountinue wheat imports. Such deliberate policy coupled with the need to have bread on the dining table threw a challenge to the research scientists to fashion out processes and products using the locally/tropically available crops, so that the populace could easily afford (Garrand 1974; Bean and Nashita 1983; Akobundu et al. 1988).

Prior to the ban on wheat imports, several Nigerian researchers have done some work to prepare bread from composite flour formulations, using local grains and tubers (Tsena and Akinrele 1974; Okaka and Potter 1977; Olatunji and Akinerele 1978). Elsewhere, miscellaneous combinations have been reported (FAO 1973; DeRuiter 1978; Knight et al. 1982; Chandrashekara and Shurpalekar 1983; Youssef and Bushuk 1986). Although some problems were encountered with non-wheat technology (Ajayi 1986), subsequent reports indicate that good quality (and nutritious) loaves could be made from non-wheat flours (Ogunsua 1987; Osutogun 1987; Akobundu et al. 1988; Misra et al. 1991).

The present study was undertaken to examine the chemical, physical, functional and organoleptic attributes of flours as well as bread formulated from wheat flour substituted with local cereals, legumes and cassava flours to the levels of 40%.

Materials and Methods

Materials : Fresh cassava tubers of the bitter variety (*Manihot esculenta*, Crantz) were procured from Research Farm, F.U.T., Owerri, Nigeria. Seeds of cowpea (*Vigna unguiculata*), soybean (*Glycine max*, Merrill), general-purpose wheat flour (Ideal Flour Mill Ltd, Kaduna, Nigeria), sugar, salt, baking fat (Special Holsum, Lever Bros. Nigeria PLC. Lagos) and dried yeast (Fleetdene Ltd, London) were used.

Preparation of plain and blended flours : Cleaned grains of maize and sorghum were separately milled in Bamford Rapid Grinding Mill (Model 982) and sieved to obtain fractions <425 mirons (plain whole flours). Cowpea and soybean seeds were blanched in boiling water for 5 min and 40 min, respectively, manually dehulled, floatationally separated the hulls in a running tap water. The cowpea and soybean cotyledons were dried in an oven for 14 h at 80°C and 16 h at 105°C, respectively and

* Corresponding Author

milled by adopting the procedure described for cereals by Patil and Shukla (1990) to obtain full-fat legume flours. Cassava tubers were cleaned, washed, peeled and cut into chips, soaked in water containing 0.05% (w/w) sodium metabisulphite for 24 h. The steep liquor was drained off and the chips washed and dried (60°C for 16 h; then 80°C for 4 h and finally 100°C for 4 h). They were cooled, milled and sieved as previously described.

Composie flours were prepared by blending the flours of wheat, cassava/other cereals and legumes in the proportions of 60:20:20 and 60:30:10.

Baking experiments : The method described by Kim and DeRuiter (1968) was generally adopted. The recipe for the dough was made up of flour mixture (100%), sugar (6); yeast (2); fat (2); salt (1.5) and water (50-60).

Chemical and physical analyses : Moisture, fat, fibre, ash and total nitrogen were determined according to AOAC (1975) methods. Protein was obtained by multiplying the total nitrogen with the factor 5.70 for wheat (Murray et al. 1981), soybean 5.71 and others 6.25 (Aykroyd and Doughty 1982). The residual gluten content was determined according to the AACC (1962) method, while water retention capacities of the all-wheat and composite flours were analyzed following the procedure of Hall-green (1984). Masses of the loaves were measured with a weighing balance, while the volumes were determined by seed displacement method (Ott 1989) upon cooling.

Sensory evaluation : A 25-member trained taste panel, selected from the University Community, assessed the organoleptic qualities of the composite bread sample (CBS) and side by side, the all-wheat bread. Surface finish, colour, taste, aroma, texture and overall acceptability attributes of CBS were descriptively evaluated on a 9-point Hedonic scale. A score of 1 indicated 'dislike extremely', while 9 indicated 'like extremely'. The panel consisted of 10 males and 15 females: 8 teenagers (13-19 years), 10 middle-aged (20-30 years), and 7 adults (31-55 years). The panel was trained as a group, but made to conduct the evaluation in two environments: sets of 3-5 persons to assess at one time and individually in another session (i.e., one sample was evaluated twice by each member of the panel).

Statistical analyses of data : The effect of compositing on the quality attributes measured for flour and bread samples were statistically analyzed through a one-way analysis of variance (ANOVA) technique as per Hayslett (1974). The data from taste panel evaluation were subjected to two-way ANOVA, while Tukey's test for multiple comparisons was used to test significant differences between samples according to the procedure of Watts et al (1989). Three-way ANOVA was done according to Steel and Torrie (1980).

Results and Discussion

Chemical and physical characteristics of the flours : Results of proximate analyses on wheat (WT), maize (MZ) sorghum (SG) cowpea (CP), soybean (SB) and cassava (CS) plain flours (Table 1) have shown that SB and CP are nutritionally superior. When these compositions were pro-rated to evaluate for the composite flour mixtures (CFMs), SB showed its superior nutrient composition, followed by CP. These results agree with the concept and practice of using grain legume flours to fortify bread flours to improve its nutritional quality (McConnel et al. 1974; Patel and Johnson 1974; Jeffers et al. 1978; Finney et al. 1980; Mabesa et al. 1983; Misra et al. 1991).

Functional characteristics of the composite flour mixture (CFMs) : Dilution of wheat flour through various cereal-legume and cassava-legume flour combinations by up to 40% has resulted in more than 15% increase in water retention capacity (WRC), while gluten content (GC) dropped by at least 36.41%. The SB-containing flour effected the greatest WRG (99.66%) and residual GC (3.86%) among CFMs relative to the CPS (Table 2). Among the CFMs, the variations in WRC were significant (P=0.05), while GCs were not. However, in comparison between CFMs and all-wheat flour, there was difference (P=0.05). In terms of the grain cereals and tuber flours, the performances are thus: MZ>SG>Cs. It has been shown that increasing the levels of cassava flour in a wheat-tuber flour blending progressively reduced water absorption capacity (Chandrashekara and Shurpalekar 1983).

TABLE 1. PROXIMATE COMPOSITION OF PLAIN FLOURS

Flour	Moisture, %	Protein[1], %	Fat, %	Fibre, %	Ash, %	Carbohydrates by difference %
Maize	15.5	11.3	5.3	2.8	2.3	78.3
Sorghum	16.3	11.3	4.7	2.3	3.0	78.7
Wheat	14.7	15.2	2.0	2.4	2.2	78.3
Cowpea	13.0	26.7	1.7	3.8	4.3	63.5
Soybean	11.4	45.4	22.6	2.5	4.6	25.0
Cassava	13.8	1.7	0.7	1.4	2.3	94.0

[1]N x 5.71 for soybean; N x 5.70 for wheat and N x 6.25 for others

The values are on dry basis and means of duplicate determinations

TABLE 2. WATER RETENTION CAPACITY AND GLUTEN CONTENT OF THE COMPOSITE FLOUR MIXTURE

Flour	Proportion	Water retention capacity, %	Gluten content, %
Wheat (control)	100	71.56[h]	6.07[a]
Wheat-Maize-Soybean	60:20:20	99.66[a]	3.86[b]
Wheat-Maize-Soybean	60:30:10	95.53[c]	3.83[b]
Wheat-Sorghum-Soybean	60:20:20	99.37[a]	3.84[b]
Wheat-Sorghum-Soybean	60:30:10	95.13[c]	3.81[b]
Wheat-Cassava-Soybean	60:20:20	96.66[b]	3.62[b]
Wheat-Cassava-Soybean	60:30:10	91.07[d]	3.49[b]
Wheat-Maize-Cowpea	60:20:20	89.64[ef]	3.86[b]
Wheat-Maize-Cowpea	60:30:10	90.51[de]	3.82[b]
Wheat-Sorghum-Cowpea	60:20:20	89.36[f]	3.84[b]
Wheat-Sorghum-Cowpea	60:30:10	90.09[ef]	3.85[b]
Wheat-Cassava-Cowpea	60:20:20	86.70[g]	3.56[b]
Wheat-Cassava-Cowpea	60:30:10	86.10[g]	3.47[b]

* Values are means of three determinations
Values are based on dry matter (db)
Means with common superscripts along columns do not differ significantly (P=0.05) according to Tukey's Test

An earlier report has indicated that the predominantly carboxyl groups in the molecule of zein could be responsible for the high water absorption of blends containing maize (Reiners 1978).

Chemical and physical quality of composite bread : The results of the physical measurements on the composite bread samples (CBSs) (Table 3) showed that both the volume and specific volumes of cowpea (CP)-containing loaves were relatively higher than their soybean (SB) counterparts as well as those of all-wheat loaves. These differences were also significant (P=0.05). Elsewhere, reduction in volume has been associated with fababean-wheat flour (Youssef and Bushuk 1986) and soy-wheat flour (Misra et al. 1991) in bread-making. In sorghum-wheat composite baking trials, Hallgren (1984) obtained bread with specific volumes that are equivalent to the ones obtained in this study.

Water retention capacity (WRC) is an index of starch damage. It is also a determinant of the varying masses of the CBSs. This inference is in line with the view held by D'Appolonia (1977). This observation also confirmed that bread made from CFMs with 60:30:10 proportion had higher mass than those with 60:20:20 for any particular formulation (Table 3).

The rates of moisture loss (RML) for the CBSs were generally more than those of the control, but statistically different (P<0.05) from each other. This underscores the crucial role of flour compositing in moisture release during baking. Doughs with higher moisture content are expected to lose more water in baking. In reality, the extent of moisture loss in the dough matrix must depend on the ease of release of water from the dough network in the presence of heat.

The residual proteins of CBS are varied. Protein of bread implies that it has interacted with water and other components that make up bread and also has passed through ambient conditioning as well as in the oven (≥230°C); These conditions will adversely influence the residual protein of the ultimate products. However, legumes, especially

TABLE 2. WATER RETENTION CAPACITY AND GLUTEN CONTENT OF THE COMPOSITE FLOUR MIXTURE

Flour	Proportion, %	Mass, g	Volume, cm^3	Specific volume, cm^3/g	Rate of moisture loss, g/min[a]	Moisture, %	Proteins, %
Wheat (control)	100	204.9 (137.7)[h]	570.9[b,c]	2.79[c,d]	4.00[f]	32.8(48.8)	6.84(10.18)[e]
Wheat-Maize-Soybean	60:20:20	153.2(102.6)[e]	431.6[f]	2.82[b,c,d]	5.73	33.0(49.3)	10.05(15.00)[a]
Wheat-Maize-Soybean	60:30:10	159.2(106.7)[c,d]	417.7[f]	2.62[d]	5.53[cc]	33.0(49.3)	6.85(10.22)[e]
Wheat-Sorghum-Soybean	60:20:20	157.3(105.4)[c,d]	459.5[d,e,f]	2.92[bcd]	5.59[bc]	33.0(49.3)	10.14(15.13)[a]
Wheat-Sorghum-Soybean	60:30:10	160.3(107.2)[c,d]	445.6[e,f]	2.78[cd]	5.49[de]	33.1(49.5)	5,91(8.84)[d]
Wheat-Cassava-Soybean	60:20:20	160.3(107.2)[c,d]	473.4[d,e,f]	2.95[bcd]	5.49[e]	33.1(49.5)	8.65(12.93)[b]
Wheat-Cassava-Soybean	60:30:10	171.30(114.6)[d]	459.5[d,e,f]	2.68[cd]	5.12[d]	33.1(49.5)	4.50(6.73)[e]
Wheat-Maize-Cowpea	60:20:20	169.0(113.2)[c,d]	515.2[c,d,g]	3.20[bce]	5.20[bcd]	33.0(49.3)	6.16(9.19)[cd]
Wheat-Maize-Cowpea	60:30:10	170.5(114.2)[c,d]	501.3[d,e,g]	2.94[bcd]	5.15[cd]	33.0(49.3)	6.25(9.33)[cd]
Wheat-Sorghum-Cowpea	60:20:20	164.8(110.4)[c,d]	529.1[b,c,g]	3.21[bc]	5.34[cc]	33.0(49.3)	6.25(9.33)[cd]
Wheat-Sorghum-Cowpea	60:30:10	116.1(111.1)[c,d]	501.3[d,e,g]	3.02[bcde]	5.30[bcd]	33.1(49.5)	4,93(7.37)[e]
Wheat-Cassava-Cowpea	60:20:20	169.8(113.6)[c,d]	501.3[d,e,g]	2.95[bcd]	5.17[cd]	33.1(49.5)	4.64(6.94)[e]
Wheat-Cassava-Cowpea	60:30:10	171.6(114.6)[d]	587.3[b]	3.42[e]	5.11[d]	33.2(49.7)	2.56(3.83)[f]

* Values are means of three baking trials
Figures in parenthesis are values on dry matter basis
Superscripts along columns indicate significant differences among means at P=0.05 in multiple comparison test

TABLE 4. SENSORY EVALUATION SCORES OF THE BREAD SAMPLES MADE FROM DIFFERENT PROPORTIONS OF FLOUR

Flour	Proportion, %	Surface finish	Colour	Taste	Aroma	Texture	Overall acceptability
Wheat (control)	100	8.0[a]	8.2[a]	8.3[a]	8.0[a]	8.4[a]	8.2[a]
Wheat-Maize-Soybean	60:20:20	6.6[b]	7.2[b]	6.5[b]	6.4[b]	6.3[b]	6.5[e]
Wheat-Maize-Soybean	60:30:10	6.4[b]	6.8[b]	6.6[b]	6.7[b]	6.4[b]	6.6[e]
Wheat-Sorghum-Soybean	60:20:20	6.2[b]	6.9[b]	6.6[b]	6.2[b]	6.0[b]	6.5[e]
Wheat-Sorghum-Soybean	60:30:10	5.9[b]	6.5[b]	6.3[b]	6.0[b]	5.7[b]	6.1[f]
Wheat-Cassava-Soybean	60:20:20	5.9[b]	6.8[b]	6.7[b]	6.0[b]	6.4[b]	6.5[e]
Wheat-Cassava-Soybean	60:30:10	6.2[b]	6.8[b]	6.6[b]	6.1[b]	6.2[b]	6.5[e]
Wheat-Maize-Cowpea	60:20:20	7.6[a]	7.5[a]	7.2[b]	7.1[b]	7.4[a]	7.4[b]
Wheat-Maize-Cowpea	60:30:10	7.2[a]	7.6[a]	7.2[b]	6.7[b]	7.1[a,b]	7.2[c]
Wheat-Sorghum-Cowpea	60:20:20	5.4[b]	5.3[c]	6.1[b]	6.0[b]	6.1[b]	5.8[g]
Wheat-Sorghum-Cowpea	60:30:10	5.6[b]	5.6[c]	5.7[c]	5.6[c]	5.7[b]	6.0[f]
Wheat-Cassava-Cowpea	60:20:20	6.1[b]	6.8[b]	6.8[b]	6.3[b]	6.0[b]	6.6[e]
Wheat-Cassava-Cowpea	60:30:10	6.8[a,b]	6.7[b]	6.8[b]	6.6[b]	6.4[b]	6.9[d]

* 25 trained judges scored each bread sample

Means with different superscripts in the same column are significantly different from each other at P=0.05 in Tukey's multiple comparison

add to the protein content of bread (Misra et al. 1991). The observed variations in the after-bake protein were significant (P=0.05), indicating that flour-blending is of vital importance to the bread protein.

Sensory attributes of the composite bread

Internal characteristics : Flour blending : The analysis of variance (ANOVA) results for the means of sensory scores by 25-member panel showed that diverse significant differences existed between the control bread and the CBSs and among the CBSs also (Table 4). For the surface finish, colour and texture, the differences between the wheat-maize-cowpea CBS (i.e., both 60:20:20 and 60:30:10) and the control sample were not significant (P=0.05), unlike in the other attributes.

Despite the superior nutrient status of the soyflour-containing CFMs and CBSs, the cowpea flour-containing CBSs were found to be more highly rated by the sensory panel.

External characteristics : Consumer social class: Results of further statistical evaluation (three-factor ANOVA) showed that, in addition to flour blending, age group and sex of panel member influenced the sensory scoring pattern of composite flour bread (CBSs) (Table 5). These variations due to different age groups were found to have significant effect on colour (P=0.10) and texture (P=0.05), while sex differences caused significant changes in surface finish (P=0.10) only. The interactions of these factors also significantly affected some of the attributes. On age group versus sex (BxC), only surface finish and aroma were affected.

Conclusion

The results from the present work have shown that composite breads fortified with legume flours in different proportions improves the physical, chemical, and sensory qualities. Organoleptic scores showed varied pattern, depending on the social standing of the panelists.

TABLE 5. THREE-FACTOR ANOVA[1] OF SENSORY EVALUATION SCORES FOR COMPOSITE FLOUR BREAD

Sources of variation	Degrees of freedom	SeNSory attributes					
		Surface finish	Colour	Texture	Aroma	Taste	Overall rating
A (flour composting)	12	3.64**	3.66**	3.14**	2.22**	2.20**	2.58**
B (age groups)	2	0.03[NS]	0.41*	3.56**	0.37[NS]	0.47[NS]	0.08[NS]
C (sex)	1	0.86*	0.28[NS]	0.18[NS]	0.13[NS]	0.11[NS]	0.17[NS]
A X B	24	0.35[NS]	0.35**	0.24[NS]	0.26[NS]	0.13[NS]	0.17[NS]
A X C	12	0.40[NS]	0.53**	0.08[NS]	0.32*	0.16[NS]	0.24[NS]
B X C	2	1.62**	0.05[NS]	3.81[NS]	0.70**	1.11[NS]	0.19[NS]
Error	24	0.29	0.15	0.46	0.17	0.47	0.20

[1] Values are mean squares (MS)

* Significant at 10% level

** Significant at 5% level

NS= Not significant

Acknowledgement

The authors gratefully acknowledge the technical and analytical assistance of Messrs C. Ndupuh, 0.0 Ewa and Mrs. U. Obaba.

References

AACC (1962) Approved Methods of Analysis. American Association of Cereal Chemists, 8th edn. St Paul, Minn

Ajayi F (1986) Setback of non-wheat flour in baking. Nigeria Daily Times, July (30), p 5

Akobundu ENT, Ubbaonu CN, Nduphuh CE (1988) Studies on the baking potential of non-wheat composite flours. J Food Sci Technol 25 (4): 211-214

AOAC (1975) Official Methods of Analysis, 12th edn. Association of Official Analytical Chemists Washington, DC

Aykroyd WR, Doughty J (1982) Legumes in Human Nutrition. Food and Agricultural Organisation (FAO), Rome, Italy

Bean MM, Nishita KD (1983) Rice flour for baking application for wheat-sensitive diets. In: Proceedings of the 6th International Congress of Food Science and Technology Vol 3, pp 5-6

Chandrashekara S, Shurpalekar SR (1983) Some chemical, pasting, rheological and textural characteristics of composite flour based on wheat and tubers. J Food Sci Technol 20(6):308-312

D'Appolonia BD (1977) Rheological and baking studies of legume-wheat flour blends. Cereal Chem 54:53-57

DeRuiter D (1978) Composite flours. In: Pomeranz Y (ed) Advances in Cereal Science and Technology. American Association of Cereal Chemists, Vol II, pp 349-385

FAO (1973) Composite Flour Programme Documentation Package Food and Agricultural Organisation Vol 1, 2nd edn (FAO), Rome, pp 85-96

Finney PL, Morad MM, Hubbard JD (1980) Germinated and ungerminated faba bean in conventional U.S. breads made with and without sugar and in Egyptian balady breads. Cereal Chem 57:257-271

Garrad ID (1974) The Story of Food. AVI Publ. Co, Inc, Westport, Conn, USA

Hallgren L (1984) Functional properties of sorghum and wheat in breadmaking. In: Proceeding of the Symposium on Sorghum and Millets: Criteria for Quality of Grains and Products for Human Foods. International Association for Cereal Chemists 11th Congress, Vienna, Austria, p 16

Hayslett HT (1974) One-way analysis of variance: another approach. In: Murphy P (ed) Statistics Made Simple WH Allen, London, pp 202-207

Jeffers HC, Rubenthaler GL, Finney PL, Anderson PD, Bruinsma, BL (1978) Pea: a highly functional fortifier in wheat flour blends. Bakers Digest 52:36-40

Kim JC, DeRuiter D (1968) Bread from non-wheat flours Composite Flour Programme. FAO, Rome

Knight E, Okoro C, Okaka JC (1982) Taste and biological responses of toasted wheat and soy-wheat bread. Nutr Reports Intl 26:827-839

Mabesa LB, Atutubo EO, Daquil MM (1983) Nutritional quality of products from flours of germinated legumes. Phil J Nutri 6:182-187

McConnell LM, Simmonds DH, Bushuk W (1974) High protein bread from wheat-faba bean composite flours. Cereal Sci Today 19:517-521

Misra P, Usha MS, Singh S (1991) Soy-wheat flour blends: chemical, rheological and baking charcteristics. J Food Sci Technol 28(2):89-91

Murray ED, Myers CD, Barker LD, Maurice TJ (1981) Functional attributes of proteins: a non-covalent approach to processing and utilizing plant proteins. In: Stanley DW, Murray ED, Lees DH (eds) Utilization of Protein Resources. Food and Nutrition Inc, Westport, Conn, USA, p 158

Ogunsua AO (1987) Process modifications in the production of bread and biscuit from composite or non-wheat flour. In: Proceedings of Symposium on The Use of Non-wheat Flour for Bread and Biscuit Production, Nigerian Institute of Food Science and Technology, Lagos, pp 84-94

Okaka JC, Potter NN (1977) Functional and storage properties of cowpea-wheat flour blends in breadmaking. J Food Sci 42:828-833

Olatunji O, Akinrele IA (1978) Comparative rheological properties and bread qualities of wheat flour diluted with tropical tuber and bread flours. Cereal Chem 55(1):1-6

Osuntogun A (1987) Policies for self-reliance in the supply of raw materials for the Nigerian baking industry. In: Proceeding of Syposium on The Use of Non-wheat Flour for Bread and Biscuit Production, Nigerian Institute of Food Science and Technology Lagos, pp 11-20

Ott B (1989) Applied Food Science Lab Manual Pergamon Press, Oxford, London

Patel KM, Johnson JA (1974) Horsebean as protein supplement in breadmaking. I. Isolation of horsebean protein and its amino acid composition. Cereal Chem 51:693-697

Patil RK, Shukla BD (1990) Studies on open sun-drying of blanched soybean. J Food Sci Technol 27(2):116-118

Perlmann GE (1977) Encyclopedia of Science and Technology, McGraw-Hill Book Co, New York, p 246

Reiners RA (1978) Zein. In: Peterson MS, Johnson AH (eds) Encyclopedia of Food Science AVI Publ Co, Inc, Westport, Conn, USA

Rychnovsky M (1990) Composite flour: alternative raw materials for making bread. World Grain Magazine 8(1):1-8

Sosulski FW (1977) Concentrated seed proteins. In: Graham HD (ed) Food Colloids. AVI Publ Co. Inc, Westport, Conn, USA

Steel RGD, Torrie JH (1980) Analysis of variance III: Factorial experiments. Principles and Procedures of Statistics-A Biometrial Approach, 2nd edn. McGraw-Hill Intl Book Co, London, pp 336-376

Tsena S, Akinrele IA (1974) Bread and confectionery baking test with composite flour, Nigerian J Sci 8:1-2

Van der Made C (1970) Bread from composite flour. Composite Flour Programme, Vol 1, 2nd edn, FAO, Rome

Watts BM, Ylimaki GD, Jeffery LE, Elias LG. (1989). Basic Sensory Methods for Food Evaluation. Intl Dev Res Centre, Ottawa, Ontario, Canada, pp 60-79

Youssef MM, Bushuk W (1986) Breadmaking properties of composite flours of wheat and faba bean protein preparations. Cereal Chem 63(4):357-361

Received 8 February 1995; revised 25 March 1997; accepted 28 March 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 316-319

Effect of Caseinates on Physico-chemical, Textural and Sensory Properties of Chicken Nuggets from Spent Hens

R.R.B. SINGH*, K.H. RAO, A.S.R. ANJANEYULU, K.V.S.S. RAO, P.C. DUBEY AND P.L.YADAV

Division of Livestock Products Technology,
Indian Veterinary Research Institute, Izatnagar-243 122, India.

Effect of addition of sodium caseinate and calcium caseinate on different quality attributes of chicken nuggets from spent hens was studied. Emulsion stability and pH of the batters containing caseinate were significantly higher than the control. Both sodium and calcium caseinates improved the product yield at 1% level and reduced the frying losses. Moisture and protein contents were significantly higher in samples containing caseinates. Sodium caseinate increased the firmness of nuggets, while calcium caseinate had no effect. Cohesiveness improved significantly in the experimental samples. Flavour, juiciness and texture scores improved in the samples except in the one with 2% sodium caseinate. A typical non-meaty flavour and excessive firmness was noticed in the samples with 2% levels of caseinates. It was suggested that sodium caseinate at 1% level could be beneficially used to improve the quality of nuggets from spent hens.

Keywords: Sodium caseinate, Calcium caseinate, Emulsion quality, Texture profile, Sensory characteristics, Chicken nuggets.

The meat industry is constantly looking for development of ground or emulsion type products for efficient utilization of tough meat from spent animals. A number of non-meat ingredients have been reported to improve the texture, appearance, flavour and nutritive value of comminuted meat products (Keeton et al. 1984; Mir Salahuddin et al. 1991; Hung and Zayas 1992). The non-meat ingredients provide a greater flexibility in formulation and minimise the processing losses, where salt soluble myofibrillar protein is limited due to the use of low quality meat or reduced amount of lean (Mittal and Usborne 1985; HoogenKamp 1985). In comminuted meat products, a considerable amount of fat is released during processing and it essentially requires an efficient ingredient to stabilize the emulsion in order to obtain the end product with acceptable sensory qualities.

Judicious utilization of milk proteins (proteinaceous products from the surplus skim milk of dairy industry) improves the sensory quality and nutritive value of comminuted meat products. The selection of milk protein is guided by the type of meat and variety of the product. Several workers attempted to improve comminuted meat products using caseinates (Jordan 1991; Hung and Zayas 1992; Visser 1984). However, reports on their use in chicken products from spent hens are scanty. Hence, the present investigation was carried out to study the effect of sodium caseinate and calcium caseinate on various quality attributes of nuggets from spent hens.

Spent laying hens were slaughtered at the experimental slaughter house. Hand deboned spent hen meat along with gizzard and heart and natural proportions of skin, abdominal fat and yolk were collected. All the materials were frozen-stored at -10°C and were used after partial thawing at 5°C for 15 h. Meat was cut into small cubes and coarse-minced, using an 8 mm grinder plate. Chicken fat, skin, gizzard and hearts were minced through a 4 mm grinder plate.

Spray-dried sodium caseinate having 81.05% protein, 5.95% moisture and 1.22% sodium content and calcium caseinate having 80.50% protein, 6.16% moisture and 1.54% calcium content were procured from Central Drug House (Pvt) Ltd., New Delhi. All other ingredients used in the formulations were obtained from local market.

Meat emulsions were prepared in a bowl chopper (Model 8418 D Hobart, USA). The nugget formulation (control) had (%) deboned chicken meat 55, SGH mix 15 (skin 77.70% + gizzard 17.57% + heart 4.73%), chicken fat 10, ice flakes 10, yolk 1.8, *maida* 2, salt 1.7, spices 1.7, condiments 2, sugar 0.5, tetrasodium pyrophosphate 0.5 and sodium nitrate 0.15. Experimental formulations were prepared by replacing yolk and *maida* by 1 or 2% sodium caseinate or calcium caseinate. In our preliminary trials, the caseinate formulations imparted bland flavour to the product and hence

* Corresponding Author

the levels of condiments were increased to 3.8% compared to 2% in control formulation.

The meat emulsions of 450 g each was filled in aluminium moulds and were cooked for 30 min, using steam to an internal temperature of 90±1°C, as indicated by the temperature probe. The meat blocks, thus formed, were immediately chilled and sliced into nuggets of uniform size for quality evaluation. The experiments were repeated thrice.

Emulsion stability was determined by the method suggested by Baliga and Madaiah (1970) with some modifications. About 25 g of emulsion taken in accurately pre-weighed small polythene bags was sealed and dipped for 20 min in a temperature-controlled water bath at 80°C. The samples were removed from the bags after draining cook fluid and the loss in weight (g) was recorded. The emulsion stability is inversely related to emulsion cooking loss (in %).

The pH values of emulsions were recorded, using digital pH meter with glass electrode (Model CP 901, Century Instruments Ltd., India) after homogenising 10 g of sample with 50 ml distilled water for 1 min. The moisture, protein and fat contents of chicken nuggets were determined, using standard procedures (AOAC 1980). The slices of chicken nuggets of uniform size (1.5x1.5x10 cm) were fried in refined vegetable oil (Dhara, NDDB, India) at 170°C for 6 min and the loss of weight was expressed as % (w/w) frying loss.

Texture profile analysis of chicken nuggets was performed on the Instron Universal Testing Machine (Model 4301, Instron Corporation, USA). Cylindrical nugget samples (1.90 cm dia, cross sectional area of 2.84 cm^2 and 2.00 cm height) were compressed to 80% of initial height at 15±1°C. Firmness, cohesiveness, springiness, gumminess and chewiness were determined following the procedure of Bourne (1978).

The nugget samples were evaluated for colour and appearance, flavour, juiciness, texture, mouth coating and overall acceptability by a 9 member experienced sensory panel, using 8-point descriptive scale (8–extremely desirable, 1=extremely undesirable). Data from 3 trials each independently designed experiments were statistically analysed by analysis of variance and critical difference between treatment means (Snedecor and Cochran 1968).

Physico-chemical attributes: Emulsion stability of the batters with sodium caseinate was significantly higher ($P<0.01$) than the control and the stability improved with increasing levels (Table 1). Calcium caseinate also improved ($P<0.05$) the emulsion stability, but sodium caseinate appeared to be more effective than the former and this may be due to the difference in their solubility in the emulsion system. The emulsifying ability of the protein derivatives is reported to be dependent on their solubility in restructured meat products (Visser 1984). The reactivity of caseinates lies in the unique distribution of electrical charges on the polymeric molecule and its richness of hydrophilic as well as lipophilic bonding sites. Both sodium caseinate and calcium caseinate significantly increased ($P<0.05$) the pH for obtaining better emulsions.

The product yield significantly improved with the incorporation of sodium caseinate ($P<0.01$) as well as calcium caseinate ($P<0.05$) at 1% level. Higher levels of addition could not improve the yield further, indicating that 1% caseinate was sufficient to reduce cooking loss in nugget formulations from chicken. This could be attributed to the ability of caseinates to bind moisture through H-bonding and entrapment, thereby enhancing the product yield. Hung and Zayas (1991) reported that milk proteins decreased the amount of free water and increased the immobilised water in sausage batter to improve the yield of final product. Interestingly, frying losses of chicken nuggets significantly reduced ($P<0.01$), on addition of caseinates to the formulations. Caseinates thickened the gravy during frying and prevented it running out from product as observed by Salavatulina et al (1983).

Moisture and protein contents of the experimental samples were significantly higher ($P<0.01$) than control. Higher moisture content of nuggets containing caseinates may be attributed to improved hydration, which is in agreement with the observations of Pipek et al (1980), while higher protein contents of nuggets are due to the greater quantity of protein in caseinates (80.50 to 81.05%). Fat contents of nuggets with sodium caseinate and calcium caseinate varied from 12.9 to 13.3% and 12.5 to 12.8%, respectively, but the variation was found non-significant.

Textural properties: Experimental samples with 1 and 2% sodium caseinate showed greater firmness ($P<0.01$) than control. It indicates that the incorporation of sodium caseinate improved the textural properties of the finished product, which agreed with the observations of Hung and Zayas (1992) in frankfurters. In contract, Cserhalmi-ormai and Czukor (1991) reported that Instron hardness

TABLE 1. EFFECT OF CASEINATES ON DIFFERENT QUALITY ATTRIBUTES OF CHICKEN NUGGETS

Quality attributes	n	Sodium caseinate			CD		Calcium caseinate			CD	
		0% Control	1% level	2% level	5%	1%	0% Control	1% level	2% level	5%	1%
Physico-chemical											
Emulsion stability, %	9	7.6±0.02	7.0±0.03	6.6±0.06	0.23	0.31	6.7±0.15	6.4±0.16	6.1±0.15	0.39	—
pH	3	6.3±0.01	6.4±0.01	6.4±0.01	0.03	—	6.3±0.01	6.4±0.01	6.4±0.01	0.03	—
Cooking yield, %	6	95.3±0.38	96.6±0.19	96.3±0.18	0.79	1.09	95.3±0.33	96.2±0.26	96.4±0.28	0.88	—
Frying loss, %	3	5.9±0.08	5.3±0.21	4.4±0.29	0.81	1.23	10.0±0.33	9.2±0.24	8.1±0.47	1.26	—
Moisture, %	6	57.4±0.17	59.0±0.62	64.8±0.34	1.27	1.75	57.2±0.25	58.0±0.32	63.2±0.46	1.03	1.47
Protein, %	6	14.1±0.28	15.2±0.40	15.7±0.23	0.94	1.30	14.5±0.23	15.8±0.25	16.3±0.20	1.02	1.42
Fat, %	6	13.3±0.47	13.0±0.41	12.9±0.39	NS	NS	12.8±0.20	12.8±0.29	12.5±0.30	NS	NS
Texture profile											
Firmness, mN	6	619.70 ±8.47	657.80 ±11.75	725.50 ±12.14	32.85	45.43	548.20 ±9.34	525.60 ±8.04	560.60 ±11.79	NS	NS
Cohesiveness	6	2.60 ±0.04	2.30 ±0.08	2.40 ±0.05	0.19	—	3.60 ±0.11	3.1±0.10	3.10 ±0.06	0.27	0.38
Springiness, mm	6	8.00 ±0.13	8.10 ±0.15	8.20 ±0.11	NS	NS	7.20 ±0.16	7.30 ±0.16	7.30 ±0.21	NS	NS
Gumminess, mN	6	1607.50 ±39.95	1570.70 ±36.83	1760.00 ±36.33	113.60	157.20	1879.60 ±40.23	1653.60 ±41.99	1617.00 ±37.51	120.39	166.49
Chewiness, mN. mm	6	12860.10 ±277.03	13718.90 ±367.98	14080.00 ±236.41	899.91	—	13685.30 ±299.15	11561.00 ±463.78	11214.80 ±311.36	1102.33	1524.44
Sensory scores*											
Colour and appearance	27	7.1±0.11	7.1±0.11	7.2±0.15	NS	—	7.1±0.11	7.0±0.17	7.0±0.17	NS	—
Flavour	27	7.0±0.17	7.3±0.15	6.7[a]±0.15	0.49	—	7.1±0.11	7.2±0.15	6.7[a]±0.15	0.42	—
Juiciness	27	6.8±0.15	7.3±0.15	7.2±0.15	0.45	—	6.8±0.15	7.2±0.15	7.3±0.15	0.45	—
Texture	27	6.8±0.15	7.4±0.18	7.0±0.17	0.48	—	6.9±0.11	7.1±0.11	7.4±0.18	0.40	—
Mouth coating	27	7.3±0.15	7.2±0.15	6.9[d]±0.11	NS	—	7.2±0.15	7.1±0.11	6.8[d]±0.15	NS	—
Overall acceptability	27	7.0±0.17	7.4±0.18	6.8±0.15	0.42	—	7.0±0.17	7.2±0.15	6.9±0.11	NS	—

a= atypical flavour, d= dryness, NS= Non-significant, CD= Critical difference

* Sensory scores based on 8 point descriptive scale, 8= extremely desirable, 1= extremely undesirable.

values significantly lowered in sausages with sodium caseinate. In the present nugget formulations, tetra sodium pyrophosphate (0.5%) was included and this must have improved the firmness of the product. Keeton et al (1984) observed that polyphosphates increased the sensory firmness and Instron hardness in frankfurters. Tetra sodium pyrophosphate appears to have synergestic effect with caseinate to improve the firmness in chicken nuggets. Interestingly, calcium caseinate had no effect on firmness of the finished product.

Cohesiveness of the experimental samples containing sodium caseinate ($P<0.05$) and calcium caseinate ($P<0.01$) significantly improved. This can be attributed to the binding capacity of caseinates. Binding of water and protein in comminuted meat formulations with sodium caseinate was reported to be better than in the formulations with other protein additives (Cserhalmi-ormai and Czukor 1991). Springiness was unaffected by the incorporation of caseinates. Cserhalmi-ormai and Czukor (1991) observed that addition of sodium caseinate decreased the elasticity in sausages, but it maintained the same in canned comminuted meat products, indicating that the effect of protein additives in different types of meat products was different. Gumminess, which depended on both firmess and cohesiveness, did not show any definite trend. Chewiness significantly improved ($P<0.05$) in the samples with sodium caseinate, while calcium caseinate had a negative effect on chewiness of the experimental samples.

Sensory scores: Colour and appearance scores were not affected by the incorporation of both the caseinates. Flavour was slightly improved in nuggets on addition of caseinates at 1% level, but atypical non-meaty flavour was noticed at 2% level of addition, resulting in significant decrease ($P<0.05$) in flavour scores. A strong atypical aroma was also reported by Hung Zayas (1992) in beef frankfurters with 2% sodium caseinate. Juiciness significantly improved ($P<0.05$) in the nuggets at 1% addition

of sodium caseinate and 2% addition of calcium caseinate. Juiciness scores, instead of increasing, adversely affected at 2% level of sodium caseinate, probably due to excessive water absorption. The present observations are in contrast to the findings of Hung and Zayas (1992), who observed that juiciness was significantly less for frankfurters, which were extended with different milk proteins.

Salavatulina et al (1983) reported that juiciness was unaffected, till sodium caseinate exceeded 5% level. Texture of chicken nuggets significantly ($P<0.05$) improved at different levels of addition of caseinates, except in samples with 2% sodium caseinate. The nuggets containing 2% caseinates were excessively firm and hence rated lower. These sensory observations on texture are in confirmity with the Instron texture profile analysis of the product. No differences were found in case of mouth coating in the samples extended with different levels of caseinates. However, dried mouth feel was noticed at 2% addition of caseinates, agreeing with the reports of Comer et al (1986). Overall acceptability scores of the nuggets prepared from formulations with 1% sodium caseinate were significantly ($P<0.05$) higher than the control, while 2% level had a negative effect on acceptability score. The experimental samples with calcium caseinate were not significantly different from the control with respect to overall acceptability.

On the basis of physico-chemical quality, textural analysis and sensory scores, it could be concluded that sodium caseinate at 1% level could be used to advantage in improving the overall quality of nuggets from spent hens. Sodium caseinate performed better than calcium caseinate in formulations for chicken nuggets.

References

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

Baliga BR, Madaiah N (1970) Quality of sausage emulsion prepared from mutton. J Food Sci 35(4):383-385

Bourne MC (1978) Texture profile analysis. Food Technol 32(7):62-66

Comer FW, Chew N, Lovelock L (1986) Comminuted meat products: Functional and microstructural effects of fillers and meat ingredients. Can Inst Food Sci Technol J 19(2):68-74

Cserhalmi-ormai ZS, Czukor B (1991) Protein additives effect on textural properties of comminuted meat products. In: Proceedings of 37th International Congress of Meat Science and Technology. Kulmbach, Germany, Vol 2, pp 704-708

HoogenKamp H (1985) Super emulsions. Meat Processing 24(2):32-36

Hung SC, Zayas JF (1991) Emulsifying capacity and emulsion stability of milk proteins and corn germ protein flour. J Food Sci 56(5):1216-1218

Hung SC, Zayas JF (1992) Functionality of milk proteins and corn germ protein flour in comminuted meat products. J Food Quality 15:139-152

Jordan P (1991) Milk proteins have a competitive edge. Meat International 1(1):18-21

Keeton JT, Foegeding EA, Patana-Anake C (1984) A Comparison of non-meat proteins, sodium tripolyphosphate and processing temperature effects on physical and sensory properties of frankfurters. J Food Sci 49(6):1462-1465

Mir Salahuddin, Kondaiah N, Anjaneyulu ASR (1991) Effect of *maida*, potato and textured soya as binders on the quality of chicken and mutton *kababs*. J Food Sci Technol 28(5):301-303

Mittal GS, Usborne WR (1985) Meat emulsion extenders. Food Technol 39(4):121-130

Pipek P, Klein S, Votrubec B, Brezina (1980) Effect of sodium caseinate addition on the binding of meat. Prumysl Potravin 31(12):670-673

Salavatulina RM, Gorbutov VM, Aliev SA (1983) Effect of milk protein concentrates on the quality and yield of boiled sausages. Myasnaya Industriya 3:34-37

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

Visser EMW (1984) Milk proteins in meat products and soups or sauces. Milk proteins-84. In: Proceedings of International Congress on Milk Proteins, Pudoc, Wageningen, The Netherlands, pp 206-216

Received 1 December 1995; revised 20 January 1997; accepted 11 February 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 320-323

Physico-chemical Characteristics of Extruded Snacks Prepared from Rice (*Oryza sativa.* L) and Chickpea (*Cicer arietinum.* L) by Single Screw Extrusion

S. BHATTACHARYYA, P. CHAKRABORTY*, D.K. CHATTORAJ AND S. MUKHERJEE

Department of Food Technology and Biochemical Engineering,
Jadavpur University, Calcutta-700 032, India.

Rice and chickpea blends were used to prepare snack products. On increasing the proportion of chickpea in the blends, the diametric expansion (DE) and water holding capacity (WHC) decreased, while peak shear force (PSF) and bulk density (BD) increased. Sodium dodecyl sulphate (SDS) plus 2-mercaptoethanol (ME), containing phosphate buffer (pH 6.9) were found to extract more protein than SDS or plain buffer solution from the extrudates. Loss of carbohydrates was documented after extrusion of feeds, containing >50% carbohydrates and >30% proteins. In products with high DE and low PSF, air cells were predominant and smooth surface morphology was evident as observed from the scanning electron microscopy (SEM).

Keywords: Extrudate, Rice, Chickpea, Water holding capacity, Protein solubility, Scanning electron microscopy.

Snacks contribute an important part of many consumers' daily nutrient and calorie intake (Tettweiller 1991). Soybean has been a popular feed material for extrusion because of its low cost, ready availability and good functional properties (Harper 1981). Extrusion of rice-soy blend has been found most suitable for puffed product (Patil et al. 1990). In this study, a cereal (rice) and a high protein legume (chickpea) were used for the production of extruded snacks, since such a combination would have higher protein content of superior nutritional quality over the traditional corn or rice snack. The nutritive value of chickpea protein is superior as compared to soybean, cowpea, lentils, faba bean, pigeon pea and blackgram (Chandrasekharappa 1979) and further, it supplements the deficient essential amino acids of rice (Chakraborty et al. 1987). The objectives of the present study were (a) to examine the changes of textural properties of extrudates, (b) to compare the protein solubility in different extracting solution and carbohydrate content of unextrudate and extrudate and (c) to examine the surface morphology of the extrudates through scanning electron microscopy.

Rice and chickpea obtained from local market, were ground to 20 mesh size. The rice and chickpea flours, thus obtained were blended in the ratios of 100:0 (set A); 90:10 (set B); 70:30 (set C); 50:50 (set D); 30:70 (set E) and 10:90 (set F) and used for experimentation. The blended mixtures tempered with water to a moisture content of 15%, were packed into polyethylene bags and equilibrated at 4°C for 24 h. These samples were extruded after the blended products attained room temperature with a single screw Brabender 20DN extruder mounted to Do-corder DEC 330. The extruder operation conditions were fixed at: 160 rpm screw speed, 4:1 screw compression ratio, 6 mm die dia, 28 g/min. feed rate. Temperature profile in the barrel zones towards the die were 120°C, 160°C and 180°C, respectively. The extruder barrel had a dia of 19 mm with a 20:1 length: dia ratio and 8 longitudinal grooves. The extrudates were collected, when the operation condition was at steady-state.

A vernier caliper was used to measure the mid-point diameter of 10 extruded samples collected from each run. Expansion was determined by dividing the mid point dia by the dia of the die hole. Residual moisture contents of extrudates were determined by drying in a cabinet drier at 110°C for 5 h, using duplicate sample of 1 g each. Water holding capacity (WHC) of samples was measured according to the method of Bhattacharya and Hanna (1988). The product specific volume was measured (in triplicate) by sand displacement procedure (Park 1976) and was calculated as the extrudate volume by the sample weight. The values of bulk density (BD) were calculated from the values of product specific volume and expressed in g/100 ml. An Instron Universal Testing Machine (Model No. 4301) with a Warner-Bratzler shear device was used to obtain PSF of the samples at test conditions: cross head speed 25 mm/min. chart speed 50 mm/min. load cell 50 N, sample length 10 cm and number of replicates 10. Carbohydrate was measured colorimetrically according to the method of Dubois et al (1956). Initial and extruded protein samples (1g each) were extracted either with 0.1M phosphate buffer solution

* *Corresponding Author*

TABLE 1. PROXIMATE COMPOSITION OF RICE AND CHICKPEA (DRY BASIS)

Food stuff	Protein, %	Fat, %	Fibre, %	Carbohydrates, %		Ash, %
				Amylose	Amylopectin	
Rice, raw, milled	8.44	1.15	0.23	22.63	66.53	1.02
Chickpea	23.08	6.22	1.38	13.41	52.91	3.02

N x 5.7 for rice and N x 6.25 for chickpea

The standard deviation for result is approximately 2% of the value in the Table

(pH 6.9) or with a buffer solution containing sodium dodecyl sulphate, (SDS), an agent known to disrupt non-covalent interaction or with a buffer solution with 1% SDS plus 1% 2-mercapto ethanol (ME), an agent known to reduce disulphide bonds for 5 h at 37°C. After centrifugation (1500 x g, 20 min., 4°C), the protein content of the supernatant was determined, using Folin-phenol reagent (Lowry et al. 1951) and was expressed as bovine serum albumin equivalent. SEM of the samples was done in Hitachi make machine model No. S415A, after coating the samples with gold by sputtering technique upto a thickness of 150Å under ion current of 10 mA and ionization voltage of 1400V (DC) in a IB2 model ion coater manufactured by Eikoeng, K.K., Japan. The proximate composition of raw material (feed components) was determined by AOAC (1984) procedures to visualize the proportions of the important constituents of rice and chickpea blends (Table 1). Bulk density (BD) and diametric expansion (DE) indicate the extent of expansion resulting from extrusion. BD, which was lowest for set A, increased steadily upto set F, accompanied with a steady decrease of DE values (Fig. 1). Since, the fat content of 1% in set A blends was gradually increased to 5.14% in set F, there was a gradual but increased interruption of the cohesive matrix or lubrication of the barrel and screw surface of the extruder during cooking of sets A to F, resulting in less puffed product as

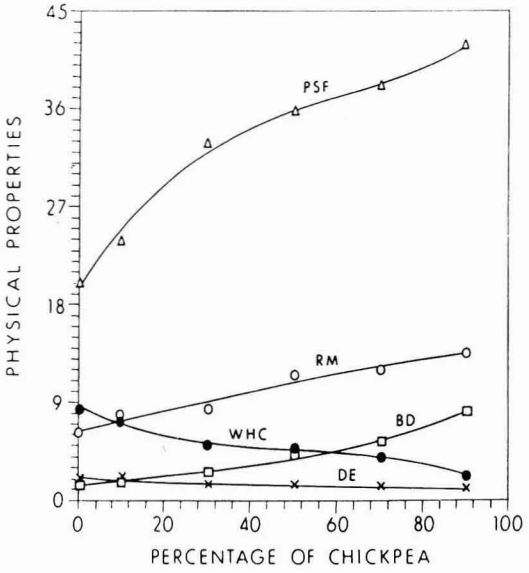

Fig. 1. Effect of increasing percentage of chickpea on the physical properties of extrudates. (PSF in Newton; RM in %; WHC in g of sample/g of H_2O); BD in g/100 ml)

reported earlier by Maxwell (1988). The BD values indicated that they were directly proportional to the fat content and inversely proportional to the carbohydrate content of the respective sets. PSF, which is the textural parameter related to puffing characteristics of the extrudates, showed lower but increasing values in sets A and B, while there was a dramatic increase in set C, followed by gradual increase upto set F (Fig. 1). A linear but inverse relation was also observed between PSF and DE (Fig. 1). Having less than 2.4% fat and balanced amylose-amylopectin ratio, extrudates of sets A, B and C expanded much, as evidenced by high water holding capacity values of these sets. From these data (Table 2), it is clear that extrusion of the rice-chickpea blends, leads to a marked decrease in the solubility of the protein constituents. Reduction in solubility is principally due to (i) non-covalent

TABLE 2. PROTEIN SOLUBILITY IN DIFFERENT EXTRACTING SOLUTIONS AND CARBOHYDRATE CONTENT OF UNEXTRUDATE AND EXTRUDATE

Sets	Unextrudate				Extrudate			
	% Protein solubility in			Carbohydrate, %	% Protein solubility in			Carbohydrates, %
	PB	PB+SDS	PB+SDS+ME*		PB	PB+SDS	PB+SDS+ME	
A	1.36	1.89	3.45	79.23	0.66	0.96	1.32	75.00
B	2.68	2.88	4.03	77.26	0.57	1.26	2.42	73.33
C	3.28	4.76	5.01	73.45	0.65	1.45	3.04	64.68
D	3.85	5.08	5.77	69.59	1.25	3.58	4.29	63.47
E	4.28	5.15	6.16	65.74	1.79	4.00	4.74	60.69
F	5.09	5.77	6.17	61.89	1.91	4.01	5.28	61.61

*PB-phosphate buffer, SDS=sodium dodecyl sulphate, ME=2- mercapto ethanol

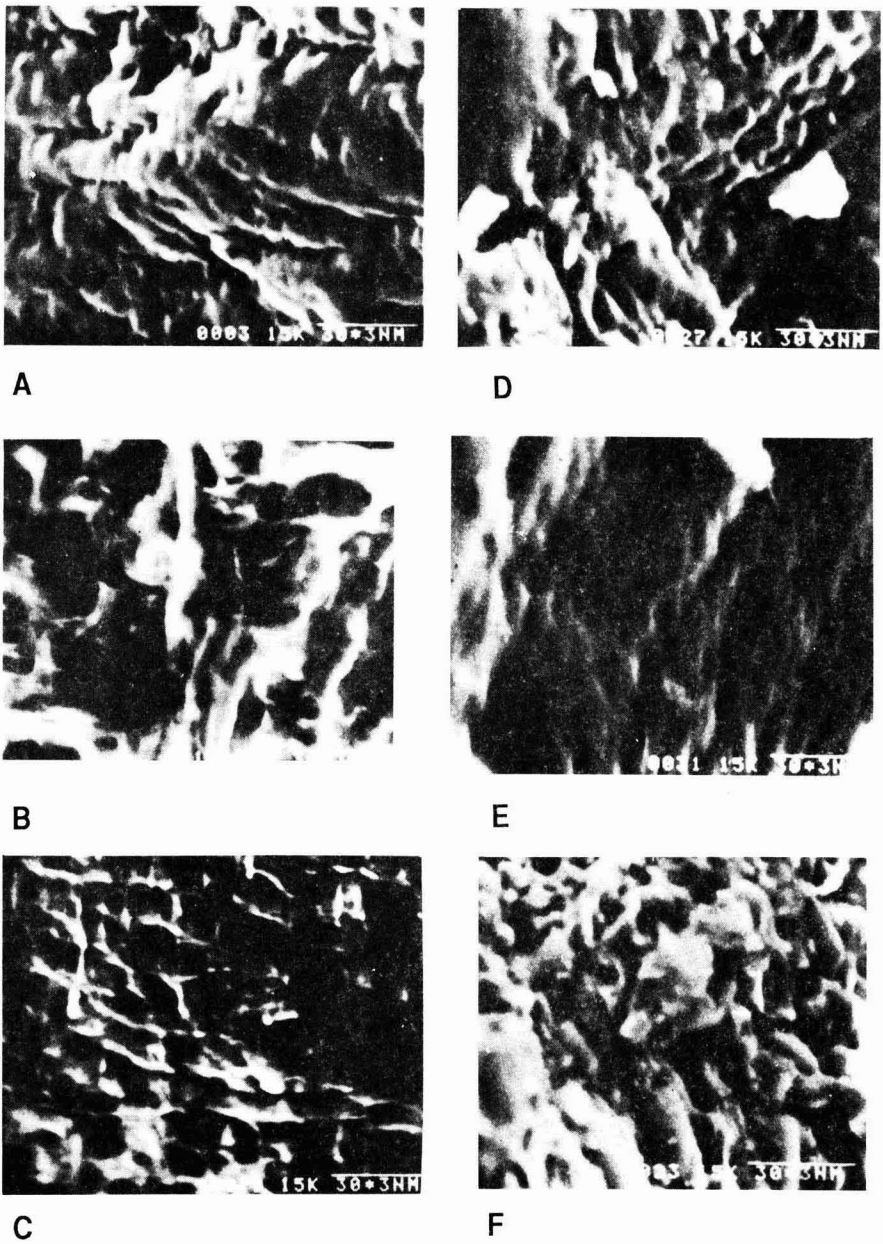

Fig. 2. Scanning electron micrographs of different sets at 1000 magnification (A = Set A; B = Set B; C = Set C; D = Set D; E = Set E; F = Set F)

interaction between polypeptide chains themselves and other constituents. Since more protein is soluble in SDS containing buffer than in plain buffer and (ii) due to the formation of new disulphide bonds either by interchange reaction or by cystine formation from cysteine, since more protein is soluble in SDS plus 2-ME buffer than in SDS buffer of the respective sets. Carbohydrate contents of the feeds and their extrudates of the respective sets as given in Table 2 indicate the loss of carbohydrate, especially in sets C and D extrudates from unextrudate values due to Maillard reaction. SEM revealed the microstructural feature of set A, which showed the numerous air cells with unequal cell wall thickness (Fig. 2A) and an elongated and parallel carbohydrate layer, resulting

in low BD and PSF and high DE values of the extrudate. A semi-continuous matrix with scattered disruption and lumpy structure of gelatinized starch components was observed in the micrograph of set B (Fig. 2B). Discontinuous structure having thick air cell wall (Fig. 2C) in set C resulted high PSF and BD in comparison to sets A and B. Rope-like protein matrix with very small number of incompletely developed air cells were apparent in the micrograph of set D (Fig. 2D). While micrograph of set E showed extremely dense structure with very small air cells and a fibrous protein matrix with a well define gaps in between the fibre layer (Fig. 2E) resulting in their low DE values, micrograph of set F showed discontinuous protein matrix, which was free from any air cells, leading to very low DE and WHC and very high PSF and BD values.

The first author is grateful to University Grants Commission, New Delhi for the award of research fellowship under DSA programme.

References

AOAC (1984) Official Methods of Analysis, 14th edn, Association of Official Analytical Chemists, Washington, DC

Bhattacharya M, Hanna, AM (1988) Extrusion processing to improve nutritional and functional properties of corn gluent. Lebensm. Wiss., u. -Technol 21:20-24

Chakraborty P, Mukherjee S, Roy A, Sengupta T (1987) Physico-chemical characteristics of legumes and oilseeds protein. J Surf Sci Tech (International) 3(2):105-114

Chadrasekharappa G (1979) Nutritional quality of the protein of blends of wheat and rice with *Bengal*gram, redgram or blackgram. Nutr Rep Int 18:401-403

Dubois M, Gillers KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350-356

Harper JM (1981) Extrusion of Food. CRC Press Inc, Boca Raton, FL USA 1:1-19

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin-phenol reagent. J Biol Chem 193:265-268

Maxell D (1988) The impact of ingredients upon extruder performance. Prepared Foods 8:177-179

Park KH (1976) Elucidation of the extrusion of puffing process. Ph.D. Thesis, University of Illinois, Urbana,Il

Patil RT, Singh DS, Tribel Horn (1990) Effect of processing conditions on extrusion cooking of soy-rice blend with a dry extrusion cooker. J Food Sci Technol 27(5):376-378

Tettweiler P (1991) Snack foods for worldwide. Food Technol 45(2): 58–61

Received 27 November 1995; revised 17 January 1997; accepted 12 February 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 324-327

Evaluation of Heat-shrinkable Film-wrapping of 'Nagpur mandarin' (*Citrus reticulata.* Blanco) for Storage

M.S. LADANIYA*[1], R.K. SONKAR AND H.C. DASS

National Research Centre for Citrus, Amravati Road, Nagpur-440 010, India.

Polyethylene and 'Cryovac' (Polyolefin material) heat-shrinkable films were evaluated for individual and tray-wrapping of bavistin-treated 'Nagpur mandarin' fruits to extend their shelf life at ambient (30-35°C; 25-35% RH) and refrigerated (6-7°C; 90-95% RH) conditions. Unwrapped fruits were unacceptable (>30% weight loss) after 3 weeks at ambient condition, while polyethylene (25 and 11 μm) and 'Cryovac' (BDF 2001 and D 955) film-wrapped fruits were firm and fresh (2-7% weight loss). Perforated (SM 250) film resulted in excessive shrivelling of fruits. Wrapping reduced weight loss during 45 days of refrigerated storage and also during post-storage holding period of 2 days. Bavistin (4000 ppm) treatment before wrapping minimized decay. Individually wrapped fruits had less decay as compared to tray-wrapped fruits. 'Cryovac' (BDF 2001 and D 955) films had better gloss, strength and clarity as compared to polyethylene. Deformation was less in film-wrapped fruits as compared to unwrapped ones. Flavour was significantly better in polyethylene, BDF 2001 and D955 film-wrapped fruits after 3 weeks at ambient condition, when compared to SM 250 film-wrapped and unwrapped fruits.

Keywords: 'Nagpur mandarin', Bavistin, Heat-shrinkable film-wrapping, weight loss, Decay, Fruit quality, Storage.

Seal-packaging of individual citrus fruits with shrinkable polyethylene film, successfully doubling the shelf-life, was first reported by Ben-Yehoshua (1978). Since then, several workers reported usefulness of shrink-wrapping in citrus fruits (Kawada and Kitagawa 1988; Zhou et al. 1990).

Delaying the deterioration of 'Nagpur mandarin', a commercially important loose jacket orange from Central India, is a prime necessity, considering its hot and dry ambient conditions. Although packaging of citrus fruit in polyethylene was reported to preserve their freshness (Choudhari and Kumbhare 1979; Ramana et al. 1988), the reports on seal-packaging particularly in 'Nagpur mandarin' are not available. The packaging of citrus fruits in polyethylene was observed to increase decay (Grierson 1969). However, reports are contradictory about shrink-film-wrapping (Ben-Yehoshua 1978; Kawada and Albrigo 1979). Eckert et al (1984) suggested fungicide treatment before shrink-wrapping. Bavistin (Carbendazim 50% WP) has been found to be effective against storage decay in 'Nagpur mandarin' and concentration of 4000 ppm was necessary in spray application as against 1000 ppm in dip treatment to get equal efficacy (Naqvi 1994). The present investigation was undertaken to evaluate heat-shrinkable film-wrapping combined with carbendazim (Bavistin) treatment for extending the shelf life of 'Nagpur mandarin' at ambient and refrigerated storage conditions.

* *Corresponding Author, Present address: NRC for Citrus, PB No. 464, Shankar Nagar PO, Nagpur-440 010*

During the 1994 'Mrig' (Monsoon blossom) crop season, mature 'Nagpur mandarin' fruits were spot picked, sorted out for uniform orange colour, size (nearly 6.5 to 7.0 cm dia), shape, free from blemishes and injuries and then subjected to fungicide treatment, shrink-wrapping and storage.

Fungicide treatment: Out of the two lots of fruits, one lot was washed with chlorine (1000 ppm) water, rinsed, treated with bavistin (4000 ppm) through sprayer on conveyor belt covering the stem-end and entire fruit surface. After surface - drying, treated and untreated fruits were taken for shrink wrapping.

Shrink wrapping: A sealer (Weldotron model 6300-L) was used to loosely pack the films around the fruits before wrapping in a heat shrink tunnel (Weldotron model 7121A). The polyethylene (LDPE, 25 and 11μm thick) and 'Cryovac' films (BDF 2001 multi-layered co-extruded polyolefin 30 μm thickness; D 955 multi-layered cross-linked polyolefin 15 μm and 25 μm thickness and SM 250 suitably oriented polyolefin 35 μm thickness with microperforations) were shrink-wrapped around the fruit individually and as tray over-wrap. Polyethylene and 'Cryovac' (BDF 2001 and D 955) films were shrink-wrapped at 106°C in 30 sec, while SM (250) film was wrapped at 147°C in 20 sec. Unwrapped fruits were compared with individually and tray-wrapped fruits. Fruit temperature was measured at inlet and outlet of the heat tunnel with Telethermometer (Model 43 TD, Yellow Springs Instruments Co. Ohio, USA), using stainless steel probe (No. 418).

Storage: Fruits were kept in vented (5% surface area on sides punched) corrugated fibre board boxes measuring 39 cm (L) x 23 cm (W) x 15 cm (H) and stored under ambient (30°-35°C; 25-35% RH) conditions for 3 weeks and refrigerated (6°-7°C; 90–95% RH) conditions for 45 days. Fruits kept at refrigerated condition were evaluated after holding for 2 days at ambient condition. Fruit weight loss and decay during storage were expressed as percent losses. General appearance and flavour were evaluated by a panel of 15 trained judges in the laboratory. Flavour scale ranged from 1 for very poor to 5 for very good.

Each treatment included 10 individually wrapped fruits and over-wrapped trays (one dozen fruits) with two replications. The data were subjected to analysis of variance.

With the time-temperature combinations as mentioned above for various films, there was no burn injury to the fruits. Seal quality was not affected and fruit pulp temperature did not rise more than 2°C.

Weight loss: The weight loss was significantly less in all shrink-wrapped fruits as compared to unwrapped and SM 250 film wrapped fruits during ambient and refrigerated storage (Table 1). The higher weight loss in SM 250 film at ambient conditions can be attributed to higher temperature (30-35°C), lower RH (25-35%) and microperforations (0.1 mm^2 perforation size; 9 perforations/cm^2) on this film with water vapour transmission rate (WVTR) 250g/sqm/24h Among 'Cryovac' films, BDF 2001 recorded least weight loss probably due to minimum WVTR, i.e., 12.6g/sqm/24 h followed by D 955 (25 µm thickness) film (13g/sqm/24 h). Shrink, wrapping with polyethylene, BDF 2001 and D955 films also minimized weight loss significantly during and after refrigerated storage. Almost 8 to

TABLE 1. EFFECT OF HEAT-SHRINKABLE FILM WRAPPING AND BAVISTIN TREATMENT ON WEIGHT LOSS AND DECAY OF 'NAGPUR MANDARIN' FRUITS STORED AT AMBIENT AND REFRIGERATED CONDITIONS

	Ambient condition, 3 weeks				Refrigerated condition, 45±2 days*			
	Individually wrapped		Tray wrapped		Individually wrapped		Tray wrapped	
Treatment	Weight loss, %	Decay %	Weight loss, %	Decay %	Weight loss, %	Decay %	Weight loss, %	Decay %
PE (25 µm)	3.9	nil	3.6	8.3 (10.3)	1.5	10.0 (17.8)	0.9	nil
Bavistin+PE (25 µm)	4.8	nil	3.6	nil	1.7	nil	0.9	4.1 (11.6)
PE (11 µm)	4.6	nil	6.3	4.1 (11.6)	1.6	20.0 (25.8)	1.2	25.0 (29.8)
Bavistin+PE (11 µm)	4.3	5.0 (12.9)	6.3	nil	1.9	10.0 (17.8)	1.3	nil
BDF 2001 (30 µm)	5.9	20.0 (26.5)	3.3	12.5 (19.7)	2.1	35.0 (36.2)	1.5	12.5 (19.7)
Bavistin+BDF 2001 (30 µm)	2.8	nil	1.7	nil	1.2	5.0 (9.2)	1.1	nil
D 955 (25 µm)	7.1	nil	2.4	16.6 (21.8)	2.6	15.0 (22.7)	1.0	20.8 (23.2)
Bavistin+D 955 (25 µm)	5.0	nil	3.0	4.1 (11.6)	1.8	5.0 (12.9)	1.3	4.1 (11.6)
D 955 (15 µm)	7.8	nil	4.9	4.1 (11.6)	2.0	10.0 (17.8)	1.5	16.6 (21.8)
Bavistin+D 955 (15 µm)	5.2	nil	6.2	nil	1.8	10.0 (17.8)	1.1	nil
SM 250 (35 µm)	39.4	nil	29.6	16.6 (21.8)	13.0	10.0 (18.4)	11.4	8.3 (16.6)
Bavistin+SM 250 (35 µm)	37.7	5.0 (12.9)	31.8	8.3 (16.6)	13.4	5.0 (9.2)	9.9	nil
Unwrapped	40.7	5.0 (12.9)	43.0	29.1 (30.8)	16.1	5.0 (9.2)	13.3	nil
Bavistin+unwrapped	39.2	nil	49.2	25.0 (29.8)	15.5	nil	13.0	25.0 (29.8)
CD at 5% level	2.5	7.4	5.9	16.3	2.4	17.0	0.9	14.8

*Storage at refrigerated conditions for 45 days followed by 2 days at ambient condition

Figures in parenthesis are Arisin transformed values

10 times reduction in fruit weight loss with shrink-wrapping was also reported by Hale et al (1986) but with other films.

Decay: Bavistin (4000 ppm) treatment significantly minimized decay in shrink-wrapped fruits under ambient and refrigerated storage conditions. (Table 1). Higher incidence of decay in tray-wrapped fruits as compared to individual fruit wrapping under ambient storage was possibly due to very high humidity (95-100%) and congenial temperature (30-35°C) inside the wrapped film, leading to growth of fungal pathogens and their spread. However, in individual seal packed fruit, decay was contained within the pack. Effect of various films on decay level was not clear although decay was higher in films with higher thickness, particularly at ambient storage conditions. Further large scale trials are warranted to confirm these inferences. Studies with lemons indicated that shrink-wraps did not increase infection by various pathogens and in fact, appeared to reduce decay (Eckert et al. 1984). Possibility of minimizing decay in shrink-wrapped fruit with pre-harvest carbendazim sprays needs to be explored, as field infection was considered to play a major role in the extent of decay. The decay losses were found to be reduced by pre-harvest treatments (Naqvi 1993). The carbendazim treatment also controlled *Penicillium* rots, besides stem-end rots. However, it was ineffective against sour rot (*Geotrichum* spp), which invaded through wounds during handling. Under these circumstances, it would be prudent to effectively use available fungicides in pre and post-harvest treatments, coupled with careful handling and due care of fungicide residues. While 0.4 ppm carbendazim residues were reported with 3 pre-harvest sprays of bavistin (1000 ppm) in 'Nagpur mandarin' (Naqvi 1993), the post-harvest dip treatment with carbendazim (1250 ppm) resulted in 4 ppm and 0.1-0.7 ppm residues on orange peel and its interior, respectively (BASF 1981). The Indian tolerance limits for carbendazim in fruits is 5 ppm (Voluntary Health Association of India 1993), (Personal communication). Residue hazard is further reduced in loose jacket orange, as the peel is removed before consumption.

General appearance and flavour: Individual and tray-wrapped fruits in polyethylene, BDF 2001 and D955 films appeared fresh and firm after ambient and refrigerated storage. (Table 1). The 'Cryovac' films provided eye-catching sparkle to the fruit. Tray-wraps appeared very attractive due to clarity of BDF 2001 and D955 films. There was no deformation in shrink-wrapped fruits. Unwrapped and SM 250 film-wrapped fruits were shrivelled and turned dark brown with tough peel after 3 weeks at ambient condition. The shrivelled fruits recorded significantly low flavour rating of 1.0 to 2.5 (unacceptable) against 3.0 to 4.0 (fair to good) in polyethylene, BDF 2001 and D955 film-wrapped fruits at ambient condition. Excessively dry peel in wrapped and SM 250 film-wrapped fruits might have possibly hindered normal gas exchange, resulting in off-flavour. Unlike ambient condition, higher humidity and lower temperature considerably minimized shrivelling in unwrapped and SM 250 film-wrapped fruits under refrigerated condition. Shrink-wrapping with polyethylene, BDF 2001 and D955 film reduced shrivelling of fruits during and after refrigerated storage.

In conclusion, it can be stated that wrapping of 'Nagpur mandarin' fruits in heat-shrinkable polyethylene (25 and 11 µm), BDF 2001 (30 µm) and D955 (15 and 25 µm) films after chlorin (1000 ppm) wash and bavistin (4000 ppm) spray treatment extended the shelf-life upto 3 weeks at ambient condition and reduced the weight loss during and after refrigerated storage without losing their natural appearance and flavour.

References

BASF (1981) Bavistin. BASF India Ltd, Bombay, pp 1-198

Ben-Yehoshua S (1978) Delaying deterioration of individual citrus fruit by seal packaging in film of high density polyethylene - General effects, In: Cary PR (ed), Proceedings of the International Society of Citriculture, Sydney, Australia, pp 110-115

Choudhari KG, Kumbhare GB (1979) Post-harvest life of sweet orange (*Citrus sinensis*. Osbeck) as influenced by various polyethylene film packs. J Maharashtra Agric Univ 4:228-229

Eckert JW, Sievert JR, Ratnayake M (1984) Decay of lemons in plastic wraps. Citrograph 70(1):11-14

Grierson W (1969) Consumer packing of citrus fruits. In: Proceedings of the First International Citrus Symposium, Riverside, California, pp 1389-1401

Hale PW, Miller WR, Smoot JJ (1986) Evaluation of a heat shrinkable copolymer film coated with imazalil for decay control of Florida grapefruit, Trop Sci. 26:67-71

Kawada K, Albrigo LG (1979) Effects of film packaging in carton air filters and storage temperatures on the keeping quality of Florida grapefruits. Proceedings of the Florida State Horticultural Society 92:209-212

Kawada K, Kitagawa H (1988) Plastic film packaged storage of citrus and some other fruits in Japan. In: Goren R, Mendel K (eds), Proceedings of the Sixth International Citrus Congress. Tel Aviv, Isrel, pp 1555-1565

Naqvi SAMH (1993) Pre-harvest applications of fungicides in Nagpur mandarin orchards to control post-harvest storage decay. Indian Phytopath 46(2):190-193

Naqvi SAMH (1994) atudies on post-harvest diseases of Nagpur mandarin (*Citrus reticulata* Blanco) and their control. National Research Centre for Citrus, Nagpur, Annual Report for 1993-94, pp 52-53

Ramana KVR, Rodrigues R, Vijayendra Rao AR (1988) Packaging of fresh fruits and vegetables, In: Souvenier, National Seminar on Packaging of Fruits and Vegetables in India. Agri-Horticultural Society, Hyderabad, pp 31-38

Zhou BY, Wang YY, Li BJ (1990) Investigations on storage of loose skin mandarin fruits sealed individually in plastics film. In: Huang B, Yang Q (eds), Proceedings of the International Citrus Symposium, Guangzhou, China, pp 791-795

Received 17 December 1995; revised 13 March 1997; accepted 19 March 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 328-330

Nutritional and Cooking Evaluation of Greengram (*Vigna radiata.* L. Wilezek) Cultivars

ANITA KOCHHAR* AND CHARANJEET K. HIRA

Department of Foods and Nutrition,
Punjab Agricultural University, Ludhiana-141 004, India.

Thirtytwo greengram genotypes were evaluated for protein, sulphur, S-containing amino acids, tryptophan and cooking quality. Protein and sulphur contents ranged from 18.12-26.87 and 0.137-0.276%, respectively. Mean methionine, cystine and tryptophan contents were 1.54, 0.88 and 1.13 g/16g N, respectively. Genotype variations for all characters studied except for sulphur were significant (P<0.01). Protein content was significantly associated with methionine (r= −0.497**), cystine (r= −0.424**) and tryptophan (r= 0.630**). Grain weight was found to be associated with cooking time (r= 0.427*) and solid dispersion (r= 0.388*). The genotypes 'Russian', 'MUG-125' and 'MUG-121' were found to be better and could be exploited to select desirable characters.

Keywords: Greengram, Protein, Sulphur, Amino acids, Cooking quality.

Among the grain legumes, greengram (*Vigna radiata.* L) is known for its easy digestibility, low flatulence potential and high protein content (Doughty and Walker 1982). At national level, efforts are being made to evolve new high yielding varieties of legumes, including greengram to increase per capita pulse availability, which, in turn, will improve the dietary quality of the population. In addition to quantitative yield, nutritional and cooking quality characters are important from consumer point of view. In terms of nutritional quality, protein content and limiting amino acids of legumes are of significance due to their supplementary effect on cereal grains. Cooking legumes in boiling water or steam is the commonest method used in most parts of the world. The shorter the cooking time required to make beans soft and greater the expansion in volume, the higher is the acceptability (Shivashankar et al. 1974; Neelakantan et al. 1977). The present study pertains to nutritional and cooking evaluation of 32 greengram cultivars grown at farms of Punjab Agricultural University, Ludhiana.

Thirtytwo greengram cultivars grown during 1989-90 under similar agroclimatic conditions were procured from the Department of Plant Breeding, Punjab Agricultural University, Ludhiana. The grains were fully mature, unbroken and free from disease and infestation. To determine cooking time, 5 g of each sample was boiled in 40 ml distilled water with continuous stirring. Cooking of seeds was judged visually by pressing between two glass slides for softness and cooking time noted. The excess cooking water was decanted to determine dissolved solids, as mentioned by Narasimha and Desikachar (1978). Weight of the cooked samples was recorded to calculate water absorption.

Samples were finely ground to 100 mesh, dried and analysed in duplicate. Nitrogen was estimated by macro-Kjeldahl method (AOAC 1980) and protein was calculated by multiplying by the factor 6.25. Sulphur was estimated after wet digestion, using gravimetric method (Raghuramulu et al. 1981). Cystine and methionine were estimated, using colorimetric methods of Liddle and Saville (1959) and McCarthy and Sullivan (1941), respectively. For tryptophan estimation, the method of Smith and Agiza (1951) was followed. The data were statistically analysed, using analysis of variance and correlation coefficient were worked out, wherever applicable.

The data presented in Table 1 revealed more than two-fold variations in 100 grain weight of cultivars with a range of 2.13 g ('MUG 125') to 4.57 g (VC 1482 Selc') and genotypic differences were significant (P<0.01). Similar variations in seed weight i.e., 2.46-4.01 g for 9 strains of greengram have been reported by Rosaiah et al (1993). The range for cooking time was recorded to be from 30 min ('Russian' and 'ML-80 x ML-131') to 37 min ('MUG-121') with significant differences (P<0.01) among cultivars. Vimala and Pushpama (1987) have reported cooking time of 27-49 min, while Rosaiah et al (1993) and Hira et al (1988) have reported cooking time of 32-48 min and 41 min for greengram, respectively. Percent water absorption of cultivars ranged from 76.85 ('ML-125 x ML-5') to 130.12 ('ML-80') with a mean of 104.67 and significant (P<0.01) genotypic differences. These values are within the range of water uptake for greengram varieties (52-150%), reported by Narasimha and Desikachar (1978) and for greengram by Vimala and Pushpama (1988). Rosaiah et al

* Corresponding Author

TABLE 1. COOKING CHARACTERS, PROTEIN, SULPHUR AND AMINO ACID CONTENTS OF GREENGRAM CULTIVARS

Cultivars	100 grain wt, g	Cooking time, min	Water absorption, %	Solid dispersion, %	Protein (Nx6.25), %	Sulphur, %	Methionine, g/16gN	Cytine, g/16gN	Tryptophan, g/16gN
'ML-5'	2.99	32.0	123.4	13.69	21.87	0.246	1.27	0.84	1.06
'ML-80'	2.89	33.5	130.1	12.60	26.25	0.230	1.28	0.73	1.24
'ML-131'	2.70	32.0	99.5	10.24	23.75	0.147	1.29	1.02	1.20
'ML-192'	2.81	32.5	112.6	14.13	24.31	0.181	1.44	0.83	1.38
'ML-197'	2.78	31.0	99.1	13.67	26.25	0.141	1.63	0.70	1.05
'ML-229'	3.02	36.0	98.7	8.52	22.50	0.270	1.50	0.51	0.90
'ML-267'	2.51	31.5	96.8	10.72	23.12	0.261	2.05	0.67	1.27
'ML-337'	3.12	36.0	93.8	9.50	20.00	0.181	1.49	0.85	1.00
'ML-353'	2.89	33.0	122.0	5.19	23.12	0.153	1.58	0.77	1.07
'ML-687'	3.20	31.0	115.1	10.86	21.25	0.159	1.47	0.91	0.95
'MUG-121'	2.65	37.0	130.0	10.27	21.87	0.264	2.09	1.23	1.27
'MUG-124'	2.62	34.0	101.8	10.16	24.37	0.228	1.43	0.54	0.96
'MUG-125'	2.13	30.5	102.7	6.60	25.00	0.276	1.40	1.04	1.26
'MUG-140'	2.87	32.5	117.3	10.43	22.43	0.222	1.60	0.73	0.99
'MUG-144'	2.62	34.0	89.7	9.62	21.87	0.246	1.44	1.17	1.12
'MUG-172'	2.92	35.0	81.9	9.08	26.87	0.194	0.99	0.66	1.31
'IS-127'	2.66	34.5	112.8	10.46	23.75	0.205	1.30	0.99	1.14
'Russian'	2.83	30.0	93.7	10.25	18.12	0.274	1.58	0.85	0.98
'V-3484'	3.14	36.5	109.1	7.89	21.48	0.220	2.08	0.85	1.19
'HYB 1-39'	3.10	36.0	110.9	8.05	23.12	0.265	1.55	0.84	1.16
'11/99'	3.57	33.0	94.0	10.78	23.75	0.207	1.31	0.91	1.06
'72'	2.64	31.0	122.4	12.52	21.25	0.175	1.37	1.14	1.00
'L2'	2.78	31.0	121.8	12.98	20.62	0.201	1.40	1.46	1.03
'ML-80 x ML-329'	2.92	35.0	90.9	16.22	19.37	0.190	2.06	0.78	1.06
'ML-80 x ML-5'	2.81	34.0	125.0	10.44	24.37	0.212	1.26	0.72	1.10
'ML-80 x ML-131'	2.04	30.0	96.8	10.16	20.62	0.194	1.71	0.73	1.36
'ML-80 x ML-267'	2.92	35.0	122.9	11.24	19.37	0.137	1.61	1.39	0.89
'ML-125 x ML-5 F1'	2.77	32.5	76.9	8.38	21.87	0.168	1.66	0.85	0.82
'MUG-125 x ML-131 F1'	2.79	32.0	79.4	8.90	27.50	0.170	1.48	0.75	1.29
'11/99 x ML-131 F1'	3.24	34.0	92.2	8.28	20.00	0.178	1.76	0.90	0.92
'UPM-79-3-4'	2.92	33.0	90.6	10.05	23.75	0.224	1.40	0.85	1.20
'VC-1482-SELC'	4.57	35.5	97.7	17.6	20.62	0..244	1.80	1.02	1.04
Mean±SD	2.89	33.26	104.67	10.62	22.51	0.200	1.54	0.89	1.13
	±0.42	±1.97	±14.88	±2.57	±2.09	±0.04	±0.26	±0.21	±0.21
F-value	17.12**	5.41**	15.60**	21.47**	30.14**	1.29^{NS}	12.36**	16.29**	22.16**

(1993) reported a slightly higher range (122-162%) in *mung*bean. The differences could be attributed to locational and genotypic variations. Solids dispersed in cooking medium ranged from 5.19 ('ML-353') to 17.86% ('VC-1482 Selc') with a mean of 10.62%. Similar value (11.3%) of solid dispersion for greengram 'ML-267' was reported by Hira et al (1988). Waldia (1996) reported a wide range of solid dispersion for chickpea genotypes (1.18-15.15%).

Nutrient analysis of data (Table 1) revealed significant differences (P<0.01) in protein contents of cultivars with a range of 18.12 ('Russian') - 26.87% ('MUG-172'). Rosaiah et al (1993) reported 20.5-25.9% protein in 9 *mung*bean varieties. Similar values for protein contents of greengram have also been reported by other workers (Neelakantan et al. 1977; Raghuvanshi et al. 1990). Sulphur contents of cultivars ranged from 0.137 ('ML-80 x ML-267') to 0.276 g % ('MUG-125') with non-significant genotypic variations. Methionine and cystine contents ranged from 0.99 ('MUG-127') - 2.09 ('MUG-121') and 0.51 ('ML-229') - 1.46 g/16 g N ('L-2'), respectively and differences were significant (P<0.01). Rosaiah et al (1993) reported slightly

TABLE 2. CORRELATION COEFFICIENTS AMONG NUTRITIONAL AND COOKING QUALITY ATTRIBUTES IN GREENGRAM

Character		1	2	3	4
100 grain weight		1.000	0.427*	-0.062	0.388*
Cooking time			1.000	0.058	-0.048
Water absorption				1.000	0.148
Solid dispersion					1.000
	Protein	Sulphur	Methionine	Cystine	Tryptophan
Protein	1.000	-0.027	-0.497**	0.424**	0.263
Sulphur	-	1.000	0.131	-0.126	NA

higher values of methionine (1.4-1.8 g/16g N) and lower value of cystine (0.4-0.7 g/16g N). These differences could probably be due to variations in analytical methods used. Like all other grain legumes, S-containing amino acids are limiting in greengram (Khan et al. 1979). These amino acids together contribute 2.42% of the protein, which is 69.14% of provisional scoring pattern (3.5 g/16g N) of FAO/WHO (1973). Tryptophan ranged from 0.819 ('ML-125 x ML-5') to 1.58 g/16g N ('ML-192') and varietal differences were significant.

The correlation coefficients between 100 grain weight and various cooking quality parameters indicated non-significant values except for those of 100 grain weight with cooking time (r=0.427*) and percent solid dispersion (r=0.388*). The values in Table 2 further revealed that sulphur was not significantly associated with protein and sulphur containing amino acids. However, methionine and cystine were negatively and significantly associated with protein content, indicating the difficulty in improvement of both the traits simultaneously. A positive, but non-significant correlation between tryptophan and protein, was observed.

Thus, wide variations among quality parameters of greengram genotypes could be used to isolate those with desirable characters. Taking into consideration, the various nutritional and cooking characteristics, it could be concluded that 'Russian' and 'MUG-125' took minimum cooking time (30 min) and also had high S- contents. Similarly, 'MUG-121' showed maximum water absorption (130%) and also had high values of total sulphur, methionine and tryptophan. 'ML-267' had high amounts of both methionine and tryptophan.

The authors are grateful to the Head, Pulses Section, Department of Plant Breeding, Punjab Agricultural University, Ludhiana for providing the samples.

References

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

Doughty J, Walker A (1982) Legumes in Human Nutrition. FAO Food and Nutrition Paper 20, FAO, Rome, p 46

FAO/WHO (1973) Energy and Protein Requirements. WHO Technical Report Series No. 522. FAO Nutrition Meetings Report Series No. 52, WHO, Geneva, FAO, Rome

Hira CK, Kanwar JK, Gupta N, Kochhar A (1988 Cooking) quality and nutritional evaluation of ricebean (*Vigna umbellata*). J Food Sci Technol 25:133-136

Khan MA, Jacobson I, Eggum BO (1979) Nutritive value of some improved varieties of legumes. J Sci Food Agric 30:395-400

Liddle HF, Saville V (1959) Colorimetric determination of cystine. Analyst 84:188-190

McCarthy TE, Sullivan MX (1941) A new and highly specific colorimetric test for methionine. J Biol Chem 141:1871-1875

Narasimha NV, Desikachar HSR (1978) Objective methods for studying cookability of *tur* pulse (*Cajanus cajan*) and factors affecting varietal differences in cooking. J Food Sci Technol 15, 47-51

Neelakantan S, Mani Meghalai G, Krishnaswamy S (1977) Cooking quality of some improved varieties of greengram *dhal*. Madras Agric J 64:748-757

Raghuramulu N, Madhavan Nair K, Kalyanasundaram S (1981) A Manual of Laboratory Techniques. National Institute of Nutrition, LCMR, Hyderabad, India, p 148

Rosaiah G, Santha Kumari D, Satyanarayana A, Rajarajeswari V, Naidu NV, Singh U (1993) Cooking quality and nutritional characters of *mung*bean, J Food Sci Technol 30:219-221

Raghuvanshi RS, Bhattacharya L, Singh OP (1990) A study on the chemical composition and protein fractions of *mung*bean. Bull Grain Technol 28:18-21

Shivashankar G, Rajendra BR, Vijay Kumar S, Sreekantanadhya R (1974). Variability of cooking characteristics in collection of greengram (*Phaseolus aureus* Roxb). J Food Sci Technol 11:232-234

Smith AM, Agiza AI (1951) The amino acids of several grassland species, cereals and bracken. J Sci Food Agric 2:503-520

Vimala V, Pushpamma P (1987) Storage changes in cookability of pulse from three regions of Andhra Pradesh. J Food Sci Technol 24:155-158

Waldia RS, Singh VP, Sood DR, Sardana PK, Mehla IS (1996) Association and variation among cooking quality traits in *Kabuli* chickpea (*Cicer arietinum* L.) J Food Sci Technol 33:397-402

Received 23 September 1995; revised 15 March 1997; accepted 19 March 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 331-334

Quality of Nuggets and Patties from Mutton with Incorporation of Milk Co-precipitates

K.H. RAO*, A.S.R. ANJANEYULU, R.R.B. SINGH, P.C. DUBEY AND P.L. YADAV

Division of Livestock Products Technology,
Indian Veterinary Research Institute, Izatnagar-243 122, India.

Effect of incorporation of different milk co-precipitates viz., low (0.6%), medium (0.8%) and high (0.9%) calcium types at 15% levels on physico-chemical and sensory quality of mutton nuggets and patties was studied. The emulsion stability, yield, moisture and protein contents of the samples were not affected, while pH ($P<0.05$), frying loss ($P<0.01$) and shear force values ($P<0.05$) were significantly affected in mutton nuggets. Co-precipitates had significant effect on flavour ($P<0.01$) and juiciness ($P<0.01$) in nuggets. None of the physico-chemical parameters except pH ($P<0.05$) was affected in mutton patties. Co-precipitates had no adverse effect on any of the sensory characteristics in patties.

Keywords: Co-precipitates, Emulsion quality, Sensory attributes, Mutton nuggets, Mutton patties.

With rapid developments in meat-based fast food industry, researchers have focussed increasing attention to utilize milk proteins as fillers, binders and extenders in comminuted meat products (Sen 1993; Kondaiah et al. 1994). Milk proteins substituted more expensive meat proteins and maximised the yield of saleable product. Dairy proteins improved emulsifying capacity, emulsion stability, water binding capacity of the batter and slicing characteristics of the product. In addition, the nutritive value of the end product is enhanced by their excellent amino acid profile (Jonas 1973), besides improving sensory characteristics of the finished product.

Milk proteins can be used in various forms such as casein and caseinates, co-precipitates, skim milk powder, whey proteins etc., in meat products. The choice of milk proteins in food system is guided by their potential functional properties, which, in turn, are monitored by their constituents, composition and processing conditions, employed for isolating them. Information available in the literature on the use of co-precipitate is scanty, patented and also confined to sausages from pork and beef. Hence, an attempt has been made in the present investigation to study the effect of incorporation of different types of co-precipitates in mutton nuggets and patties.

Spent female sheep (aged 5-6 years), were slaughtered at the experimental slaughter house, according to the standard procedure (Gracey and Collins 1992) to obtain mutton. The carcasses were hand-deboned within 3 h of post-mortem and the mutton samples were collected from deboned meat and were frozen at -10°C. The frozen samples were tempered at 5°C for 12 h before utilization in the trials.

Fresh cow skim milk obtained from Dairy Technology Section of Indian Veterinary Research Institute was used for the preparation of co-precipitates by the method of Fox (1992). Skim milk heated at 90°C was precipitated at pHs 4.8, 5.6 and 5.9 after addition of 0.03, 0.06 and 0.2% calcium chloride to obtain low, medium and high calcium co-precipitates, respectively.

Mutton and co-precipitates were coarse-minced. Mutton emulsions were prepared in a bowl chopper (Model 8418D, Hobart, USA). The control formulations had (%) lean mutton 70, vegetable oil 10, *maida* 2, salt 1.8, sugar 0.5, sodium tripolyphosphate 0.5, ice flakes 10, condiments 3, spices 1.2 and sodium nitrate 0.1. In experimental formulations, 15% lean mutton was substituted with milk co-precipitates.

Emulsion was formed into blocks of 450 g each in aluminium moulds, which were then steam-cooked for 30 min to an internal temperature of 90±2°C. Chilled meat blocks were cut into nuggets of similar size. For preparation of patties, the emulsion was hand-moulded by using petri-dish (77x19 mm) into patties, each weighing 75g, which were then cooked at 180°C for 25 min in a pre-heated oven, to obtain an internal temperature of 75°C, as recorded by a temperature probe. The experiments were replicated thrice.

Emulsion stability was determined by the method of Baliga and Madaiah (1970), as modified by Kondaiah et al (1985). pHs of co-precipitates and emulsions were determined by using a digital pH meter with glass electrode. The yield of co-precipitates was expressed as per cent weight of

* Corresponding Author

skim milk from which they were prepared, while the yield of meat products was determined as % of raw emulsion weight. For frying loss of nuggets, the nugget slices (15 x 15 x 100 mm) were fried in vegetable refined oil and the weight loss during frying was expressed as % weight frying loss.

AOAC (1980) procedures were followed to determine moisture, protein, fat, ash and calcium contents of the samples. Shear force value of nuggets and patties (15x15 mm size pieces) was determined, using Warner Brazier shear force press (GR Electric Manufacturing Co., USA).

A seven member experienced sensory panel evaluated the nuggets and patties for appearance, flavour, juiciness, texture and overall palatability, using an 8-point descriptive scale (Keeton 1983). The data were subjected to analysis of variance and critical differences (Snedecor and Cochran 1968) were calculated to determine any significant differences between treatment means.

Results presented in Table 1 indicated that the variations in processing conditions viz., level of calcium chloride, heat treatment and pH of precipitation employed for the manufacture of different types of co-precipitates brought about significant differences ($P<0.05$) in their composition profile. As the amount of calcium chloride increased for coagulation, the moisture content increased in the precipitates, which, in turn, inversely influenced the protein content. The ash contents ranged between 2.6 and 2.8%, the highest being in high calcium co-precipitate ($P<0.01$). The yield of high calcium co-precipitate was significantly ($P<0.01$) higher, indicating the strong calcium cross linkages between casein micelles to form the close matrix, holding more moisture (Ling 1956). The pH of the co-precipitates were also affected significantly ($P<0.01$) with the level of calcium chloride used in their preparation.

The emulsion stability and yield of the experimental samples were not significantly affected by the type of co-precipitate incorporated in the formulations (Table 2). However, pH of emulsion containing high calcium co-precipitate was significantly higher ($P<0.05$), which was in agreement with the observations of Rogov et al (1980) and Zhuravaskaya and Perkel (1981). Interestingly, frying loss was lowest for the nuggets with high calcium co-precipitate ($P<0.01$), which may be due to the improved, water binding capacity of high calcium co-precipitate in the emulsion. Thomas et al (1978) and Rogov et al (1980) also reported that the water binding capacity of meat and pork emulsion improved when co-precipitate was added at 20-30% levels. Moisture and protein contents of the nuggets were not significantly ($P>0.05$) affected by type the of co-precipitate. The present results are in contrast to the findings of Rudolph and Hansen (1986) on yield, moisture and protein contents of the products. This could be attributed to the method of preparation of co-precipitates, used in their experiments. The moisture and protein contents of the co-precipitate used in the present trials were similar to those of mutton. The fat contents varied from 11.0 to 12.5% but within the normal range. Type of co-precipitate significantly ($P<0.05$) influenced the shear force value and was found highest in the samples with high calcium co-precipitate.

TABLE 1. COMPOSITIONAL QUALITY, YIELD AND PH OF DIFFERENT TYPES OF MILK CO-PRECIPITATES

Parameters	Type of milk co-precipitate				
	Low calcium	Medium calcium	High calcium	CD 5%	CD 1%
Moisture, %	64.5±0.97	64.6±0.57	67.0±1.10	2.01	—
Protein, %	29.6±0.87	29.4±0.51	27.2±0.09	1.76	—
Ash, %	2.6±0.04	2.6±0.03	2.8±0.02	0.09	0.13
Calcium, %	0.6±0.02	0.8±0.02	0.9±0.01	0.04	0.06
Yield, %	13.5±0.36	13.8±0.20	15.8±0.60	1.26	1.75
pH	5.5±0.03	5.5±0.03	5.8±0.05	0.12	0.17

n = 6, CD = Critical difference

With respect to the sensory characteristics, the flavour and juiciness of the samples were significantly ($P<0.01$) affected by the type of co-precipitate. However, samples with low calcium co-precipitates obtained equal scores for all the sensory attributes, as compared to those of control. The whey protein casein complex of low calcium co-precipitate might have provided the suitable matrix in the emulsion for the preparation of nuggets (Hynd 1970). High calcium co-precipitate imparted non-meaty flavour to the products and adversely affected the juiciness of nuggets. In contrast, Sen (1993) and Kondaiah et al (1994) reported the suitability of high calcium co-precipitate in chicken loaves and nuggets on the basis of sensory scores. Texture of all the experimental samples did not differ significantly, while overall palatability of the nuggets were significantly affected ($P<0.05$).

It was observed that barring pH, all the parameters viz., emulsion stability, yield, moisture, fat, protein and shear force value of patties were not significantly influenced by the type of co-precipitate. All the sensory attributes of mutton patties were unaffected by the incorporation of any of the co-precipitates. Mann (1989), Goldman (1974),

TABLE 2. QUALITY OF MUTTON NUGGETS AND PATTIES INCORPORATED WITH MILK CO-PRECIPITATES

Parameters	n	Type of milk co-precipitate				CD 5%	CD 1%
		Control	Low calcium	Medium calcium	High calcium		
Mutton nuggets							
Emulsion stability, %	9	6.3±0.07	8.4±0.36	6.4±0.20	6.6±0.25	NS	—
pH	6	6.3±0.02	6.3±0.02	6.3±0.01	6.4±0.01	0.04	—
Yield, %	9	97.0±0.44	96.9±0.23	97.0±0.26	96.8±0.27	NS	—
Frying loss, %	6	11.7±0.35	13.1±0.58	11.7±0.23	9.5±0.68	1.46	1.99
Moisture, %	9	61.9±0.11	61.5±0.12	60.9±0.19	61.1±0.18	NS	—
Protein, %	9	15.2±0.09	15.9±0.03	15.4±0.07	15.7±0.04	NS	—
Fat, %	6	12.5±0.19	11.0±0.28	12.1±0.21	11.5±0.24	0.69	0.94
Shear force value, kg/1.5 cm^3	12	0.4±0.04	0.3±0.01	0.5±0.03	0.5±0.03	0.13	—
Appearance	7	7.3±0.13	7.2±0.12	7.3±0.15	7.3±0.11	NS	—
Flavour	7	7.5±0.14	7.2±0.25	6.5±0.34	6.0±0.23	0.73	0.99
Juiciness	7	7.3±0.17	7.1±0.19	6.6±0.20	6.0±0.24	0.59	0.80
Texture	7	7.3±0.25	7.1±0.29	6.6±0.29	6.3±0.35	NS	—
Overall palatability	7	7.4±0.19	7.2±0.28	6.6±0.32	6.2±0.30	0.81	—
Mutton patties							
Emulsion stability, %	9	6.3±0.01	8.4±0.36	6.4±0.20	6.6±0.25	NS	—
pH	6	6.4±0.02	6.4±0.02	6.4±0.02	6.5±0.01	0.04	—
Yield, %	15	91.7±0.60	92.2±0.66	91.8±0.84	90.9±0.58	NS	—
Moisture, %	6	59.7±0.27	60.2±0.29	59.6±0.34	59.8±0.40	NS	—
Protein, %	6	16.3±0.37	17.4±0.39	16.8±0.42	16.7±0.41	NS	—
Fat, %	6	12.6±0.30	12.7±0.38	12.5±0.36	11.9±0.33	NS	—
Shear force value, kg/1.5 cm^3	12	0.7±0.04	0.8±0.03	0.7±0.03	0.7±0.03	NS	—
Appearance	7	7.4±0.11	7.3±0.13	7.3±0.13	7.3±0.13	NS	—
Flavour	7	7.1±0.13	6.9±0.10	6.9±0.10	7.2±0.15	NS	—
Juiciness	7	6.9±0.04	6.8±0.09	6.9±0.09	7.0±0.04	NS	—
Texture	7	7.0±0.11	6.5±0.26	7.0±0.13	7.0±0.11	NS	—
Overall palatability	7	7.2±0.11	6.9±0.13	6.9±0.12	7.2±0.12	NS	—

NS = Non-significant, CD = Critical difference

Sensory scores were based on 8-point scale, wherein 8 = extremely desirable and 1 = extremely undesirable

Bartekova et al (1985) and Malyshko (1986) reported in different experiments that all types of co-precipitates could replace upto 5-30% of meat without affecting the sensory quality of sausages, meat cutlets, luncheon meat and meat paste. The sensory scores of experimental samples were comparable to those of control, indicating the suitability of all types of co-precipitates for the manufacture of patties.

References

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

Baliga BR, Madaiah N (1970) Quality of sausage emulsion prepared from mutton. J Food Sci 35(4):383-385

Bartekova Z, Dubravicky J, Strmiskova G, Simunicova A, Buchtova V (1985) Effect of added soybean and milk proteins on technological and sensory properties of a pork luncheon meat product. Bulletin Patravinarskeho Vyskumu 24(1): 19-31

Fox PF (1992) Manufacture of casein, caseinates and co-precipitates. Developments in dairy chemistry, Vol. I Proteins, Applied Science Publishers, London, pp 328-329

Goldman A (1974) Applications for dairy products in small goods manufacture. Food Technol New Zealand 9(7):25-27

Gracey JF, Collins DS (1992) Meat hygiene. 9th edn. Bailliere Tindall, London, pp 143-167

Hynd J (1970) Utilization of milk proteins. J Soc Dairy Technol 23(2):95-99

Jonas JJ (1973) Utilization of dairy ingredients in other foods. J Milk Food Technol 36(6):323-332

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

Kondaiah N, Anjaneyulu ASR, Rao VK, Sharma N, Joshi HB (1985) Effect of salt and phosphate on the quality of buffalo and goat meats. Meat Sci 15:183-192

Kondaiah N, Anajaneyulu ASR, Lakshmanan V, Rao KH, Rao KVSS, Yadav PL (1994) Effect of milk co-precipitate and eggs on the quality of nuggets from spent hens. Indian J Poult Sci 29(1):116-117

Ling ER (1956) A Text Book of Dairy Chemistry. Vol. I. Chapman and Hall Ltd., London

Malyshko TS (1986) Use of milk proteins in the manufacture of a meat paste from by-products. Pischevaya Promyshiennost 32:85-87

Mann EJ (1989) Dairy ingredients in meat products. Dairy Ind Int 54(2):9-10

Rogov IA, Zhuravskaya NK, Yasyreva VA, Roslova AP, Pismenskaya VN, Perkel TP, Kulikoya VV (1980). In: Proceedings of European Meeting of Meat Research Workers No. 26. Vol. I, G-11:347-350

Rudolph U, Hansen R (1986) Use of milk proteins in mixed meat products - A contribution towards finding substitutes for meat. Mathematisch-Naturwissenchaftliche Reihe 35(7):671-672

Sen AR (1993) Effect of some extenders on physico-chemical and sensory attributes of chicken loaf. M.V.Sc. Thesis, Indian Veterinary Research Institute, Izatnagar

Snedecor CW, Cochran WC (1968) Statistical Methods, 6th edn. Oxford and IBH Publishing Co., Calcutta

Thomas MA, Baumgartner PA, Hyde KA (1974) A study on some of the functional properties of calcium co-precipitates in a model system. Aust Dairy Technol 11:59-64

Zhuravskaya NK, Perkel TP (1981) Colour formation in combined boiled sausages containing milk co-precipitate. Pischevaya Technologiya 1:38-40

Received 12 October 1995; revised 15 March 1997; accepted 19 March 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 335-336

Standardization of Jelly Preparation from Grape : Guava Blends

POONAM AGGARWAL*, G.S. PADDA AND J.S. SIDHU**

Department of Food Science and Technology,
Punjab Agricultural University, Ludhiana-141004, India.

Coloured jelly was prepared by using 3 grape varieties namely, 'Lomanto', 'Madeleine Royal' and 'Beauty seedless - Ruby red hybrid'. It was observed that all the 3 varieties separately as well as in mixed form were unable to form a desirable jelly due to their inadequate pectin content and high acidity. Therefore, grape juice was mixed with guava extract in 20:80, 40:60 and 60:40 ratios. Of these, grape:guava blend in the ratio of 40:60 scored the highest for colour, flavour, consistency and overall acceptability by sensory evaluation.

Keywords: Jelly, Grape varieties, Grape:guava blends, Organoleptic quality, Pectin, Sugar and acid percentage.

Grape is one of the commercial fruits of importance produced in the world. It grows over a wide range of the north temperate region. There are only a few grape varieties, which are processed commercially. Most of the grape juice and grape jellies made in United States come from concord grapes (Anon 1983). Some of the coloured grape varieties namely, 'Lomanto', 'Madeleine Royal' and 'Beauty seedless – Ruby red hybrid' are being grown in the orchards of Punjab Agricultural University, Ludhiana. Due to their high acidity and low sugar content, these varieties are not suitable for table purpose. In order to use the coloured grapes into suitable product, an attempt has been made to prepare an acceptable quality jelly.

Procurement of raw materials : Three cultivars of grapes viz., 'Lomanto', 'Madeleine Royal' and Beauty seedless – Ruby red hybrid' were procured from the Department of Horticulture, Punjab Agricultural University, Ludhiana. Commercial guavas were purchased from the local market.

Extraction of juice : Grape juice was extracted through the juicer of the Lady Chef Food Processor (Singer make) and was stored in a refrigerator. Fully ripened guava, after washing were cut into thin slices. As per procedure of Lal et al (1986), pectin extract was prepared and kept in the refrigerator, till further use.

Preparation of jelly : Various proportions of grape-guava extract were used along with various ingredients like sugar, pectin, citric acid, calcium chloride etc. for the preparation of desirable jelly. Juice was concentrated to 2/3 by quick heating. Pectin was mixed with sugar to dissolve completely in the boiling extract. Then, acid was added. In some of the recipes, calcium chloride was added in the later stages of cooking. The heating was stopped, when the product attained 70°Brix. The finished product was immediately poured into sterilized glass jars (450 g). The product was allowed to cool and jars were sealed air-tight. Cut out examination was done after 24 h for every recipe made.

Physico-chemical and organoleptic evaluation : All the three grape varieties were analysed for their physical characteristics. Total soluble solids, pH, acidity, pectin, reducing and total sugars were determined in grape juice, guava extract and selected grape-guava jelly blends, employing standard methods (AOAC 1980). Pectin strength of the juice was also estimated by spirit test (Lal et al. 1986). Sensory evaluation of the product was done by a semi-trained panel, using a 7-point Hedonic scale. Values were analyzed statistically by analysis of variance (Snedecor and Cochran 1967).

Physical characteristics : All the varieties were round in shape. The fruit colour of the variety 'Lomanto' and 'Madeleine Royal' was purplish black and that of 'Beauty seedless – 'Ruby red hybrid' was dark purple (Table 1). The highest juice yield of 84.5% was recorded in 'Beauty seedless - Ruby red hybrid'.

Chemical composition of juice : Variety 'Lomanto' was found to have the lowest TSS (10°Brix) and maximum acidity (2.03%), whereas 'Beauty seedless-Ruby red hybrid' was sweeter than other two varieties, because of its low acid content (Table 1). Pectin quality by spirit test gave fibre-like structure in case of variety 'Lomanto' and fragmented clots in other two varieties. Guava extract was found to have 0.36% acidity and 0.5% pectin content. A thick lump appeared in pectin quality test of guava

* *Corresponding Author, ** Present Address – Central Institute of Post-Harvest Engineering, ICAR, PAU, Ludhiana*

TABLE 1. PHYSICO-CHEMICAL CONSTITUENTS* OF VARIOUS CULTIVARS OF COLOURED GRAPES AND GUAVA JUICE

Varieties	Size	Colour of fruit	Juice yield, %	Colour	TSS, °B	pH	Acidity, %	Pectin, %	Reducing sugars, %	Total sugars, %	Pectin quality by spirit test
'Lomante'	Small	Purplish black	73.5	Dark purple	10.0	2.91	2.03	0.19	8.7	9.2	Small granular clots
'Medeleine Royal'	Small	Purplish black	76.9	Dark purple	13.8	3.25	1.80	0.28	10.2	12.3	Fragmented small clots
'Beauty Seedless-Ruby red hybrid'	Medium	Dark purple	84.5	Purplish pink	12.0	3.01	1.43	0.32	8.5	9.7	Fragmented less firm clots
Mixed grape juice	-	-	78.3	Purple	12.9	3.45	1.22	0.23	10.4	11.3	Fragmented clots
Guava juice	Medium	Yellowish green	57.5	Cream	9.0	4.30	0.36	0.50	3.8	6.3	Lump formation

* Average of 3 values

TABLE 2. BIOCHEMICAL* AND SENSORY EVALUATION** OF SELECTED GRAPE: GUAVA JELLY BLENDS

	pH	Acidity %	TSS, °B	Reducing sugars, %	Total sugars, %	Colour	Flavour	Consistency	Sugar acid ratio	Overall acceptability
	3.40	0.80	68.0	19.68	65.9	5.57^{c}	6.29	6.14^{ab}	6.14	6.14^{ab}
	3.39	1.00	68.5	23.15	68.9	5.71^{bc}	6.57	6.29^{ab}	6.14	6.14^{ab}
	3.43	1.03	71.5	20.03	68.2	6.00^{abc}	5.85	5.14^{bc}	5.57	5.15^{bc}
	3.45	0.98	71.0	22.16	71.6	6.57^{abc}	6.14	4.42^{c}	5.71	4.85^{c}
	3.45	1.00	71.0	20.00	70.2	6.85^{a}	6.42	6.71^{a}	6.42	6.92^{a}
	3.46	1.00	70.5	22.06	70.6	6.64^{ab}	6.28	5.57^{abc}	6.00	6.21^{ab}
F value	ND	ND	ND	ND	ND	4.78***	0.96 (NS)	5.49***	1.03 (NS)	5.10***
LSD	ND	ND	ND	ND	ND	1.05	NS	1.47	NS	1.16

* Average of 3 values

** Mean scores of 7 panelists

*** The values are significantly different at 5% and 1% points of distribution of F. Any two values not followed by similar superscript are significantly different at 5% level

extract by addition of rectified spirit, showing an adequate amount of pectin for jelly making (Table 1).

Biochemical and sensory analysis of selected grape-guava jelly blends : All the three varieties separately as well as in the mixed form were tried into jelly formation, but these were made out in a honey-like product. Calcium chloride was also used upto 1% in the grape juice for the formation of good jelly. But, the finished product appeared in a precipitated structure. So, guava extract was combined with grape juice. About 20 combinations of grape-guava juice were tried, using various levels of ingredients. Out of six selected combinations, 20:80 grape-guava blend had excellent jelly setting property. Combinations 60:40 and 40:60 grape-guava juice were found to have slightly soft setting property. These later combinations were improved by the addition of 1% pectin and made into excellent jelly referred as No. 5 and 6 in Table 2. Acidity of finished product was 1% in almost all the six recipes. Total soluble solids varied from 68 to 71% and pH from 3.39 to 3.46. Reducing sugars were minimum in guava jelly and maximum in 20:80 grape-guava blend (Table 2).

Sensory evaluation showed the highest scores for No. 5 grape : guava blend. The values for colour, consistency and overall acceptability of jelly blends were found significantly different at both 5 and 1% levels of significance.

References

Anon (1983) The New Book of Knowledge. G. Rolier Inc, Danbury Conn. Copyright G, 7: 476

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

Lal G, Siddappa GS, Tandon GL (1986) Preservation of fruits and vegetables, Publications and Information Division, Indian Council of Agricultural Research, New Delhi

Snedecor GW, Cochran WG (1967) Statistical Methods, 6th edn. Oxford and IHB Publishing Co. New Delhi

Received 12 April 1995; revised 13 March 1997; accepted 19 March 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 337-339

Studies on the Incorporation of Bovine Plasma in Emulsion Type of Meat Product

E. MARQUEZ*, Y. BARBOZA DE M, P. IZQUIERDO AND G. TORRES

Unidad de Investigacion en Ciencia y Tecnologia de los Alimentos,
Universidad del Zulia, Venezuela.

Bovine plasma was used to replace part of the lean as a source of protein in the formulation of a meat emulsion-type product. Formulation included one of all lean (control) and three test formulae, one with all lean and plasma added for water and two where lean was reduced to a half and plasma was or was not added to substitute half of lean and water. Results have shown that the addition of plasma improved emulsion stability, yield and protein content. Phenylalanine and valine contents increased with the addition of plasma. There were no differences in overall or colour acceptabilities due to addition of plasma.

Keywords: Meat emulsion, Plasma, Stability, Yield, Amino acids, Acceptability.

Comminuted meat products are made by the emulsification of animal fat in water, using meat proteins as emulsifying agent (Schut 1978). Incorporation of other components like vegetable fat to increase polyunsaturated fatty acids (Marquez et al. 1989), vegetable proteins or plasma protein isolate to increase protein content or as a substitute of meat has been tried (Sofos and Allen 1977; Dill 1975; Terrel et al. 1979; Rusig 1979; Hazarika and Biro 1993).

The utilization of bovine blood plasma in food for human consumption is becoming increasingly popular in devloping countries due to their nutritional and functional properties and to their relatively low cost. Bovine plasma contains approximately 7% of protein. Its major protein is albumin, which is rich in all the essential amino acids (Tybor et al. 1975).

Plasma and globulin protein isolates prepared from slaughter blood are excellent emulsifiers under optimum conditions of pH and protein concentration (Tybor et al. 1973).

Relatively few studies on the use of blood as human food are reported in the literature. Terrell et al (1979) formulated frankfurters, containing meat plus 5% plasma protein isolated and reported increase in the elasticity of the outer skin and in the strength of the body. Rusig (1978) formulated sausages in which 20 or 40% of the meat protein was replaced by plasma alginate fibres and reported that the products were not liked by the taste panel members in terms of their flavour and colour, while they liked their texture.

In Venezuela, only a small amount of the blood from slaughtered animals is used in foods intended for human consumption and it is used in an empirical way. The objective of this work was to evaluate emulsion stability, protein, essential amino acid content, yield and acceptability of an emulsion-type product with added plasma to replace part of the meat as a source of protein.

Ingredients and formulation : Major ingredients viz., lean, fat and plasma contained protein 20, 4 and 7%, fat 5 and 80%, moisture 70, 14 and 92%, respectively. Once the proximate analysis for fat, lean meat and plasma were determined and smoke house yield (90%) calculated, a low cost microcomputer software formulation was used to determine the quantities of ingredients to be used in the formulation. The final fat content was adjusted to 20% and moisture content to 4%.

Formulation included one of all lean (control) and 3 test formulas, one with all lean and plasma added for water and two, where lean was reduced to a half and plasma was or was not added to substitute half of lean and water.

Products were manufactured (6 replications each of 4 formulations), using conventional manufacturing procedures.

Treatments : Treatment A (all lean, as control). Treatment B was similar to A, but with plasma to substitute water. Treatment C was same as A, but lean was reduced to half in the formulation. Treatment D was same as C, but with bovine plasma to substitute water. Treatment ingredients are presented in Table 1.

Processing procedure : Lean and fat after thawing (0°C), were mixed with the spices, curing ingredients and ice water or plasma for 15 min in a Fatosa bowl chopper model KF-70, until an emulsion was obtained. Final temperature of the emulsified batter

* Corresponding Author

was 16°C. The emulsified mixture was transferred into a (Fatosa) stuffer, where products were stuffed into 9.5 cm cellulose casing.

All the batches were weighed separately before the products were smoked and cooked in a Jose Lisondon smokehouse, until the internal temperature reached 70°C. Products were showered for 15 min and chilled (2°C) for 24 h. After chilling, the products were weighed, sliced, vacuum-packaged and stored at 4°C for 1 week.

Emulsion stability : Emulsion stability was performed by the test developed by Townsend et al (1968). Protein and moisture were determined using Kjeldahl and oven-drying methods (AOAC 1980), respectively.

Amino acid analysis : Amino acids were analyzed by high performance liquid chromatography. A Shimadzu model LCC-6A HPLC equipped with a FLD-6A fluorescence detector, two LC 6A pumps, a SCL-6B auto injector, CTO-6A column oven and a C-R4A chromatopack integrator was used throughout the experiments.

An altex ultrasphere ODS, C-18, 15 cm length x 4 mm ID, 5 um column was used. Two solvent systems were used. Solvent A was composed of acetate buffer (0.05 M), methanol and tetrahydrofurane (80:19:1). Solvent B was composed of methanol and acetate buffer (80:20). A Sigma laboratory standard solution 50 n mol/ml amino acid concentrations was used as a reference.

A precolumn derivatization of the amino acids was performed. Samples of 0.02 ml were injected onto the column. Flow rate was 1 ml/min. Fluorescence was read at 470 nm with an excitation wave length of 350 nm. Peak areas were used for quantitative calculations.

Acceptability : A 200 member scale consumer panel evaluated the overall and colour acceptability of each treatment. Panel members ranged from 18 to 30 years of age and were regular consumers of meat. Products were oven-dried and presented in randomized order to the panelists along with ketchup and mustard. Condiments were served separately and panelists were instructed to use them according to their food eating habit. For colour acceptability, products (sliced and vacuum-packaged) were evaluated during retail display. Panelists were asked to indicate as to what extent they liked or disliked each of the products on a 9-point Hedonic scale, 1 = dislike extremely and 9 = like extremely.

Statistical analysis : Data obtained were subjected to ANOVA technique, using SAS PROC GLM (1987). For each response, the average values for main effects of the different treatments were subjected to pairwise comparison procedures (Duncan 1955) to determine as to which pairs were different.

Emulsion stability and chemical composition for the different treatments are presented in Table 1. Significant differences ($P<0.05$) were observed in emulsion stability, when expressed as either ml of water released or ml of fat released. Results indicated that as lean content decreased from 5.26 (treatments A and B) to 2.63 (treatments C and D) water and fat released from the emulsion increased. They further indicated that the addition of plasma to treatment with low lean content (treatment D) improved emulsion stability, when compared to treatment with low lean content without the addition of plasma (treatment C).

The present results show that it is possible to reduce an important portion of lean in an emulsion-type product and compensate with addition of plasma, taking advantage of the emulsifying

TABLE 1. INGREDIENTS (kg), EMULSION STABILITY* CHEMICAL AND AMINO ACID COMPOSITION OF PRODUCTS WITH DIFFERENT TREATMENTS

Attributes	Treatments			
Ingredients	A	B	C	D
Lean	5.26	5.26	2.63	2.63
Beef fat	2.15	2.15	2.15	2.15
Spices	0.39	0.39	0.29	0.29
Water	3.06	0.00	3.06	0.00
Plasma	0.00	3.06	0.00	3.06
Emulsion stability				
Water	0.88[a]	0.98[a]	5.52[c]	2.03[b]
Fat	0.10[a]	0.10[a]	0.29[c]	0.19[b]
Yield	91.47[a]	90.81[a]	79.30[b]	87.65[c]
Chemical composition				
Fat	20.03[a]	20.32[a]	20.45[b]	25.92[b]
Protein	11.77[a]	14.83[b]	9.19[c]	11.27[a]
Moisture	63.20[a]	59.85[b]	59.36[b]	58.31[b]
Amino acids				
Lysine	8.91[a]	-	-	8.88[a]
Leucine	9.11[a]	-	-	9.12[a]
Isoleucine	2.72[a]	-	-	2.49[a]
Phenylalanine	4.00[a]	-	-	5.67[b]
Valine	5.62[a]	-	-	7.00[b]
Methionine	1.62[a]	-	-	1.57[a]
Histidine	2.89	-	-	3.24[a]

* Expressed as ml lost per 34 g emulsion.
A = Control; B = Same as control but with plasma to substitute water; C = Same as A but with half of lean; D = Same as C but with plasma to substitute water. [a, b, c] Means in a row having different superscripts are significantly different ($P<0.05$)

capacity of the plasma protein, as reported by Satterlee et al (1973) and Tybor et al (1973).

Rusig (1979) reported excellent emulsion stability, when 40% of the meat protein was substituted by isolated plasma protein. In the present study, plasma without any treatment to simplify the process was used. Treatment C (half lean without plasma) was highly unstable and presented the lowest yield (79.30) and protein content (9.19). When plasma was added (treatment D), products were stable and yield increased up to 87.65% and protein up to 11.27%. Treatment B (all lean plus plasma) presented the highest protein, while treatment D had lower yield than treatment A.

In general, these results indicate that the addition of plasma improved yield and protein content, when half of the lean was used in the formulation.

Mean values for essential amino acid profile of treatments A and D were expressed as a percentage of the total essential amino acid present. It can be observed that the addition of plasma at the amount used in these experiments to substitute half of lean and total water had a significant effect on the total essential amino acid content and on some individual essential amino acids. Addition of plasma did not change lysine, leucine and isoleucine contents, while it increased phynylalanine, valine and histidine and decreased methionine contents. These results indicate that (i) with the exception of methionine, all the essential amino acids either remained the same or increased and (ii) plasma protein, being rich in all the amino acids, can be an excellent substitute for lean protein.

There were no differences in overall or colour acceptabilities due to addition of plasma. All the products were evaluated as acceptable (scores >6) by the panelists. It is important to point out that products with half of lean plus plasma had lighter colour than the others. However, this difference did not influence consumer acceptability. The lack of differences in consumer acceptability may be related to the wide range of colours for emulsion-type products, presently available in the market.

The authors acknowledge with thanks the financial support extended by CONDES and PTU.

References

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

Dill CW (1975) Use of blood protein in processed meats and sausage. The National Provisioner. 173: 14–17

Duncan DB (1955) Multiple Range and F test. Biometrics 11: 1–42

Hazarika M, Biro G (1993) Effect of incorporation of blood proteins into sausage. J Food Sci Technol 30: 380–381

Marquez E, Ahmed E, West R, Johnson D (1989) Emulsion stability and sensory quality of beef frankfurters produced at different fat or peanut oil levels. J Food Sci 4: 867–873

Rusig O (1979) Evaluation of plasma and and plasma-alginate fibers for use in sausages. Meat Sci 3: 295–307

SAS PROC GLM (1987) SAS User's Guide Statistics, 5th edn, SAS Institute Inc., Carry, NC

Satterlee L, Free B, Leven B (1973) Utilization of high protein tissue powder as binders and extenders in meat emulsion. J Food Sci 38: 306–309

Schut J (1978) Basic meat emulsion In: Proceedings of Meat Industry Research Conference, American Meat Institute pp 1–6

Sofos J, Allen C (1977) Effect of lean meat source and levels of fat and soy protein on the properties of wiener type products. J Food Sci 42: 875–878

Terrel R, Winblatt P Smith G, Carpenter Z, Dill C, Morgan R (1979) Plasma protein effects on physical characteristics of all meat and extended frankfurters. J Food Sci 44: 1041–1048

Townsend W, Witnauer L, Riloff J, Swift C (1968) Comminuted meat emulsions, differential thermal analysis of fat transitions. Food Technol 22: 319–338

Tybor P, Dill C, Landmann W (1973) Effects of decolorization of lactose incorporation on the emulsification capacity of spray dried blood protein concentrates. J Food Sci 38: 4–7

Tybor P, Dill C, Landmann W (1975) Functional properties of proteins isolated from bovine blood by a continuous pilot process. J Food Sci 40: 155–159

Received 17 March 1995; revised 11 March 1997; accepted 19 March 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 340-342

Selection of Level and Type of "LAB" Starter in the Preparation of Dietetic *Shrikhand*

B. SURESH SUBRAMONIAN*, C. NARESH KUMAR, R. NARASIMHAN, A.M. SHANMUGAM AND M. MOHAMEDHABIBULLA KHAN

Department of Communication and Entrepreneurship, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai - 600 052.

The level and type of lactic acid bacteria starter (LAB) required for making "chakka" with better yield, total solid recovery and sensory scores was assessed, based on the time required by the single and combined cultures of *Lactobacillus acidophilus* and *Streptococcus salivarius* ssp. *thermophilus*, at different levels of inoculum to develop a required acidity of 0.9% to 1% and a pH of 4.5 to 4.6. A 2% combined culture of *L. acidophilus* and *S. salivarius* ssp. *thermophilus* each at 1% level took a minimum time of 10 h to develop the required acidity and pH, a better yield of 28.65% and a total solid recovery of 54.56%. *Shrikhand* prepared using 2% combined culture as starter scored the maximum total score of 24.23, indicating that 2% combined culture is the optimum level and type of starter for the preparation of *shrikand*.

Keywords: Dietetic *shrikand*, LAB starter, Level of starter, Acidity and pH, Sensory evaluation, Yield and total solid recovery.

Shrikhand, a fermented sweetened dairy product of pasty consistency is popular in western India. It has a good market potential in other states also (Ramachandra Rao et al. 1987). This product is made from "chakka" its western analogue "Quarg", which is an intermediate product obtained by draining of whey from *dahi*. "chakka" obtained is kneaded with cream, sugar and other ingredients to prepare *shrikhand*. Skimmed milk, a by-product of creameries is having poor acceptability and is available at cheaper rates. This by-product is being utilised in the manufacture of dietetic *shrikhand*. Probiotic starters like *L. acidophilus* and *S. salivarius* ssp. *thermophilus* are used instead of routine cultures.

Fresh whole buffalo milk procured from Livestock Research Station, Kattupakkam was separated to obtain skim milk. Ingredients such as sugar, saffron and cardamom of good quality were procured locally. They were powdered to fine form separately. Starter cultures like *Lactobacillus acidophilus* (NDRI-AHI) *S. salivarius* ssp. *thermophilus* (NDRI-YHS) and *Streptococcus lactis* (NDRI) were used for culturing the skim milk. *Streptococcus lactis* at 1% (C_1) level was used as control. Nine combinations of *Lactobacillus acidophilus* and *Streptococcus thermophilus* viz., *S. salivarius* ssp. *thermophilus* at 1% (C_2) *S.salivarius* ssp. *thermophilus* at 1.5% (C_3), *S.salivarius* ssp. *thermophilus* at 2% (C_4), *Lactobacillus acidophilus* at 1%(C_5), *Lactobacillus acidophilus* at 1.5% (C_6), *Lactobacillus acidophilus* at 2% (C_7), *S.salivarius* ssp.*thermophilus* at 0.5% and *Lactobacillus acidophilus* at 0.5% (C_8), *S. salivarius* ssp. *thermophilus* at 0.75% and *Lactobacillus acidophilus* at 0.75% (C_9) and *S. salivarius* ssp. *thermophilus* at 1% and *Lactobacillus acidophilus* at 1% (C_{10}) were used as starter cultures. The total solid contents of skim milk and "chakka" were analysed as per the procedure given in BIS:SP: 18 Part XI - 1981. The yield of "chakka" was estimated on weight basis. Titratable acidity as percent lactic acid and pH were estimated at 0 h, 8 h, 10 h and 12 h.

The required quantity of skim milk was heated to 72°C for 5 min and cooled to 37°C. Equal quantity of it was transferred to 10 sterilized beakers and inoculated with the above said 10 combinations of the starter culture. The cultured samples were incubated at 37°C. for 10 to 12 h, till the required acidity of 0.9 to 1% or a pH of 4.5 - 4.6 was reached. After that, the curd samples were drained, using sterilized muslin cloth to obtain the "chakka". Finely powdered sugar, cleaned cardamom powder and saffron powder were added to the 'chakka' by weight at the rate of 0.8%, 0.4% and 0.1% levels, respectively. They were kneaded to pasty consistency in a blender to obtain *shrikhand*. Thus, 10 samples of *shrikhand* (S_1 to S_{10}) were obtained using the "chakka" prepared, using starter culture C_1 to C_{10}, respectively. The final product was subjected to organoleptic evaluation by a panel of 5 trained judges, using a 9-point Hedonic scale of Quarter Master of United States of America.

Acidity and pH : Statistical analysis revealed that there was significant difference between the development of acidity and pH at different hours

* *Corresponding Author*

TABLE 1. ACIDITY AND PH DEVELOPMENT IN CULTURED MILK*

Cultures	0 h		8 h		10 h		12 h	
	Acidity	pH	Acidity	pH	Acidity	pH	Acidity	pH
C_1	0.15^a	6.63^b	0.39^c	6.25^c	0.45^d	5.87^f	0.53^e	5.75^g
C_2	0.16^a	6.60^b	0.39^c	6.21^c	0.47^d	5.81^f	0.55^e	5.72^g
C_3	0.16^a	6.59^b	0.41^c	6.14^c	0.48^d	5.62^d	0.59^{de}	5.61^f
C_4	0.16^a	6.58^a	0.43^c	5.93^d	0.58^f	5.48^d	0.63^d	5.42^e
C_5	0.16^a	6.57^a	0.81^d	5.24^c	0.89^{de}	5.04^{bc}	0.97^c	4.82^d
C_6	0.17^a	6.48^a	0.89^c	5.05^b	0.95^{cd}	4.96^b	1.00^{bc}	4.64^c
C_7	0.17^a	6.44^a	0.96^{ab}	4.84^a	1.04^{ab}	4.60^b	1.11^b	4.49^{ab}
C_8	0.17^a	6.57^a	0.82^{cd}	5.11^b	0.83^e	4.87^b	0.98^c	4.67^c
C_9	0.17^a	6.46^a	0.89^{bc}	4.98^{ab}	0.98^{bc}	4.69^b	1.08^b	4.43^a
C_{10}	0.18^a	6.43^a	0.98^a	4.99^a	1.10^a	4.43^a	1.22^a	4.11^a
Interaction	Acidity	ph						
'F' value	15.98**	34.39**						
SEM	0.0288	0.0166						
CD	0.799	0.1457						

* Value of 6 trials analysed by two factor statistical analysis

a, b, c, d, e and f indicate existence of significant differences

of incubation by starter culture, C_1 to C_{10} (Table 1).

After 8 h of incubation, significant differences in acid production and pH were noticed between single culture at 1%, 1.5% and 2%, levels (C_2, C_3, C_4, C_5, C_6, C_7) and combined cultures at 1%, 1.5% and 2%, levels (C_8, C_9, C_{10}). Combined cultures C_8, C_9 and C_{10} developed higher acidity at 8 h, 10 h and 12 h of incubation compared to the other cultures. This was in agreement with the findings of Goh et al (1990). Starter cultures of *Lactobacillus acidophilus* (C_5, C_6, C_7) developed higher acidity than starter culture of *S. Salivarius* ssp. *thermophilus* (C_2, C_3, C_4) and starter culture of *S.lactis* (C_1). This was in concurrence with the findings of Ziden et al (1990) and Kim and Harmon (1968).

The minimum incubation time required to reach the optimum required acidity of 0.9 to 1% (Upadhayay 1982) for draining of curd was reached by C_8, C_9 and C_{10} in 10 h. The acidity developed by C_{10} was significantly different ($P<0.01$) from others at 10 h. The required pH of 4.5 to 4.6 (Sharma and Reuter 1992) was reached at the earliest in 10 h by C_{10} (4.43). It took 12 h to reach the same pH by C_6 (4.6) and C_8 (4.8).

The reduction in pH values was noticed both with the increase in the level of inoculum viz., 1%, 1.5% and 2%, as observed Deane and Van patten (1971). The 2% combined culture (C_{10}) and 2% single culture (C_7) reached the required pH of 4.5 to 4.6 in 10 h. However, there were significant differences ($P<0.01$) in pH values between C_7 and C_{10}.

Sensory evaluation of shrikhand *prepared from buffalo skim milk using starter cultures :* Table 2 shows the organoleptic scores given for *shrikhand* samples S_1 and S_{10} from buffalo skim milk. The statistical analysis revealed significant differences ($P<0.01$) in flavour, consistency, acceptability and total sensory scores among the samples.

For flavour, the maximum score of 8.23 was obtained by S_{10} and minimum score of 7.30 was obtained by S_3. There were significant differences among the scores of different samples ($P<0.01$). The better scores given for *shrikhand* samples obtained by using combined cultures may be due to the increased production of flavour compounds by the symbiotic activity of cultures (Hunger 1987).

For consistency, the maximum score of 8.07 and minimum score of 7.10 were scored by S_{10} and S_2, respectively. There was a significant difference between the *shrikhand* samples grouped separately as S_5, S_8, S_9, S_{10} and S_1, S_2, S_3, S_6, S_7. The lower scores by S_1, S_2, S_3 and S_4 may be attributed to the poor texture with coarseness and low acid production.

For acceptability, the maximum score of 7.97 and minimum scores of 6.27 were given for samples S_{10} and S_4, respectively. There were significant differences ($P<0.01$) in the scores between the treatments grouped separately as S_9, S_{10} and S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8.

For total sensory scores, the maximum score of 24.23 and minimum score of 20.93 were obtained by S_{10} and S_4, respectively. The total sensory scores were influenced by flavour, consistency and

TABLE 2. SENSORY EVALUATION OF *SHRIKHAND* PREPARED FROM BUFFALO SKIM MILK USING STARTING CULTURES*

Shrikhand	Flavour	Consistency	Acceptability	Total scores
S_1	7.73^{ab}	7.32^{b}	6.27^{b}	21.47^{c}
S_2	7.50^{b}	7.10^{b}	7.10^{b}	21.67^{c}
S_3	7.30^{c}	7.27^{b}	7.27^{b}	21.83^{bc}
S_4	7.37^{c}	7.33^{b}	6.43^{c}	20.92^{c}
S_5	7.83^{b}	7.80^{a}	7.13^{bc}	22.77^{b}
S_6	7.47^{c}	7.33^{b}	6.73^{b}	21.30^{c}
S_7	7.43^{c}	7.37^{b}	6.60^{b}	21.40^{c}
S_8	7.93^{b}	7.60^{ab}	7.33^{b}	22.90^{ab}
S_9	8.10^{ab}	8.03^{a}	7.77^{ab}	24.00^{a}
S_{10}	8.23^{a}	8.07^{a}	7.97^{a}	24.23^{a}
'F' value	3.52**	2.39**	3.58**	4.68**
SEM	0.17	0.25	0.26	0.53
CD	0.48	0.68	0.72	1.47

* Values of 6 trials and 6 replications analysed by CRD

a, b, c, d, e and f indicate existence of significant differences

acceptability scores and significant differences ($P<0.01$) were noticed. Since S_{10} scored the maximum points, it can be rated as the most acceptable *shrikhand* sample with the starter culture C_{10} as the most preferred starter.

Yield and total solid recovery : Table 3 shows that high significant differences existed in parameters like yield and total solid recovery in "chakka" obtained by using different cultures C_1 to C_{10}. There was significant difference ($P<0.01$) between the "chakka" groups separately as follows; (C_{10}, C_9, C_7, C_3), (C_6, C_5, C_4, C_2, C_1) and C_8. The yield was high with C_3 though C_{10} did differ significantly. This values are in agreement with Aneja et al (1977).

The total solid recoveries in "chakka" obtained by using culture C_{10} and C_9 were significantly different from others. The values were in agreement with those reported by Aneja et al (1977). C_{10} had a total solid recovery of 54.46%, which was in agreement with that reported by Patel and Chakraborthy (1985).

Shrikhand prepared using 2% combined culture C_{10} containing *Lactobacillus acidophilus* 1% and *S.salivarius* ssp. *thermophilus* 1% (1:1 ratio) got the maximum scores. The required acidity of 0.9 to 1% and a pH of 4.5 to 4.6 was reached at the earliest of 10 h by culture C_{10}. The yield and total solid recovery were good in "chakka" obtained, using culture C_{10}.

It can be concluded that culture C_{10} containing *Lactobacillus acidophilus* 1% and *S.salivarius* ssp *thermophilus* 1% is the preferred culture for the preparation of "chakka" and its subsequent use in the manufacture of *shrikhand*.

TABLE 3. YIELD AND TOTAL SOLIDS RECOVERY IN CHAKKA*

Chakka obtained using cultures	Yield	Total solids recovery
C_1	24.17^{b}	50.23^{b}
C_2	24.72^{b}	48.62^{bc}
C_3	29.59^{a}	47.21^{c}
C_4	24.15^{b}	45.56^{c}
C_5	23.69^{b}	48.17^{c}
C_6	23.89^{b}	48.29^{c}
C_7	28.10^{a}	44.28^{c}
C_8	21.58^{c}	51.81^{b}
C_9	28.40^{a}	52.45^{ab}
C_{10}	28.65^{a}	54.46^{a}
SEM	0.64	2.611
'F' value	9.15**	11.63**
CD	1.82	4.50

* Values of 6 trials analysed by CRD

a, b, c, d, e and f indicate existence of significant differences

References

Aneja RP, Vyas MN, Karan Nawda, Thareja VK (1977) Development of industrial process for the manufacture of *shrikhand*. J Food Sci Technol 14:159-163

BIS:SP:18 (Part XI) 1981 Handbook of food analysis. Procedures 56, 21, 112, 124

Deane DD, Van patten MM (1971) Effect of malathion and tricholfon on growth and morphology of lactic culture organism. J Milk Food Technol 34(1) : 16-22

Goh JS, Kim GY, Saito J (1990). Stimulated fermentation of ultra filtration retentate by *L. acidophilus and S. thermophilus*. Korean J Dairy Sci 12 (2): 181-189

Hunger N, (1987) Starter culture for the food industry. New developments in industry. IDF Bulletin 227

Kim SC, Harmon LG (1968) Effect of insecticide on growth and fermentation ability of lactic culture organisms. J Milk Food Technol 2 (1) : 97-100

Patel RS, Chakraborthy (1985) Use of different sources of milk solids for *shrikhand* making. Egyptian J Dairy Sci 13:79:84

Ramachandra Rao HG, Atmaram K, Nambudripad VKN (1987) Factors affecting the quality of *shrikhand*. Cheiron 16:(1), 31:34

Sharma DK, Reuter M (1992) Ultra filtration technique for *shrikhand* manufacture. Indian J Dairy Sci 45:209-213

Upadhayay SM (1982) Assessing the suitability of different microbiological and chemical tests on keeping quality of *shrikhand*. M.Sc. Thesis, Gujarat Agricultural University, Ahmedabad, India

Ziden ZA, Fayed AE, Shenany El, Abovarab AK (1990) Susceptibility of some lactic acid bacteria to different insecticides. Egyptian J Dairy Sci 18: 11-22

Received 26 September 1994; revised 10 March 1997; accepted 19 March 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 343-344

X-ray Diffraction Study of Ragi *(Eleusine coracana)* Starch

G.T. SAMANTARAY*[1] AND B.K. SAMANTARAY[2]

[1]Botany Department, Government College, Angul - 759 145, India.
[2]Department of Physics, Indian Institute of Technology, Kharagpur - 721 302, India.

X-ray crystallography study has been carried out on starch isolated from 6 varieties of ragi. A quantitative analysis of data on their crystallinity and crystallite size have also been correlated with the chemical characteristics of the isolated starch and the nature of the grains.

Keywords: Ragi, Starch, X-ray, Diffraction, Crystallinity, Crystallite size.

X-ray diffraction technique is an efficient method for identification of materials and their structural characterization (Kaelble 1967). It is used to reveal the structure and arrangement of molecules in materials. Several other characteristics such as crystallite size, orientation, microstrain, degree of crystallinity and amorphocity can also be determined by the x-ray diffraction technique (Wang and Harrison 1980) The method is also non-destructive. In the present investigation, the x-ray method has been applied for indentifying and characterizing starch isolated from 6 different varieties of ragi *(Eleusine coracana)*. The varieties "B7-7-43", "B4-10-56", "T-20" and "Indaf-5" were brown-grained, while "CO9 Mut(118)" and "CO9 Mut(23)" were white-grained ragi.

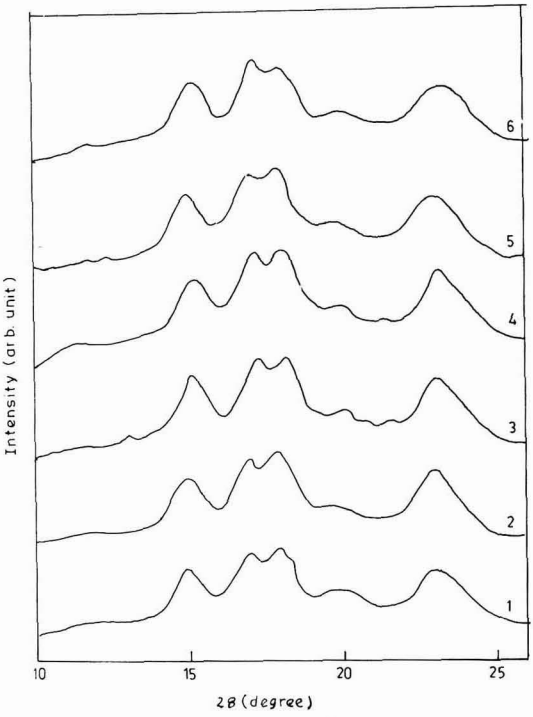

Fig. 1 Portion of the diffractograms for the different starch samples (1) "C09 Mut 18", (2) "T20", (3) "Indaf 5", (4) "B-4-10-56, (5) "C09 Mut 23" (6) "B-7-7-43".

Materials : Six different varieties of *ragi (Eleusine coracana)* were collected from O.U.A.T., Bhubaneswar. Starches from the ragi grains were isolated, following the method of Beleia et al (1980). The isolated starches were analyzed for proteins by micro-Kjeldahl method (Jackson 1973), amylose (Knutson 1983), ash, moisture and alcoholic acidity (Jackson 1973).

X-ray analysis : X-ray diffraction of starches was recorded with a Rigaku X-ray diffractometer, using nickel filtered $C_uK\alpha$ radiatioin the angular range 5° to 45°, 2θ at a scanning speed of 1/2°X

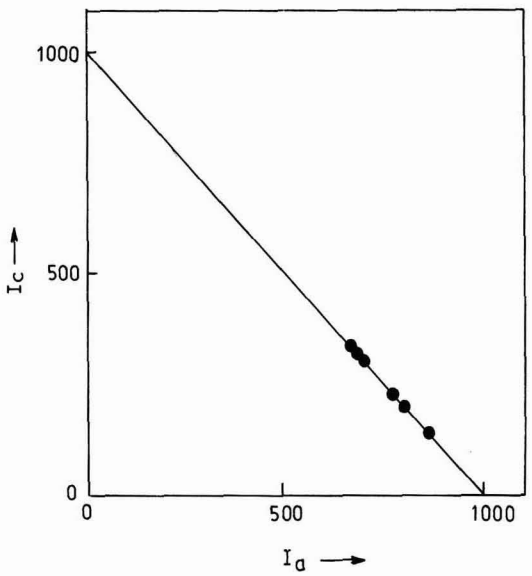

Fig. 2 A plot of x-ray intensities scattered by amorphous and crystalline regions of different samples

* *Corresponding Author*

TABLE 1. PHYSICO-CHEMICAL ANALYSIS, DEGREE OF CRYSTALLINITY AND CRYSTALLITE SIZE FOR STARCH FROM DIFFERENT VARIETIES OF RAGI *(ELEUSINE CORACANA)*

Varieties	Protein, %	Amylose, %	Moisture, %	Alcoholic acidity, %	Ash %	Degree of crystallinity, %	Crystalline size, AU
'C09 Mut (18)'	1.04	26.5	4.8	0.441	2.4	23.0	120.0
'T20'	1.24	28.0	3.2	0.440	2.5	21.0	90.0
'INDAF 5'	1.89	28.0	3.3	0.440	2.8	31.0	102.0
'B4-10-56'	1.76	26.0	3.8	0.400	2.1	32.0	130.0
'C09 Mut (23)'	1.15	26.0	4.2	0.318	2.1	15.0	98.0
'B7-7-43	1.75	28.0	4.3	0.465	3.1	33.0	120.0

(2θ) per min. The samples were pressed into the sample holder and the conditions of measurement were kept identical for all samples. The x-ray tube was maintained at 30KV and 10mA. A portion of the range 10° to 30°, 2θ for the different starch samples is shown in Fig 1. Table 1 indicates that the percentage of total protein ranges from 1.04 to 1.89%. White-grained ragi samples were low in proteins, compared to brown-grained ragi varieties. Similarly, starches isolated from brown-grained ragi were richer in amylose than those from white-grained ragi.

X-ray studies : Peaks were observed at about 15°, 17°, 18°, 20°, 23°, 26°, 2θ with slight sample to sample variation (Fig. 1). Beleia et al (1980) have also observed slight variation in the crystallinity, but have not made any quantitative measurements. Observation of peaks at similar positions showed that all the varieties of ragi contained "A" starch.

The degree of crystallinity has been determined from the diffracto-grammes, following the method of Hermans and Weidinger (1961). A plot of the X-ray intensities scattered by the crystalline and amorphous regions of different samples is shown in Fig. 2. The different points nicely fit into a straight line, establishing that the crystalline peaks have been properly separated from the amorphous halo in each case. However, the size of the crystalline regions is related to the sharpness of the profiles. The x-ray diffraction peaks are very sharp for samples, containing large crystallites. A quantitative estimation of the size of the crystalline domains was made from the half width of the line profiles by using Scherrer formula (Wang and Harrison 1980).

$$\beta(1/2) = K\lambda \; / \; t. \; Cos\theta$$

where β (1/2) is the half width (in radians) of the x-ray peaks, λ the wavelength of the radiation, and θ the Bragg angle, "t" the crystallite size and "K" is a constant taken as unity.

From the results presented in Table 1, it is observed that though the starch samples obtained from different ragi varieties have the same type of structure and give diffraction peaks at same angles, their extent of crystallinity and size of the crystalline regions vary. The ragi varieties viz., "B 4-10-56", "B7-7-43", "T-20" and "Indaf-5" have higher crystallite sizes and crystallinities than white-grained ragi i.e., "Co 9 Mut (23)" and "Co 9 Mut (18)".

Although ragi varieties show insignificant variations in chemical characterstics, the degree of crystallinity may be affected to some extent by the amylolytic activity of the ragi starch. Due to the high water binding as well as imbibing capacity, ragi starch is recommended for weaning foods and other food products. Brown-grained ragi varieties are found to be superior in this regard.

References

Beleia, A. Marston, EV, Hoseney RC (1980) Characterisation of starch from pearl millets, Cereal Chem 57: 300-303

Hermans PH, Weidinger A (1961) Determination of crystalline fraction from X-ray diffraction. Makromol Chem 44: 24-36

Jackson ML (1973) Soil Chemical Analysis, Prentice Hall (India), New Delhi

Kaelble EF (1967) Hand book of X-Rays, McGraw Hill Pub. Co., New York

Knutson, CA, (1986) A simplified colorimetric procedure for determination of amylose in maize starches. Cereal Chem 63: 89-92

Wang JI, Harison R (1980) Crystallite size and lamelar thickness. In: Fave RA (ed) Methods of Experimental Physics, Academic Press, New York

Received 25 September 1993; revised 20 March 1997; accepted 21 March 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 345-347

Changes in Solubility and *In vitro* Digestibility of Rice Proteins Subjected to Heat Processing

KAMINI DEVI, P. GEERVANI*[1] AND S. SUMATHI

Department of Foods and Nutrition, Post-graduate and Research Centre, Andhra Pradesh Agricultural University, Hyderabad - 500 030, India.

Changes in solubility and *in vitro* digestibility of rice proteins, after milling, parboiling and boiling of milled raw rice and parboiled rice, have been investigated. Solubility of rice proteins in different solvents revealed differences in extractability due to processing. Parboiling increased solubility of fraction 1 (albumin-globulin and fraction 4 (glutelin-like), whereas fraction 2 (prolamine), fraction 3 (cross-linked prolamine) and fraction 5 (glutelin) were reduced by this treatment. Recovery of soluble proteins decreased considerably, when parboiled rice was subjected to boiling. The *in vitro* protein digestibility (IVPD) of parboiled rice, both raw and upon boiling, was significantly lower ($P<0.05$), as compared to raw milled rice.

Keywords: Rice proteins, Processing, Solubility, Nitrogen, Protein fractions, *In vitro* digestibility.

Proteins present in food are a mixture of several fractions and each fraction differs in its solubility, amino acid composition and digestibility. The nitrogen distribution in different protein fractions of rice was listed by Juliano (1972). Preparation of cereals, millets and pulses for consumption involves processing by both primary and secondary methods. Heat processing of pulses has been reported to improve protein digestibility (Friedman 1975; Singh and Jambunathan 1981), due to inactivation of protease inhibitors and opening of the protein structure through denaturation. However, heat processing of cereals and millets has been found to reduce protein digestibility (Hamaker et al. 1986), as a result of non-enzymatic browning reaction and thermal cross-linking. Heat processing was also shown to alter the solubility of proteins in rice (Bradbury et al. 1984), but the effect of alteration in solubility on protein digestibility has not been reported earlier. Hence, an attempt has been made to identify the changes in solubility of protein fractions and their effect on *in vitro* protein digestibility upon subjecting rice cultivars to different heat processing methods.

Three popular varieties of rice (*Oryza sativa* L.) viz., 'Tella Hamsa', 'Sona Mashuri' and 'BPT 5204', grown in Andhra Pradesh, India, were selected for the study. The samples grown during the kharif season were processed for analysis. In order to ensure uniformity of milling in rice samples, paddy was procured and milled in the laboratory. Parboiling of paddy was done by steeping 2 kg samples of each variety overnight in water at 40°C and subsequently subjecting to boiling for 30 min. The parboiled grain was air-dried for 12h, in shade (30-32°C, RH 50-55%) and milled. Milling was carried out in a local rice mill. Polish (10%) was applied to all grains, using 'Satake' rice polisher. After standardization of cooking time, 1 kg each of milled raw rice and parboiled rice were subjected to boiling at atmospheric pressure for 19 and 17 min, respectively. The cooked rice samples were freeze-dried and powdered using a Udy mill.

Moisture was estimated in all the samples by AOAC (1984) method. Protein and nitrogen (N x 6.25) were determined, using the Technicon Auto Analyser (TAA), by the method of Singh and Jambunathan (1980). Fractionation of proteins in raw and processed samples of 'Tella Hamsa' variety of rice was carried out according to the methods of Landry and Moureaux (1970) and Jambunathan et al (1975), respectively. The individual fractions were obtained by extracting the finely ground material successively with 0.5 M sodium chloride (fraction 1), 70% isopropyl alcohol (fraction 2), 70% isopropyl alcohol, containing 0.3% 2-mercaptoethanol (fraction 3), borate buffer (pH 10), containing 2-mercaptoethanol (fraction 4), borate buffer (pH 10), containing 2-mercaptoethanol and sodium dodecyl sulphate (fraction 5) and 0.1 N NaOH (fraction 6).

In vitro protein digestibility (IVPD) was determined by the method of Axtell et al (1981). Statistical analysis of the data was carried out to determine the correlation coefficients and also the significance of difference by subjecting the data to analysis of variance (Snedecor and Cochran 1968).

Solubility of rice proteins in different fractions revealed differences in extractability (Table 1), due to processing. According to Nwasike et al (1979),

* *Corresponding Author* [1]*Present address : Sri Padmavati Mahila Visvavidyalayam, Tirupati 517 502, India*

TABLE 1. PROTEIN DISTRIBUTION OF RAW, PARBOILED AND BOILED SAMPLES OF RICE VARIETY 'TELLA HAMSA' (% N BASIS)*

Nature of sample	Protein, %	Protein fractions						Recovery, %
		F_1	F_2	F_3	F_4	F_5	F_6	
Milled, raw	8.95	1.7	0.5	0.4	1.8	9.8	0.8	93.5
Parboiled	8.96	1.8	0.3	0.3	2.6	8.9	1.3	94.9
Milled, boiled	9.50	0.7	0.2	0.3	3.2	6.7	1.8	80.7
Parboiled-boiled	9.43	0.4	0.2	0.3	3.3	5.5	2.1	74.6

* Means of duplicate analysis

the Landry Moureax fractions are constituted as fraction I (F_1) - albumin and globulin, fraction II (F_2 - true prolamine, fraction III (F_3) - prolamine - like, fraction IV (F_4) - glutelin - like fraction V (F_5) - glutelin and fraction VI (F_6) - alkali soluble residue. Fractions IV and V constitute the major proteins in raw milled rice. On parboiling, the albumin-globulin fraction (F_1) was increased marginally, fractions IV and the alkali-soluble fraction (F_6) were increased considerably, while fractions II and V were reduced to some extent. Raghavendra Rao and Juliano (1970) reported that parboiling of rice reduced extractability of all protein fractions by an average of 45% with the globulin fraction, being the most affected on % decrease basis and glutelin on weight basis. The difference in solubility observed in the present study could be due to differences in time and temperature adopted during parboiling process. Dimopoulos and Muller (1972) also reported that protein extractability in many solvents decreased in direct proportion to the severity of steaming during parboiling.

Except fractions 4 and 6, all other fractions reduced in solubility on boiling of milled rice. Contrary to the increase in fraction 1 in raw parboiled rice, boiling of the parboiled grain resulted in a reduction in solubility of this fraction from 1.7 to 0.4% on nitrogen basis. There was a decrease in extractability of fractions 2 and 5, whereas fractions 4 and 6 showed an increase. A redistribution of proteins in different fractions was observed on processing by this method, with an increase from 1.3 to 2.1% nitrogen in alkali-soluble residue. There was also a significant ($P<0.05$) reduction in % recovery from 94.9 to 74.6.

Table 2 indicates the *in vitro* protein digestibility (IVPD) of raw and processed rice cultivars. The mean *in vitro* digestibility value was higher for 'Tella Hamsa' and lower for 'BPT 5204'. Digestibility values decreased to different levels, following different processing methods applied. Boiling of milled rice resulted in a drop in digestibility value to an extent of 14%. Decrease in true digestibility (TD) in uncooked rice to 85% in cooked rice was reported by Eggum (1979). Bradbury et al (1984) and Rajyalakshmi (1986) also observed a reduction in digestibility by 11 and 12%, respectively, on boiling of rice. Parboiling of rice resulted in reduction in IVPD by 13.4%. Upon parboiling, the unbound lipid and free fatty acids in milled rice decrease, whereas lipids bound to starch and protein and free phenolic acid increase, thereby reducing the extractability of proteins in the grains (Kato et al. 1983), resulting in reduction in protein digestibility. IVPD of raw parboiled rice was 65.5%, which decreased to 55.4% on boiling the parboiled grain. This difference in IVPD observed on processing suggests that the method of cooking is of critical importance with regard to protein digestibility and further, time and temperature of heat treatment may be the factors, which affect the digestibility of processed grains. Hsu et al (1977) have made a similar observation and concluded that high temperature short time heat process, as adopted in puffing of rice, gelatinizes the starch, opens the carbohydrate protein structure and makes it easier for proteolytic enzymes to hydrolyze the proteins.

Correlation coefficients between the different protein fractions and IVPD have revealed that *in vitro* protein digestibility is negatively correlated with the glutelin - like fraction (F_4) and insoluble residue (F_6) (Table 3). Upon processing by parboiling and boiling, increases in these two fractions were observed with a concomittant reduction in IVPD values. The higher the proteins in these two fractions, the lower was the IVPD. Thus, proteins

TABLE 2. *IN VITRO* PROTEIN DIGESTIBILITY (IVPD) OF RICE CULTIVARS SUBJECTED TO DIFFERENT PROCESSING METHODS, %

Nature of sample	Cultivars			SE
	'Tella Hamsa'	'Sona Mashuri'	'BPT 5204'	
Raw	77.7	75.8	73.5	1.214
Parboiled	65.0	65.5	66.0	0.289
Milled-boiled	61.5	64.8	69.0	2.170
Parboiled-boiled	54.3	52.6	59.4	2.043

Means of duplicate analysis

TABLE 3. CORRELATION BETWEEN PROTEIN FRACTIONS, IVPD AND PROTEIN CONTENT IN RICE

	F_1	F_2	F_3	F_4	F_5	F_6
Protein	-0.9677*	0.6257	-0.4983	0.8685	-0.9297	0.8861
IVPD	0.8080	0.8786	0.7537	-0.9521*	0.9358	-0.9800*

* 'r' values for 2 df. at 5%

in processed rice foods may be assumed to have a lower digestibility, depending on the processing applied.

Since *in vitro* studies have been supported earlier by *in vivo* experiments to confirm that a simple cooking treatment has a deleterious effect on protein digestibility (Bookwalter et al. 1987), the results of the present *in vitro* investigation may help to indicate the *in vivo* protein digestibility of rice grains, subjected to common processing methods such as parboiling and boiling. There is, however, a need to study the changes in solubility and digestibility of grain protein due to commercial processed foods such as breakfast foods and infant food formulations, which are mostly based on cereals like rice.

References

AOAC (1984) Official Methods of Analysis, 14th edn, Association of Official Analytical Chemists, Washington DC

Axtell JD, Kirleis AW, Hassen MM, Mason ND, Mertz E, Munck L (1981) Digestibility of sorghum proteins. In: Proceedings of the National Academy of Science, Washington DC, USA, pp 1333–1335

Bookwalter GN, Kirleis AW, Mertz E (1987) *In vitro* digestibility of protein in milled sorghum and other processed cereals with and without soy fortification. J Food Sci 52 (6): 1577–1619

Bradburry JH, Collins JG, Pyliotis NF (1984) Digestibility of proteins of the histological components of cooked and raw rice. Brit J Nutr 52: 507–513

Dimopoulos JS, Muller HG (1972) Effect of processing conditions on protein extraction and composition and on some other physico-chemical characteristics of parboiled rice. Cereal Chem 49: 54–62

Eggum BO (1979) The nutritional value of rice in comparison with other cereals : Chemical aspects of rice grain quality. In: Proceedings of the Workshop of International Rice Research Institute, Los Banos, The Philippines, pp 91–111

Friedman M (1975) Protein Nutritional Quality of Food and Feeds : 2: Quality Fectors – Plant Breeding, Marcel Dekker Inc., New York

Hamaker BR, Kirleis AW, Mertz ET, Axtell JD (1986) Effect of cooking on the protein profiles and pepsin digestibility of sorghum and maize. J Agric Food Chem 34:647–649

Hsu HW, Vavek DL, Satterlee LD, Miller GA (1977) A multi-enzyne technique for estimating protein digestibility. J Food Sci 47(5): 1269–1273

Jambunathan R, Mertz ET, Axtell JD (1975) Fractination of soluble proteins of high lysine and normal sorghum grain. Cereal Chem 52 (1): 119–121

Juliano BO (1972) Recent Developments in Rice Grain Research. Report of 7th Working and Discussion Meetings : International Association of Cereal Chemists, Vienna

Kato H, Ohta T, Tsugita T, Hosaka Y (1983) Effect of parboiling on texture and flavour components of cooked rice. J Agric Food Chem 31: 818–823

Landry J, Moureaux T (1970) Heterogeneite des glutehies du grain de mais : extraction selective et composition on acids amines des trois fractions isolees. Bulletin Soc Chem Biol 52: 1021–1037

Nwasike CC, Mertz CT, Pickett RC, Glover DV, Chibber BAK, Van Scoyoc SE (1979) Lysine level in solvent fractions of pearl millet. J Agric Food Chem 27: 1329–1331

Raghavendra Rao SN, Juliano BO (1970) Effect of parboiling on some physico-chemical properties of rice. J Agric Food Chem 18: 289–294

Rajyalakshmi P (1986) A study on qualitative and quantitative aspects of foods consumed by the tribals of Vizianagaram, Andhra Pradesh. Ph.D. Thesis, Andhra Pradesh Agricultural University, Hyderabad, India

Singh U, Jambunathan R (1980) Evaluation of rapid methods for the estimation of protein in chickpea (*Cicer aroetomi*, L.). J Sci Food Agric 231: 247–254

Singh U, Jambunathan R (1981) Studies on *desi* and *kabuli* chickpea (*Cicer arietinum* L.) cultivars : The levels of protease inhibitors, levels of phenolic compounds and *in vitro* protein digestibility. J Food Sci 46: 1364–1367

Snedecor GW, Cochran WG (1968) Statistical Methods, 6th edn. Iowa State University Press, USA

Received 23 March 1993; revised 12 August 1996; accepted 25 March 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 348-350

A Density Prediction Model in Relation to Shrimp Freezing Process

S.K. MISHRA, S. BANDYOPADHYAY* AND B. KARUNAKAR

Department of Agricultural and Food Engineering,
Indian Institute of Technology, Kharagpur-721 302, India.

Temperature dependent density of shrimp (*Penaeus* spp.) was experimentally determined at different initial moisture contents for both the frozen and unfrozen conditions. The data were fitted to empirical polynomial equations, taking both the product temperature and moisture content as input parameters. The density values predicted by the present model, existing empirical and theoretical models were in close agreement for a typical product condition. The present empirical model has got wider applicability than the previous ones.

Keywords: Density of shrimp, Prediction model, Freezing.

Density is one of the important thermo-physical properties of the food products, widely used for process calculations such as in food freezing. Solutions to heat conduction equation to predict time temperature profiles, freezing time and rate require reliable models for prediction of thermo-physical properties. The change of state of water inside the food product during freezing causes drastic changes in these property values, particularly in the phase change zone and varies non-linearly with fall in product temperature in the frozen state (Heldman 1982). The assumption of constant density for food products throughout the freezing process obviously leads to inaccuracies in the final freezing time prediction (Ramaswamy and Tung 1984). Compilations of density data are abundant in the literature for various food products under varying conditions (Dickerson 1968; Polley et al. 1980; ASHRAE 1981; Sanz et al. 1987), but prediction models, based on the experimental results are rare. The existing density prediction models are based on either empirical correlations (Rahman and Driscoll 1994; Succar 1984) or the theoretical models, based on component additive principle (Miles et al. 1983; Choi and Okos 1985). The theoretical models are rarely experimentally verified for their prediction accuracy. The main drawback of the empirical models is the use of different empirical parameters for food products at different initial moisture contents, thus precluding their general application even for a specific food product. Among the high moisture content seafoods, the density prediction models have been rarely cited in the literature (Rahman and Driscoll 1994). In the present work, the density of shrimp has been determined experimentally under varying product conditions, prevalent in the shrimp freezing process and an empirical model has been proposed for prediction of density, using initial moisture content and temperature as the independent variables. The accuracy of the experimental results has been validated with that obtained from the existing theoretical prediction model. Variations among the theoretical, empirical and proposed model have also been compared for a typical product condition.

Prediction models: The prediction models used in the present study were the additive model as proposed by Chois and Okos (1985) and the modified Schwartzberg's empirical equation for the lean fish meat, as given by Succar (1984). The additive model is given as:

$$\frac{1}{\varrho(t)} = \frac{(x_w - x_{ice}(t))}{\varrho_w} + \frac{x_{ice}(t)}{\varrho_{ice}(t)} + \frac{x_s}{\varrho_s} \quad \ldots\ldots (1)$$

$$\varrho \text{ ice } (t) = 916.9 - 0.156\ t \quad \ldots\ldots (2)$$

where ϱ is the density in kg/m^3, x is the mass fraction of components, t is the temperature in °C and subscripts ice, s and w denote ice, solids and initial water, respectively.

The mass fraction of ice used for the additive model can be calculated by its fraction prediction equation proposed by Schwartzberg (1976) as:

$$x_{ice}\ (t) = (x_w - x_b)\ (1 - t_{if}/t) \quad \ldots\ldots (3)$$

$$x_b = 0.4\ x_p \quad \ldots\ldots (4)$$

where subscripts b and p denote bound water and protein, respectively and t_{if} is the initial freezing temperature of the product in °C.

The modified Schwartzberg empirical equation for the initial moisture level of 82% is given as:

$$\varrho(t) = 985 + 0.2036\ (t_{if} - t) + C\ 1065 - 985)\ (t_{sw} - t_{if})/(t_{sw} - t) \quad \ldots\ldots (5)$$

* *Corresponding Author*

where t_{sw} is the freezing temperature of pure water in °C.

Experimental method: Shrimp (*Penaeus* spp.) was used for the experimental study. Samples of 20-25 g size were procured from the local market, peeled, deveined, beheaded and stored in polyethylene packets under refrigeration for use. Proximate composition of samples was determined following AOAC (1980) methods. Ranges of values for proteins, fat and minerals were 15.19-19.75%, 2.74-3.56% and 0.61-0.83%, respectively. The samples were dried under controlled conditions to arrive at the desired initial moisture content of the shrimp, which varied between 75.05 and 80.81% (wb).

The density of the shrimp at different temperatures was measured by liquid displacement method. To measure the density in the frozen range, 10 g of chopped samples were put in each of the two 25 ml specific gravity bottles and were frozen to -40°C, by dipping the bottles in ethyl alcohol-liquid nitrogen mixture, kept in an insulated stainless steel container. After freezing, the bottles were transferred to an insulated chamber, where the temperature of samples in one of the bottles was monitored by a temperature scanner. When the temperature of measurement was attained, the other specific gravity bottle was filled with toluene precooled at the same sample temperature. The weights were noted immediately and the density of shrimp calculated. The above procedure was repeated to find out the density of shrimp at different temperatures for both unfrozen and frozen range and for different initial moisture contents. The values reported were average of three replications at each temperature.

The experimental density of shrimp at different temperatures and initial moisture levels are presented in Fig. 1. The variation of density at different initial moisture contents justified the inclusion of moisture content of the product as a prediction parameter. The results also showed that for all the moisture levels, the product density remained constant and independent of the tempertaure during the unfrozen state. The density decreased with lowering of the product temperature below the initial freezing temperature of -1°C. It indicated that the fraction of water, frozen changed continuously, when temperature fell below the initial freezing point. Since the product density is closely related to the state of water in the product, it is evident that the density would vary with temperature and more specifically to the extent at which the phase change of water has occurred during freezing.

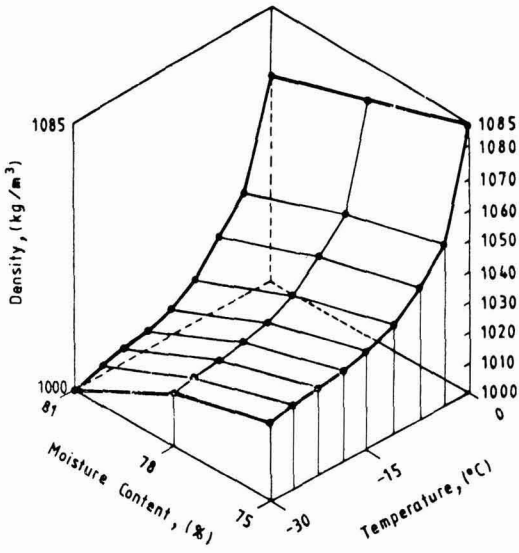

Fig. 1. Variation of density with temperature and moisture content

The comparison of the experimental values and those predicted by the theoretical model showed a very close agreement between them. The absolute mean deviation error at the various product temperatures and moisture levels varied between 0.64 and 0.89%. One set of values at moisture content of about 81% is shown in Table 1. Statistical analysis in the form of paired 't' test between the experimental and predicted values from the theoretical model showed no significant difference in the overall temperature range at 5% level of significance. However, 't' test only in the phase change zone i.e., -1 to -10°C showed a significant difference between the values. The deviation may be attributed to the validity of the model for prediction of ice fraction, which has been used in the theoretical model. The negligence of the excess volume due to interaction of phases in the multiphase system, particularly in the phase change zone, might have also led to the difference between the experimental and the predicted values (Rahman and Driscoll 1994).

The experimental density values were fitted to an empirical model by the multiple regression analysis for both the unfrozen and frozen state of the shrimp. The unfrozen state had only the initial moisture content as the dependent parameter and the first order equation with correlation coefficient of 0.98 was obtained as,

$$\varrho(x_w) = 1302.30 - 314.7\, x_w \qquad \ldots\ldots (6)$$

TABLE 1. COMPARISON OF SHRIMP DENSITY BETWEEN EXPERIMENTAL AND PREDICTED FROM THEORETICAL AT 81% MOISTURE CONTENT

Temperature, °C	Density, kg/m³	
	Experimental	Theoretical
30.0	1065.24	1065.00
20.0	1064.54	1065.00
10.0	1064.48	1065.00
-1.0	1065.05	1065.00
-2.5	1034.61	1020.38
-5.0	1019.70	1006.61
-10.0	1010.95	1000.39
-15.0	1006.78	998.78
-20.0	1003.28	998.34
-25.0	1000.61	998.34
-30.0	998.44	998.57

For the frozen state, below the initial freezing temperature of -1°C, the following second order polynomial equation with a correlation coefficient of 0.97 was fitted

$$\varrho\,(T, x_w) = 3397.84 - 29.09\,T + 3538.3\,x_w + 0.01527\,T^2 + 4.735\,x_w^2 - 3285\,x_w\,T \quad ....(7)$$

where 'T' is the temperature of the product in K.

The developed model was compared with the theoretical model as well as with the established empirical density prediction equation for a particular product condition. The density values from the model during the freezing range of the product were plotted in Fig. 2. The results showed close agreement among the model values, except in the phase change zone (-1°C to -5°C), where the proposed model differed slightly from the two other models. The analysis of variance ('F' test) among the model values did not show any significant difference at 1% level of significance, although the agreement was better between the proposed and theoretical models.

From the results, it is evident that the proposed model matches closely with both the additive and modified Schwartzberg models (for a typical product condition). The proposed empirical density prediction model has wider applicability than the existing empirical models by including both moisture content and temperature, as its prediction parameters. The proposed model overcomes the disadvantage of the existing empirical models as using different empirical parameters for different product initial moisture levels. The model can be used satisfactorily to predict the density variation of shrimp under various freezing conditions.

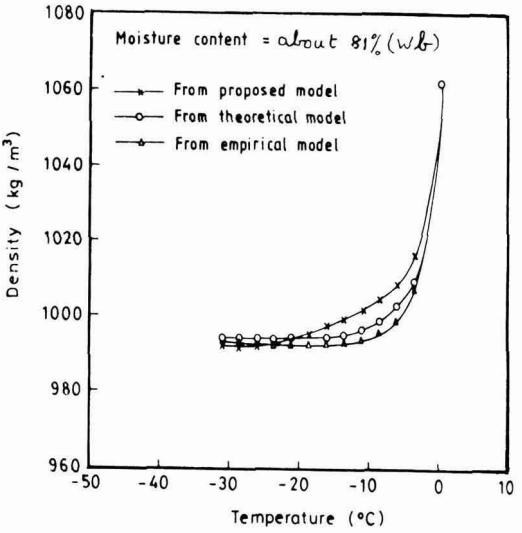

Fig. 2. Comparison of density of shrimp among the three prediction models

References

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

ASHRAE (1981) Thermal properties of foods. In: Fundamentals Handbook, American Society of Heating, Refrigeration and Air Conditioning Engineers, New York

Chois Y, Okos MR (1986) Effect of temperature and composition on the thermal properties of foods. In: Le Magure M, Jelen P (eds) Food Engineering and Process Applications, Elsevier, New York, p 93

Dickerson RW (1968) Thermal properties of foods. In: Tressler DK, Van Arsdel WB, Copley MR (eds). The freezing Preservation of Food, Van Nostran Reinhold/AVI, New York, p 26-51

Heldman DR (1982) Food properties during freezing. Food Technol 36:92-96

Miles CA, Van Beek C, Veerkamp CH (1983) Calculation of thermophysical properties of foods. In: Jowitt R (ed) Physical Properties of Foods, Vol. I, Applied Science, London, pp 269-312

Polley SL, Synder OP, Kotnour P (1980) A compilation of thermal properties of foods. Food Technol 34(11):76-94

Rahman MS, Driscoll RH (1994) Density of fresh and frozen seafood. J Food Process Engg 17:121-140

Ramaswamy HS, Tung MA (1984) A review on predicting freezing times of foods. J Food Process Engg 7:169-203

Sanz PD, Alonso MD, Mascheroni RH (1987) Thermophysical properties of meat products: General bibliography and experimental values. Trans ASAE 30(1):283-289

Schwartzberg HG (1976) Effective heat capacities for the freezing and thawing of foods. J Food Sci 41:153-156

Succar J (1984) Heat transfer during freezing and thawing of foods. In: Thorne S (ed.) Developments in Food Preservation, Vol. 5, Applied Science Publishers, London, p 253

Received 26 December 1995; revised 25 March 1997; accepted 1 April 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 351-353

Freeze-Thaw Stability of Oil-Water Emulsions Stabilised by *Detarium microcarpum* Flour and Gum

OKEY ASOTIBE, JANE ONWELUZO* AND KRIS ONUOHA

Department of Food Science and Technology,
University of Nigeria, Nsukka, Nigeria.

Oil-water emulsions stabilised by the flour and gum from *Detarium microcarpum*, a lesser known tropical legume, were subjected to weekly freeze-thaw cycles alongside commercial salad dressing, egg powder and gum tragacanth emulsions. Ability of the emulsions to remain stable to freezing and thawing was evaluated through oil-separation and syneresis. Emulsions of gum tragacanth, egg powder and mixture of seed gum and egg powder showed earliest and highest instability within the study period. The commercial emulsion and the emulsions stabilised by *D. microcarpum* flour and gum were stable to syneresis. *D. microcarpum* seed flour exhibited better compatibility with egg powder than the seed gum.

Keywords: *Detarium microcarpum*, Freeze-thaw cycle, Creaming index, Syneresis, Coalescence, Emulsion stability.

An emulsion is said to be stable, when it is slow at undergoing certain processes that would lead to the separation of the phases (Pearce and Kinsella 1978). These processes vary and include creaming, flocculation, coalescence and oiling off (Pearce and Kinsella 1978; Stainsby 1986). Except in some extreme cases and under certain conditions, these processes do not occur commonly, but certain factors predispose an emulsion to exhibit these signs of instability. One of the predisposing factors is freezing. Generally freezing has an adverse influence on emulsions (Hanson and Fletcher 1961). Since the damage of emulsions at frozen temperature is related to the influence of freezing on the emulsifier or stabiliser in the emulsion system, there is a need to search for an emulsifier or stabiliser that is stable to frozen storage.

A preliminary investigation with *D. microcarpum*, a lesser known tropical legume, suggested that flour and gum of this legume has potentials as functional ingredients in food emulsion systems. The present communication reports investigations on the stability of oil-water emulsions of *D. microcarpum* flour and gum under freeze-thaw cycles. The compatibility of these legume products with egg powder is also assessed.

Detarium microcarpum seeds were procured from Nsukka local market, while commercial salad cream (Heinz brand, England) was obtained from a supermarket in Nsukka town. Gum tragacanth was from Merck Scientific Supplies Co (Merck, W. Germany). Egg powder from National Egg Products Corporation, USA, while olive oil from Robert Laboratories, England were the other ingredients used in the study.

* *Corresponding Author*

Preparation of D. microcarpum *flour:* Flours were prepared from two differently treated seeds of *D. microcarpum*. Cleaned *D. microcarpum* seeds were divided into two equal portions. One portion was dehulled after soaking in water at room temperature (25°C), as described by Onweluzo et al (1994). The other portion was parboiled for 10 min at 100°C prior to dehulling. The dehulled seeds were dried in a hot air oven at 65°C for 48 h, pulverised in a hammer mill and screened through a 300 µm pore sized sieve. The flours were designated as SSF and BSF from the soaked and parboiled treatments, respectively.

Extraction of D. microcarpum *gum:* Gums were extracted from the defatted flours of both seed treatments by a modified of the method of Udeala and Uwaga (1981). A 10% (w/v) dispersion of the defatted flours was prepared and hydrated for 2 h. The dispersions wee centrifuged at 20,000 **g** for 30 min at room temperature. The supernatant was decanted and excess volume of cold 95% ethanol was added to it to precipitate the gum. The precipitated gum was redispersed in water 2 times and reprecipitated with excess cold ethanol. The gum extracts were dried in a hot air oven at 65°C for 48 h, pulverised in a hammer mill and screened through a 300 µm pore sized sieve as the seed flour. The gum extracts were designated as SSG and BSG from the soaked and parboiled seed flours, respectively.

Preparation of oil-water emulsions: Following preliminary tests, oil-water emulsions were prepared by the modified method of Hanson and Fletcher (1961). Eight batches of emulsions were prepared using each treatment seed flour (BSF and SSF), seed gum (BSG and SSG), gum tragacanth, egg

powder, combination of the seed flour and egg powder and a combination of the seed gum and egg powder. A dispersion of (3%) of each of these was prepared with part of the aqueous phase (54%) and allowed to hydrate for 24 h. The spices (2%), salt (2%), sugar (3%) and acetic acid (2%) were mixed with 0.5 ml of 0.2% sodium benzoate and added to the hydrated dispersions. Each mixture was homogenised for 10 min in a laboratory homogeniser. The dispersed phase - olive oil (34%) was then added to each mixture with blending at the rate of 3 ml per min. The oil addition was alternated with 2 min of homogenisation.

Emulsion stability studies: A 15 ml portion of each emulsion system was poured into 20 ml labelled calibrated test tubes and stored in a deep-freezer at about -20°C. The emulsions were subjected to weekly freeze-thaw cycles for 9 weeks. Within the storage period, the emulsions were monitored weekly for instability, using oiling-off (oil-separation) and syneresis as indices.

An index of 100% was used to designate a complete emulsion breakdown, while zero percent index represented stable or unbroken emulsion. Table 1 shows the weekly oiling-off index of the emulsions. Oil separation was observed to occur in all the emulsions, but at different degrees. Emulsion containing a combination of *D. microcarpum* seed gum and egg powder and the gum tragacanth emulsion started oil separation very early with 0.67% index each. The level of oil separation increased subsequently with storage period, giving indices of 27% and 17% for the gum tragacanth and seed gum - egg powder emulsions respectively. Comparing these two emulsion systems, the emulsion of the composite seed gum - egg powder exhibited better overall freeze-thaw stability than the tragacanth emulsion.

Emulsion containing *D. microcarpum* seed gum showed moderate stability. Both seed gum emulsions started oil-separation from the fifth week with equal index of 0.67% each. The commercial salad cream, seed flour and egg powder emulsions appeared to show equal freeze-thaw stability, as they all started oil separation from the seventh week. However, the emulsions containing boiled *D. microcarpum* seed flour showed lower average index of about 1.6% than the emulsions of the soaked seed flour, egg powder and the commercial salad cream, with average indices of 1.8%, 1.8% and 3.6%, respectively. Differences in the stability of the seed flour emulsions may be due to the different treatments.

By the ninth week, the emulsions containing tragacanth, egg powder, and a combination of seed gum and egg powder had suffered serious changes in appearance and texture. The tragacanth emulsion showed the highest level of graininess. This characteristics agreed closely with the observations of Hanson and Fletcher(1961), who noted that 5% to 10% oil separation in frozen emulsion was usually accompanied by a change in appearance and it was characterised by sponginess, when thawed. The boiled seed flour containing emulsion showed relatively better homogeneity. The commercial salad cream showed about equal stability to freeze-thaw cycle as the seed flour emulsions, but by the ninth week of storage, it was observed to have undergone serious colour change towards creamy brown with the emulsion, containing the soaked seed flour.

Results of the syneresis index over nine weeks freeze-thaw cycle table showed that there was no

TABLE 1. OILING-OFF (%) AND SYNERESIS (%) OF THE EMULSION SYSTEM AS INDICES OF FREEZE-THAW STABILITY

	Oiling off index, %									Syneresis index, %		
Time, weeks	Boiled seed gum[a]	Soaked seed gum[b]	Boiled seed flour[c]	Soaked seed flour[d]	Soaked seed flour +egg powder[e]	Soaked seed gum+ egg powder	Egg powder	Gum traga-canth	Comm-ercial salad cream[f]	Soaked seed gum +egg powder	Egg powder	Gum traga-canth
1	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.33	1.33	3.33
2	0	0	0.0	0.0	0.0	0.67	0.0	0.67	0.0	3.33	3.33	6.67
3	0	0	0.0	0.0	0.0	2.00	0.0	3.33	0.0	6.67	4.53	13.33
4	0	0	0.0	0.0	0.0	3.33	0.0	6.67	0.0	10.00	5.00	20.00
5	0.67	0.67	0.0	0.0	0.0	6.67	0.0	13.33	0.0	13.33	5.00	23.33
6	0.67	0.67	0.0	0.0	0.67	6.67	0.0	16.67	0.0	16.67	6.67	26.66
7	1.33	0.67	0.67	0.67	3.33	10.00	0.67	23.33	0.67	16.67	6.67	30.00
8	2.67	2.00	0.67	1.33	6.67	13.33	1.33	25.33	3.33	20.00	6.67	30.00
9	3.33	3.33	3.33	3.33	10.0	16.67	3.33	26.66	6.67	20.00	10.00	30.00

Emulsions of (a, b, c, d, e, f) samples showed zero syneresis index

instability due to syneresis in the commercial salad cream, as well as the emulsions, containing *D. microcarpus* seed flours and gums. However, emulsion, containing a combination of egg powder and seed gum, showed highly positive syneresis with an index of 1.33% in the first week in contrast to the emulsions, containing a combination of the seed flour and egg powder, which showed zero syneresis index all through the study period. Among the emulsions that syneresed, tragacanth emulsion exhibited the highest syneresis index and the degree of syneresis increased with increase in the storage period. It is evident from the oiling-off and syneresis characteristics of these emulsions that *D. microcarpum* seedflour had better compatibility with egg powder than the seed gum. This may be attributed to the formation of insoluble complexes between the seed gum and the egg protein. Tolstoguzov (1988) reported that anionic polysaccharides had limited compatibility, when complex formation was inhibited. The presence of acetic acid in the system may have induced opposite net charges on the egg protein and the seed gum and consequently led to phase separation that was evident from the first and second week of the study.

References

Hanson HL, Fletcher LR (1961) Salad dressings stable to frozen storage. J Food Technol 15:256-262

Onweluzo JC, Obanu ZA, Onuoha KC (1994) Composition of some lesser known tropical legumes. J Food Sci Technol 31(4):307-310

Pearce KN, Kinsella JE (1978) Emulsifying properties of proteins: Evaluation of a turbidimetric technique. J Agric Food Chem 28(3):716-722

Stainsby G (1986) Foaming and emulsification. In: Mitchell JR, Ledward DA (eds.) Functional Properties of Food Macromolecules. Elsevier Applied Sci Publ, London, pp 315-353

Tolstoguzov VB (1986) Functional properties of protein-polysaccharide mixture. In: Mitchell JR, Ledward DA (eds.) Functional Properties of Food Macromolecules. Elsevier Applied Sci Publ, London, pp 365-415

Udeala OK, Uwaga UN (1981) Some emulsifying and suspending properties of a polysaccharide gum derived from *Mucuna flagellipes*, Papilionaceae. J Pharm Pharmacol 33:75-77

Received 23 March 1994; revised 2 April 1997; accepted 3 April 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 354-356

Nutritional Evaluation of Rabi French Beans

JAI SINGH AND D.R. SOOD*

Department of Chemistry and Biochemistry,
CCS Haryana Agricultural University, Hisar-125 004, India.

Growth studies and nutritional evaluation of 'PDR-14', 'HUR-137' and 'HUR-15' French beans were carried out in rats. Moisture, crude protein, ether extracts, crude fibre, mineral matter, carbohydrates and energy of the varieties of beans ranged from 9.90 to 10.71; 20.65 to 22.75; 3.54 to 4.23, 1.54 to 1.63; 3.58 to 8.65; 57.70 to 59.77% and 376.46 to 378.94 Kcal/100g, respectively. Feed efficiency ratio did not differ much. Protein efficiency ratio varied from 1.37 to 1.47 as compared to 2.46 for casein. All the three cultivars exhibited an excellent balance of essential amino acids, except sulphur containing amino acids and tryptophan. 'PDR-14' was found to be superior in respect of protein efficiency ratio, biological value and net protein utilization.

Keywords: French bean, Proximate composition, Protein efficiency ratio, Biological value, Essential amino acids, Net protein utilization.

French bean (*Phaseolus vulgaris* L), commonly known as 'rajmash' is an important pulse grown worldwide mainly as a kharif crop and a potential source of proteins, carbohydrates and minerals. The nutritional value of dried bean protein is, however, poor and is attributed to its low content and limited availability of essential amino acids, the presence of heat-stable residual heat-labile antinutritional factors, (Deshpande et al. 1982) and/or due to poor protein digestibility (Thompson and Gabon 1987). The pigments, in general, and phenolic compounds in particular, are likely to interact with bean proteins, thereby decreasing their digestibility and utilization (Sgarbieri et al. 1979). The present study was aimed to assess the nutritional value of 3 new varieties of French beans.

Seeds of French beans varieties ('PDR-14', 'HUR-15', 'HUR-137') were procured from Indian Institute of Pulse Research, Kanpur, India. After proper cleaning and drying, the seeds were ground to 0.2 mm particle size, using Udytec Cyclone sample mill and stored in air tight plastic bottles. AOAC (1984) methods were followed for determining proximate composition. Essential amino acid composition except tryptophan was determined after acid hydrolysis as per the procedure of Singh (1982), using CL-6-Schimadzu, high pressure liquid chromatography, connected with sodium NA type amino acid analysis column. Tryptophan was estimated after enzymatic hydrolysis according to Spies and Chamber (1949). Energy value was computed from proximate principles, assuming that proteins, carbohydrates and fats yield 4, 4 and 9 Kcal/g, respectively.

Male Wister strain weanling rats weighing between 12-22 g obtained from the disease – and germ-free animal house of the University were divided into 4 groups, consisting of 6 rats each in a complete randomised design. The rats were housed individually in aluminium metabolic cages and were kept in an air conditioned room, maintained at 22-24°C. Food and water were given *ad libitum*. Rats were fed on nutritionally complete diets of raw French bean/casein at 10% protein level, containing salt mixture 3.5%, vitamin mixture 2%, vitaminized groundnut oil 5%, cellulose 5% and corn starch to equal 100% (AOAC 1984). Diet consumption was monitored daily. The rats were finally weighed after 4 weeks. Feed efficiency ratio (FER) and protein efficiency ratio (PER) were calculated. For biological value determination, the rats were first acclimatized for 4 days. Urine and faeces were collected for 6 days and pooled. Faecal samples were dried at 70°C. A few drops of H_2SO_4 (1N) were added to urine to prevent loss of ammonia. Nitrogen was determined in urine and faeces by micro-Kjeldahl method (AOAC 1984). Biological value (BV), net protein utilization (NPU) and utilizable protein were calculated from the data obtained. Essential amino acid contents and requirements in animal product, as reported by Zarkadas et al (1993) were used for comparison.

Proximate composition : The data on the proximate composition are presented in Table 1. Moisture contents of the French beans ranged from 9.90 to 10.7; proteins from 20.65 to 22.75; crude fibre from 3.54 to 4.23; ether extracts from 1.54 to 1.63; mineral matter from 3.58 to 3.65; carbohydrates from 57.70 to 59.77% and calculated energy from 376.36 to 378.94 Kcal/100 g. The values reported by different workers (Phogat et al.

* *Corresponding Author*

TABLE 1. PROXIMATE AND ESSENTIAL AMINO ACID COMPOSITION OF 'RAJMASH' VARIETIES

	'PDR-14'	'HUR-17'	'HUR-15'
Moisture, %	10.23	10.71	9.90
Proteins, %	20.65	22.40	22.75
Crude fibre, %	4.23	3.97	3.54
Ether extractives, %	1.54	1.63	1.54
Mineral matter, %	3.58	3.59	3.65
Carbohydrates, %	59.77	57.70	58.62
Energy, Kcal/100g	376.46	377.91	378.94
Each value is an average of 3 determinations.			
Amino acids (mg/g total proteins)			
Histidine	20.7	18.3	30.7
Isoleucine	24.9	23.2	26.0
Leucine	53.6	57.2	61.1
Lysine	72.0	71.8	76.3
Methonine + Cystine	14.1	13.2	12.6
Phenylalanine + Tyrosine	61.9	63.7	48.7
Threonine	35.2	44.1	41.3
Tryptophan	9.6	13.0	12.0
Arginine	67.1	55.4	72.5
Valine	51.6	50.0	58.2
Total	354.99	352.57	395.54

1984) are comparable to the present reported values. However, the values for proximate nutrients were different in comparison to earlier 'rajmash' varieties, grown in USA (Salunkhe and Kadam 1989). This may be due to variations in agroclimatic conditions and genetic factors.

Amino acids: Data presented in Table 1 indicate that the evaluated French bean cultivars contain all the essential amino acids (EAA), except sulphur containing amino acids (methionine and cysteine) and tryptophan, which are limiting amino acids. All the three beans contain lower values of individual as well as total essential amino acids in comparison to egg and cow's milk. However, the contents of essential amino acids fail to take into account the difference in the digestibility. Moreover, the essentiality of amino acids is dependent upon the requirements of the specific species in question. The present values of essential amino acids are not in conformity with the findings of Antunes and Sgarbieri (1980) in beans.

Table 2 presents data on the food intake, protein consumed, weight gain, feed efficiency ratio, protein efficiency ratio and relative protein efficiency ratio. FER did not vary much in beans and ranged between 1.37 and 1.47, as compared to 2.46 for casein, 'PDR-14' was found to have slightly higher PER (1.47). Kakade and Evans (1966) observed mortality in rats, when fed on raw navy beans at 10% protein level and autoclaving navy beans for 5 min and then feeding to rats gave PER of 1.57, but during the present study, no mortality was found.

Data on nitrogen intake, urinary nitrogen, endogenous urinary nitrogen, faecal nitrogen, endogenous faecal nitrogen, true protein digestibility, biological value, net protein utilization and utilizable protein presented in Table 2 show that French bean had biological value between 48.3 and 65.5% and net protein utilization between 30.2 and 46.4% as compared to the corresponding values of 86.2 and 66.9% for casein. Utilizable protein and true protein digestibility varied from 6.8 to 9.6% and 62.6 to 70.8%, as compared to 61.5 and 77.7% for casein

TABLE 2. EFFECT OF FEEDING 'RAJMASH' ON RAT GROWTH, TRUE PROTEIN DIGESTIBILITY, BIOLOGICAL VALUE AND NET PROTEIN UTILIZATION

	'PDR-14'	'HUR-137'	'HUR-15'	Casein
Food intake, g	154.33±2.60	155.17±5.42	153.50±3.08	154.20±4.75
Protein consumed, g	15.43±1.06	15.52±1.14	15.35±2.10	15.42±1.18
Weight gain, g	22.67±0.88	21.33±0.33	21.00±0.58	37.90±0.33
Feed efficiency ratio	0.15±0.007	0.14±0.009	0.14±0.01	0.25±0.02
Protein efficiency ratio	1.47±0.006	1.37±0.008	1.37±0.03	2.46±0.05
Relative protein efficiency ratio, %	59.75±0.45	55.69±0.63	55.69±0.26	100.00±0.00
Nitrogen intake, mg	182.2 ±2.41	172.8 ±3.36	173.9 ±3.91	182.0 ±0.53
Urinary nitrogen, mg	56.0 ±0.94	62.3 ±0.72	64.8 ±1.59	40.2 ±3.80
Endogenous urinary nitrogen, mg	11.6 ±0.41	9.2 ±0.52	8.5 ±0.67	20.7 ±1.52
Faecal nitrogen, mg	71.8 ±0.97	73.4 ±1.08	74.8 ±0.71	67.6 ±2.44
Endogenous faecal nitrogen, mg	18.6 ±0.48	10.2 ±0.52	98.0 ±0.66	27.1 ±1.45
True protein digestibility, %	70.8 ±1.10	63.4 ±1,59	62.6 ±1.09	77.7 ±1.10
Biological value, %	65.5 ±1.35	51.5 ±2.25	48.3 ±1.15	86.2 ±1.57
Net protein utilization, %	46.4 ±0.27	32.6 ±0.52	30.2 ±0.26	66.9 ±1.46
Utilization protein, %	9.6 ±0.98	73.0 ±0.86	6.8 ±0.64	61.5 ±1.02
Each value is an average of 6 determinations.				

diet. 'PDR-14' emerged superior on the basis of nitrogen balance studies conducted under present conditions. Literature reports indicate that the protein digestibility of beans vary from 43.6 to 74% (Sathe et al. 1984). The poor digestibility of bean proteins as compared to casein diet may be due to its interaction with phenolic compounds and phytates or carbohydrates, conformational changes and enhanced nitrogen loss. Similar explanation was also given by several workers (Artz et al. 1987). Biological value of legumes varied from 45 to 74% (Phansalkar 1960) and of kidney bean and soybean ranged from 62 to 68% and 64 to 80% (Milner 1972), which are comparable to the present findings. The low NPU may be due to more nitrogen loss through urine and faeces.

It may be concluded from the present study that though 'HUR-137' had better chemical composition, 'PDR-14' exhibited higher nutritive value, as judged by growth and nitrogen balance studies on rats.

References

AOAC (1984) Official Methods of Analysis. 14th edn. Association of Official Analytical Çhemists, Washington, DC

Antunes PL, Sgarbieri VC (1980) Effect of heat treatment on the toxicity and nutritive value of dry bean (*Phaseolus vulgaris*) var. 'Rosinha G_2' proteins. j Agri Food Chem 22: 935-938

Artz WE, Bishop PD, Dunker AK, Schancus G, Swansan BG (1987) Interaction of synthetic proanthocyanidin dimer and trimer with bovine serum albumin and purified bean globulin fraction G-1. J Agri Food Chem 35:417-421

Deshpande SS, Sathe SK, Salunkhe DK, Cornforth DP (1982) Effect of dehulling on phytic acid, polyphenols and enzyme inhibitors of dry beans (*P. vulgaris* L.). J Food Sci 47:1846-1850

Kakade ML, Evans RJ (1966) Effect of soaking and germination on the nutrive value of navy beans. J Food Sci 31:781-786

Milner M (1972) Nutritional improvement of food legumes by breeding. In: Proceedings Symposium sponsored by Protein Advisory Group of United Nations System, New York

Phansalkar SY (1960) Nutritive evaluation of vegetable proteins. In: Proceedings of Symposium of Proteins. Chemical Research Committee and Society of Biological Chemists, Mysore, India

Phogat SR, Kumar S, Phogat SS, Dahiya IS (1994) Soybean - A highly nutritious food legume. Farmer and Parliament 29:19-20

Salunkhe DK, Kadam SS (1989) Handbook of World Food Legumes, Nutritional Chemistry, Processing Technology and Utilization. Vol. I, CRC Press Baca Raton, Florida

Sathe SK, Deshpande SS, Salunkhe DK (1984) Dry beans of *phaseolus*. A Review. Part I. Chemical Composition Proteins, CRC Crit Rev Food Sci Nutr 21:1-40

Sgarbieri VG, Antunes PL, Almeida LD (1979) Nutritional evaluation of four varieties of dry beans (*Phaseolus vulgaris* L) J Food Sci 44:1306-1308

Singh U (1982) Grain quality and biochemistry. Progress Report 22, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India

Spies UR, Chambers DC (1949) Chemical determination of tryptophan in proteins. Anal Chem 21:1249-1266

Thompson LV, Gabon JE (1987) Effect of Kectins on salivary and pancretic amylase activities and the rate of starch digestion. J Food Sci 52:1052-1053

Zarkadas CG, Yu Z, Voldeng HD, Amador AM (1993) Assessment of the protein quality of a new protein soybean cultivar by amino acid analysis. J Agri Food Chem 41:616-623

Received 14 December 1995; revised 7 April 1997; accepted 9 April 1997

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 357-359

Reliability of Rapid and Routine Quality Control Tests for Grading Raw Milk Under Indian Conditions

S.R. GARG* AND USHA V. MANDOKHOT

Department of Veterinary Public Health and Epidemiology,
CCS Haryana Agricultural University, Hisar-125 004, India.

The reliability of titrable acidity (TA) test, methylene blue reduction (MBR) test and standard plate count (SPC) test in grading raw milk was examined. The TA test was found to be unreliable in view of the prevalent practice of adulteration of milk with neutralizers. The grading of milk differed in 56 out of 86 samples, when evaluated separately by MBR and SPC tests, according to the recommendations of the Bureau of Indian Standards, as the former test placed 54 samples in higher and 2 in lower grades, as compared to the latter. The SPC test graded 64 samples out of 86 samples as poor, whereas MBR test graded only 17 samples as poor. The results indicated poor hygienic conditions in the region.

Keywords: Milk, Quality control tests, Adulteration, Methylene blue reduction, Standard plate count.

To ensure the supply of unadulterated milk to the consumers, various quality control tests are performed at dairy farms, milk collection centres and milk plants. In India, determination of titrable acidity (TA) is a commonly used platform test at the milk collection centres to detect the developed acidity and in turn, judge the bacteriological stability of milk. Another usually recommended platform test is methylene blue reduction (MBR) test. The present investigations were undertaken to study the reliability of these two tests in judging the quality of milk sold in the local market. The effect of addition of neutralizers like carbonates/ bicarbonates on the TA of milk and the comparative grading of raw milk according to the standards suggested by the Bureau of Indian Standards (BIS) for methylene blue reduction time (MBRT) and standard plate counts at 37°C (SPC) were studied. In addition, the quality of the milk was evaluated by other quality control tests like thermoduric bacteria counts and pH of milk.

A total of 86 samples of raw milk were collected, which included 67 from local vendors, 6 from vendors of an organized dairy unit and 13 from a local milk plant. The samples, collected in sterile containers, were transported in ice box immediately to the laboratory and processed within 1 h of collection. The samples were analysed for SPC, MBRT, TA (as % lactic acid) and adulterants like carbonates/bicarbonates by the methods recommended by the BIS (ISI 1960, 1978). The pH values of the samples were determined with a pH meter (Systronics). Twenty six samples were examined for thermoduric bacteria by the method suggested by the BIS (ISI 1978). The criteria prescribed by the BIS (ISI 1978) for SPC and MBRT were used separately to grade the milk samples.

The frequency distribution of the milk samples according to pH, TA and adulteration with carbonates/bicarbonates is depicted in Table 1. Thirtynine out of 80 samples (51%) were found to be non-adulterated with carbonates/bicarbonates. These samples, when compared with 41 adulterated samples, had lower pH and higher TA. The pH and TA in the adulterated samples varied from 6.4 to 7.2 and 0.036 to 0.156%, respectively and in the non-adulterated samples from 6.1 to 6.95 and 0.05% to 0.19%, respectively.

The results have shown that the practice of adulteration of milk with carbonates/bicarbonates was quite common in the area, though the practice is considered to be illegal under the Prevention of Food Adulteration Act (1954). The studies further reflected that the addition of alkali would help the sub-standard samples to pass the TA test. All the samples containing carbonates/bicarbonates showed TA between 0.036 and 0.158% (Table 1), which were well below 0.17%, the limit prescribed by the

TABLE 1. FREQUENCY DISTRIBUTION OF MILK SAMPLES ACCORDING TO PH, TA* AND ADULTERATION WITH CARBONATES/BICARBONATES

Test	Range	Adulteration with carbonates/bicarbonates	
		Adulterated (41 samples)	Unadulterated (39 samples)
pH	6.1-6.5	3 (7.3%)	20 (51.3%)
	6.5-6.8	26 (63.4%)	17 (43.6%)
	Above 6.8	12 (29.3%)	2 (5.1%)
TA	Below 0.1%	36 (87.8%)	11 (28.2%)
	0.1-0.17%	5 (12.2%)	20 (51.3%)
	Above 0.17%	0	8 (20.5%)

* Total titrable acidity

* *Corresponding Author*

TABLE 2. ANALYSIS OF SAMPLES SHOWING COMPARATIVE GRADING BASED ON SPC AND MBR TESTS

No. of samples	Grade on SPC basis	Corresponding grade on MBRT basis (No. of samples)			
		Very good	Good	Fair	Poor
2	Very good	2 (100.0%)	0	0	0
12	Good	1 (8.3%)	9 (75.0%)	1 (8.3%)	1 (8.3%)
8	Fair	0	5 (62.5%)	3 (37.5%)	0
64	Poor	3 (4.7%)	11 (17.2%)	34 (53.1%)	16 (25.0%)

BIS (ISI 1960). It was obvious that the use of alkali was made to neutralize and mask lactic acid produced due to the growth of bacteria present in the milk. Use of such milk for domestic consumption or for preparation of milk powder, 'chhenna' or cheese can be a potential source of health hazard, associated with toxic microbial metabolites of pathogens like *Staph. aureus*. Ghosh and Laxminarayana (1973) have reported the presence of enterotoxin in the skim milk powder and cheddar cheese marketed in India. These observations make the suitability of TA test, as a platform quality control test at milk collection centres, questionable in the absence of testing the milk for the presence of neutralizers.

Similar observations were made with the pH of milk. In case of non-adulterated samples, 51.3% samples had pH values below 6.5 (probably due to the developed acidity), whereas 92.7% of the adulterated samples had pH 6.5 and above and 29.3% samples had pH above 6.8 and up to 7.2 (Table 1). High pH of milk can be attributed either to the addition of alkali to the milk or to the mastitic milk. In the present circumstances, the former appears to be the reason, though the latter cannot be completely ruled out, since mastitis is well prevalent in the country. No test for mastitis was, however, carried out on the milk samples in the present investigations.

The comparative grading of milk samples based on the BIS guidelines for MBRT and SPC independently is presented in Table 2. The grading of majority of the samples (56 out of 86), based on the results of the SPC and MBR tests was not in agreement. MBR test, as compared to SPC, placed 54 out of 86 (62.8%) milk samples in higher and 2 samples (2.3%) in lower catagories. In the remaining 30 samples (34.9%), the grading was similar by both the tests.

Thus, grading of 65.1% samples differed, when the guidelines suggested by the BIS for MBRT and SPC were applied separately to the same milk samples. The MBR test judged only 17 samples (19.8%) as poor, whereas, the SPC test graded 64 samples (74.4%) as poor. These results indicated differences in the stringency of the two tests for grading raw milk. These observations are supported by earlier studies on the rough correlations of MBRT and SPC, where the corresponding values of MBRT for $5x10^5$, $4x10^6$ and $2x10^7$ bacterial counts per ml of milk have been reported to be 5 h and 30 min, 2 h and 20 min, respectively (ICAR 1977). A careful analysis of the BIS standards reveals that the specified values of SPC for different grades of milk are towards lower side against the corresponding MBRT values. For example, the samples showing SPC over $5x10^6$ per ml are graded 'poor', but the corresponding MBRT for 'poor' grade milk has been fixed at 30 min, though according to the previous correlation studies, the corresponding MBRT should be around 2 h for SPC of $5x10^6$ per ml.

As far as the hygienic quality of the samples is concerned, the SPC in the 86 samples ranged from $7x10^4$/ml to $2x10^{10}$/ml. Sixty four out of 86 samples (74.4%) had SPC even above $5x10^6$/ml, of which 16 samples (18.6%) had SPC above 10^8/ml. The counts for thermoduric organisms varied from 14/ml to $3x10^6$/ml in the 26 samples examined. Only 6 (23%) of these samples had low thermoduric counts varying from 14/ml to 200/ml, the remaining 77% samples had the counts as high as $1.2x10^6$/ml. All the six samples with low thermoduric counts had high SPC, varying from $2.9x10^7$/ml to $3.5x10^9$/ml.

Animal faeces, soil and water are rich sources of organisms. The high SPC (over $5x10^6$/ml) and thermoduric organisms count in majority of milk samples indicated easy accessibility of these to milk due to poor hygienic practices followed at the dairy farms in the region. Unhygienic quality of milk sold in this area was also reported by Gahlot et al (1973) and Garg et al (1977). The present investigations revealed no improvement in the quality of milk indicating lack of awareness and efforts among the consumers and the producers. Another important reason for high bacterial load (SPC) may be long interval between production and supply of milk and its storage at ambient temperature during the period, leading to multiplication of the contaminating bacteria in the absence of adequate chilling facilities.

From these findings, it can be concluded that in the absence of testing the milk for adulteration with neutralizers, the TA test used to assess bacteriological stability of milk, leading to its acceptance or rejection, cannot be depended upon. Further, SPC and MBR tests for grading raw milk lack similarity, the MBR test being comparatively more lenient. An inter-laboratory collaborative work on these aspects is desirable in different climatic regions of the country to evaluate the given methods of testing raw milk.

References

Gahlot DP, Pal RN, Kapoor CM (1975) Microbiological quality of market milk in Hisar city. Food Sci Technol 12:68-70

Garg DN, Bhargava DN, Narayan KG (1977) Pathogenic bacterial flora of raw market milk. Indian J Dairy Sci 30:36-39

Ghosh SS, Laxminarayana H (1973) Detection of preformed enterotoxins of staphylococci in dairy products employing serological method. Indian J Exp Biol 11:457–458

ICAR (1977) Handbook of Animal Husbandry, 2nd edn. Indian Council of Agricultural Research, New Delhi, p 651

Indian Standards Institution (1960) Indian Standard Methods of Test for Dairy Industry. Part I. Rapid examination of milk, ISI, New Delhi, pp 29-36

Indian Standards Institution (1978) Indian Standard Methods of Test for Dairy Industry. Part III. Bacteriological analysis of milk, ISI, New Delhi, pp 4-6

Prevention of Food Adulteration Act (1954) Ministry of Law, Government of India, New Delhi

Received 12 January 1995; revised 11 April 1997; accepted 15 April 1996

J. Food Sci. Technol., 1997, Vol. 34, No. 4, 360-362

Drying Kinetics of Greengram

N.K. JAIN* AND JABAR SINGH

Post - Harvest Technology Scheme,
College of Technology and Agricultural Engineering,
Udaipur-313 001, India.

Experiments on thin layer drying of greengram (*Phaselous aureus* Roxb.) were conducted at 40, 50, 60, 70 and 80°C in a batch type cross flow dryer at an air flow rate of 3.45 m^3/min. Drying rate and moisture ratio were calculated from the moisture loss data. It was observed that drying of greengram occurs in falling rate period and is governed by moisture diffusion. A mathematical relationship has been established between moisture ratio and drying time.

Keywords: Greengram, Drying air temperature, Moisture content, Drying rate, Diffusion, Moisture ratio, Drying constant.

Pulses being nutritionally vital, occupy a very important place in the average diet of an Indian. Pulses pass through several unit operations before being converted into *dhal* (Kurien 1977). Processing of pulses is a major milling industry in India, next to rice and flour milling (Chakravorty and De 1980). Drying of pulses is an integral step of conditioning operation in pulse milling (Kurien 1971; Chakravorty and De 1980). Kurien (1977) has reported that traditional process of sun-drying is inherently slow, non-uniform, labour intensive and restricts the turnover, as the process is dependent on climatic conditions. In India, greengram accounts for about 7 to 8% of the production of pulses. The grains (whole and split) are used as *dhal* or made into flour. Unlike other pulses, it does not produce heaviness or flatulence (Anon 1980).

Mechanical drying kinetics of different pulses, which ascertain the drying parameters for mechanical drying, have not been studied in depth as yet. Narain et al (1982) and Kulkarni (1986) recommended priorities for establishment of drying parameters for mechanical drying of pulses and development of appropriate drying equipment for proper drying of pulses. Therefore, the present work was undertaken to study the drying characteristics of greengram (*Phaselous aureus* Roxb.) at different temperatures.

Drying experiments were conducted in a laboratory level cross flow type batch dryer. The temperature of heated air inside the dryer was controlled through a contact breaker. A digital temperature indicator with resistance type sensing element was used for measurement of air temperature and the air velocity in drying chamber was measured by a hot-wire anemometer (Beckwith and Buck 1982).

Samples were prepared by soaking greengram (Cultivar 'Pusa Baisakhi') grains for 30 min in tap water and then tempering for 12 h between moist jute bags to achieve uniform absorption of water inside the kernels (Jain 1988). The moist grains were packaged in polyethylene bags and stored in a refrigerator during experimentation. The initial moisture content of sample was determined by standard oven-drying method (AOAC 1975).

Grain samples were spread in thin layer (single kernel), covering the perforated area of drying tray, and placed in the drying chamber after weighing. The moisture loss from greengram was determined by periodical weighing of the tray, till the moisture content of grains reached 10 to 11% (db), as required for milling (Chakravorty and De 1980).

The experiments were conducted in three replications at 40, 50, 60, 70 and 80°C drying air temperatures. Average values were taken for final calculations. The air flow rate was kept constant at 3.45 m^3/min in all the experiments, as recommended by Hall (1970) for most of the foodgrains and considering the terminal velocity of greengram, as reported by Sivasani (1971).

The moisture content of greengram grain, as a function of time at drying air temperature 40, 50, 60, 70 and 80°C, is shown in Fig. 1. It is evident that the total time for drying decreased from 420 to 50 min with the increase in air temperature from 40 to 80°C. The moisture content of greengram decreased very rapidly during the first 60 min of drying, in comparison to later part of drying, because of more availability of free water during the initial period of drying (Hall 1970; McCabe and Smith 1967).

Fig. 2. Illustrates the effect of drying air

* *Corresponding Author*

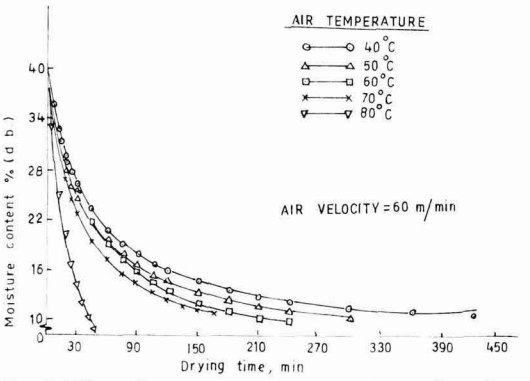

Fig. 1. Effect of air temperature on moisture loss from greengram

temperature on drying rate of greengram. It was observed that greengram do not have any constant rate drying period and that the entire drying takes place only in falling rate period. The drying rate increased with the increase in air temperature from 40 to 80°C.

A semi-logarithmic plot of moisture ratio (MR) with drying time yielded a straight line for all the drying air temperatures (Fig. 3), thereby revealing that the moisture transfer from greengram was governed by diffusion mechanism, as envisaged by Hall (1970).

McCabe and Smith (1967) reported a drying equation based on diffusion mechanism, as:

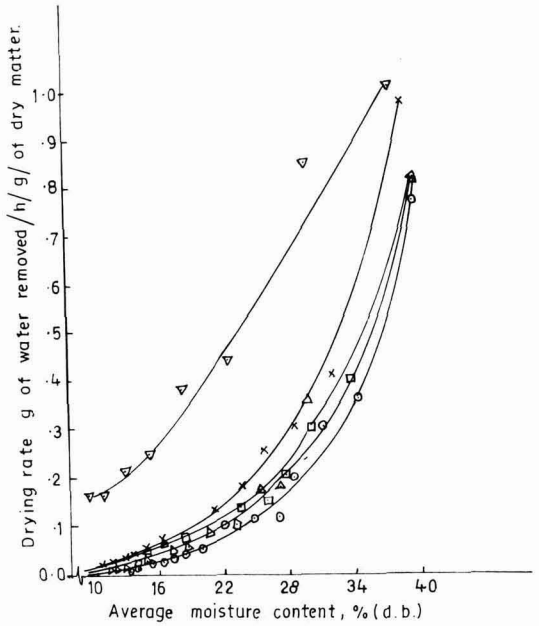

Fig. 2. Effect of air temperature on drying rate of greengram

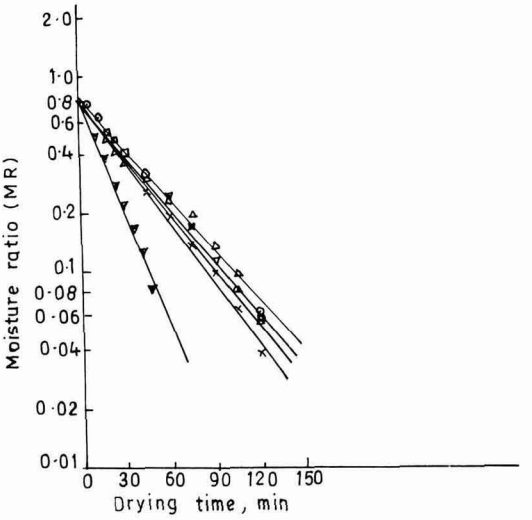

Fig. 3. Moisture ratio and drying time for greengram at different air temperatures

$$MR = \frac{M - M_e}{M_o - M_e} = A\, e^{-KQ} \qquad .....(1)$$

where M and Q represent moisture content (db) and time (h), respectively. Subscripts o and e stand for initial and final equilibrium values. Parameter A is shape factor and the parameter K is drying constant.

The values of A and K for each temperature were graphically determined as shown in Fig. 3 and are presented in Table 1. It is evident that the shape factor Å, does not change with temperature, whereas drying constant K is dependent on temperature.

The functional relationship between drying constant and air temperature was ascertained graphically, as:

$$K = 3.970 \times 10^{-4} \times (1.1034)^t + 1.191 \qquad ... (2)$$

where t is drying air temperature in °C.

Hence, the drying equation (1) derived from experimental observations becomes:

$$\frac{M - M_e}{M_o - M_e} = 0.80 \exp\left[-(3.970 \times 10^{-4} \times (1.1034)^t + 1.191)\, Q\right] \quad ..(3)$$

TABLE 1. DRYING CONSTANT AND SHAPE FACTOR* FOR GREENGRAM AT DIFFERENT TEMPERATURES

Air temp (t), °C	Drying constant (K), /h
40	1.2026
50	1.2970
60	1.3313
70	1.4628
80	2.6920

* Shape factor of 0.80 is same for all air temperatures

Thus, it can be concluded that the drying of greengram takes place in falling rate period and is governed by moisture diffusion. The total drying time for greengram decreases with increase in drying air temperature because of increase in drying rate at higher temperatures. The data are useful in design and development of dryer and improvement in processing techniques of greengram.

References

Anon (1980) Hand Book of Agriculture, Indian Council of Agricultural Research, New Delhi, India

AOAC (1975) Official Methods of Analysis, 12th edn. Association of the Official Analytical Chemists, Washington, DC

Beckwith TG, Buck NL (1982) Mechanical Measurement, 2nd edn. Measurement of Fluid Flow. Wesley Publishing Co., Inc., New York

Chakravorty A, De DS (1980) Post-Harvest Technology of Cereals and Pulses, 1st edn. Milling of Corn, Wheat and Pulses. Oxford and IBH Publishing Co., Calcutta, India

Hall CW (1970) Drying of farm Crops, 1st edn. System for Grain and Ear Corn Drying. Lyall Book Depot, Ludhiana, India

Jain NK (1988) Effect of temperature on drying characteristics of *Bengal*gram and greengram. M.E. Thesis, Rajasthan Agricultural University, Bikaner, Udaipur, India

Kulkarni SD (1986) Pulse Processing in India - A Status Report, Indian Agricultural Research Institute, New Delhi, India

Kurien PP (1971) Traditional and improved techniques of milling pulses. Paper Presented at the Conference of *Dhal* Millers, Central Food Technological Research Institute, Mysore, India

Kurien PP (1977) Grain Legume Technology. Paper presented at the FAO Experts Consultants Meeting on Grain Legume Processing, Central Food Technological Research Institute, Mysore, India

McCabe WL, Smith JC (1967) Unit Operation of Chemical Engineering, 2nd edn. Drying of Solids. McGraw Hill Book Co., Inc., New York

Narain M, Saxena RP, Singh BPN (1982) Development in pulse milling technology - A review. Paper APE: 82-103. Presented at Indian Society of Agricultural Engineers. Annual Convention, College of Technology and Agricultural Engineering, Udaipur, India

Sivasani S (1971) Terminal velocity of solids, M.Tech. Thesis, GB Pant University of Agriculture and Technology, Pantnagar, India

Received 28 August 1995; revised 10 April 1997; accepted 16 April 1997

HANDBOOK OF FOOD ANALYSIS, VOLUME 1 — PHYSICAL CHARACTERISATION AND NUTRIENT ANALYSIS. Edited by Leo M.L. Nollet, Published by Marcel Dekker, Inc. 270, Madison Avenue, New York 10016, 1996, pp: 1088; Price: Vol. 1 and 2 as a set US $ 390/-

This is a handbook of methods of analysis, in which recently developed, automated analytical techniques as well as classical ones are compiled. The book intends to get ingredient - and nutrient -information and knowledge of chemical composition.

Volume 1 comprises 25 chapters distributed in three parts:

Part 1: Sample preparation and chemometrics (Chapters 1 and 2) Part 2: Physical characterisation (Chapters 3 to 6) and Part 3: Nutrient analysis (Chapters 7 to 25).

The chapters are structured, first giving information on physical and chemical properties, followed by step by step procedures of sample preparation, extraction and clean-up methods of each analytical technique. While giving special attention to automated and recent methods of analysis, it is heartening to note that classical methods are not neglected. Regulations in food industry and future trends in the analysis of food components have been highlighted. Data and some references are represented in tabular form. All these aspects render this volume a mega source of reference for the students of food science.

Chapter 1 deals with sampling and sample preparation. Sampling can often be the greatest source of error in chemical analysis and therefore, various aspects of sampling such as heterogenicity of the population, homogenization by particle size reduction and mixing have been described. Also, due attention is given to the degradation and contamination of the sample and sample integrity.

Chapter 2 deals with the chemometrics and throws light on the importance of proper planning of chemical experiment to obtain maximum chemical information at minimum cost, optimization of analytical process and extraction of useful chemical information. Important chemometric tools, validation procedures and multivariate techniques are covered.

Chapter 3 gives information on determination of moisture and ash contents of food. Properties of water, mechanism of moisture loss, factors affecting rate of moisture removal, difficulties encountered in complete separation of all water from a food samples and methods of moisture determination are described. As ash content is an useful criterion in identifying the authenticity of a food, besides serving as a reliable index of metabolism of yeast, its determination by dry ashing, wet ashing and conductometric methods for foods high in sugar are explained in detail.

Chapter 4 deals with mechanical properties of food. This chapter gives an insight, regarding - measuring the size and distribution of elements in complex mixtures and how these elements interact and behave under mechanical stress and developing processes to maximise the formation and retention of useful structures. The data also aid the food scientist in developing suitable process parameters, packaging materials and storage conditions for food products. Fundamental principles such as viscosity of fluid products, non-Newtonian behaviour of fluid products, concentration effects on flow behaviour, rheological evaluation of solid foods, viscoelastic behaviour, dispersion and suspensions and challenges associated with rheological analysis of food materials are adequately covered.

Chapter 5 describes optical properties such as colour, gloss, translucency and their uniformity on the surface of the food products. Various colour measurement systems such as 1931 CIE system, Hunter system, and CIE Lab system have been mentioned. Instruments ranging from simple reflectance spectrophotometers to computer controlled models for the measurement of optical properties are illustrated.

Chapter 6 describes sensory evaluation techniques. Procedures for screening the panel members and to check their performance ability to carry out sensory evaluation along with (a) difference and (b) descriptive tests, which cover most applications of practical use in sensory analysis, have been described.

Chapter 7, 8 and 9 deal with amino acids, peptides and proteins in foods, respectively. Relevance of amino acid determination particularly with respect to legal issues, formulation problems and nutritional importance is mentioned. Modern instruments based on HPLC cat-ion exchange separations, taking 30-60 min. for separation and application of enzymes such as amino acid carboxylases and proteases, which have transformed amino acid analysis from slow and cumbersome to quick and easy, have been illustrated. Analytical determinations of peptides in complex matrix,

requiring sample preparation, isolation and fractionation by ultrafiltration, low pressure LC, solid phase extraction and determination by HPLC, electrophoresis and capillary electrophoresis have been elucidated. A mention about future developments in HPLC-MS, CE-MS for peptide and protein determinations and for hydrolyzates for sequence determination, robotization in sample preparation and its on-line coupling with high resolution and detection limits is made.

Chapter 10 gives attention to the most widely used enzymes in foodstuff chemistry, namely, oxidoreductases and hydrolases, which greatly affect the quality of processed foods and their products and to the most common methods for measuring them. In some cases, recently developed methods that offer substantial improvements to existing ones are discussed.

Chapters 11, 12 and 13 deal with analysis of fatty acids, triacylglycerols and unsaponifiable matter, respectively. Apart from physical and chemical properties of fatty acids, their separation by column chromatography, TLC, HPLC and analysis by (a) GC-MS particularly for locating the position of double bond in poly-unsaturated fatty acids, (b) GC-FTIR for determining *cis*-trans configuration, (c) UV spectroscopy for detection of conjugated double bonds and (d) IR spectroscopy for the detection and quantification of *trans* fatty acids in lipid mixture have also been dealt with in detail.

Structural analysis of triacylglycerols involving (a) study of the constituent fatty acids and (b) positional distribution of fatty acids is also referred.

Gas liquid chromatography, adsorption chromatography involving silicic acid, and argentation chromatography, HPLC analysis, mass-spectroscopy, giving qualitative information such as molecular weight, empirical formula, and structure and quantitative information on triacylglycerol composition and supercritical fluid chromatography used in the study of constituent fatty acids of triacylglycerols have been extensively covered.

Positional distribution of fatty acids involving (a) determination of fatty acids esterified in Sn-2 position and (b) stereospecific analysis of triacyl-glycerols are illustrated in chapter 12.

Unsaponifiable matter and its determination and fractionation into pigments, hydrocarbons, higher aliphatic alcohols and sterols are mentioned in chapter 13.

As phospholipid quantification is of utmost importance to lecithin industry, chapter 14 is separately devoted to phospholipids - their occurrence, properties and analysis, involving extraction and fractionation. Determination of phospholipid content by classical methods of conversion of organic to inorganic phosphates and other recent methods and determination of phospholipid composition by TLC, TLC-FID, HPLC and NMR are described.

Chapter 15 deals with carbohydrates. Their determination by traditional methods and recent methods such as GC, HPLC, biochemical methods and flow injection analysis (FIA) is given. FIA coupled with enzymatic assays will be the most common analytical practice for rapidity, precision, accuracy and ease with which more complex and new sugars and their derivatives that could be analysed are also mentioned.

The determination of alcohols in foods and beverages elicits interest from market classification to quality control, from research of adulterations to understanding of natural processes, which control their formation or the effect of technological problems on products intended for human consumption. An attempt is made to cover some of these aspects in chapter 16.

Chapters 17 and 18 deal with fat soluble - and water soluble vitamins, respectively. Chemical and biological nature of vitamins, namely, biological activity, dietary sources, expression of dietary values are described for vitamin A, provitamin A carotenoids, vitamin D, vitamin C and vitamin K. The analysis of vitamins involving (a) sample preparation, (b) extraction procedures such as alkaline hydrolysis, enzymatic hydrolysis, direct solvent extraction, supercritical fluid extraction, (c) clean-up or fractionation procedures viz., sterol precipitation, open column chromatography, solid phase extraction and high pressure gel permeation chromatography and (d) determination by HPLC, RP-HPLC, and 2-dimentional HPLC is also extensively dealt with.

Chapter 19 deals with organic acids in which their determination by classical, enzymatic and HPLC methods have been discussed. Chapter 20 is devoted to the identification and determination of organic bases. A brief review and published data in tabular form for various amines and alkaloids are given. Analytical methods, experimental conditions, detection limits, kind of food investigated and amount for each species are reported.

Chapter 21 deals with phenolic compounds, which contribute to the sensory quality of fresh

fruits and processed products. Analysis of phenolics in fruits and fruit-products, peel oil, and flavourings, honey, tea and coffee, cereals and alcoholic beverages involving sample preparation, solvent extraction, and hydrolysis and determination by colorimetric, enzymatic, paper chromatography, TLC, UV-visible absorption spectroscopy, counter-current chromatography, GC, HPLC, electrophoresis, size exclusion chromatography and their detection and identification by UV-visible fluorescence, photo diode assay, colormetric assay, MS-NMR and FTIR methods is given.

Chapter 22 deals with bittering substances in which their sources of occurrence in foods and properties are indicated. Colorimetric and HPLC methods for the analysis of bitters in beverages such as beer, cider, apple juice, fruit juices, tea, wine etc. are given.

Chapter 23 main pigments viz., 1. carotenoids, 2. chlorophylls, 3. anthocyanins, 4. betalains, and 5. myoglobin. Descriptions regarding physical and chemical properties, properties in food, regulation aspects, analysis-detection-separation methods, further developments and applications in food analysis for each of these five pigments are given in this chapter.

Chapter 24 describes aroma compounds. Isolation and concentration techniques involving distillation, simultaneous distillation and extraction (SDE), liquid-liquid extraction (LLE) and fractionation techniques are mentioned. Various headspace techniques such as equilibrium headspace sampling, multiple headspace, extraction, dynamic headspace sampling, followed by separation techniques such as GC and LC and finally the methods of identification of volatiles by mass and IR absorption spectra, NMR, and Korats indices on polar and non polar columns are described in this chapter.

Chapter 25 deals with dietary fibre. Methods for analysing the dietary fibre have proliferated in the last 25 years making the task of selecting a method, understanding the meaning of the results that it provides and understanding how they differ from those of another method increasingly difficult. This chapter aims to provide some clarification by describing the range of methods available, their origins and inter-relationships. Physical and chemical nature of dietary fibre constituents, food regulations, choice of methods and limitation of methods have been extensively covered.

Overall, this volume will be of immense value to the food analysts, public analysts and students of food science and technology. One major lacuna in volume 1 is that it is devoid of subject index, a cumulative index, however, appears in Volume 2.

V.D. SATTIGERI
CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE, MYSORE-570 013

HANDBOOK OF FOOD ANALYSIS, VOLUME 2 – RESIDUES AND OTHER FOOD CONTAMINANT ANALYSIS. Edited by Leo M.N. Nollet, Published by Marcel Dekker, Inc. 270, Madison Avenue, New York, 10016, 1996, pp 2041, Price Vol. 1 and 2 as a set US $ 390/-

This book is an excellent compilation of the work on food analysis with particular reference to contaminants and food component analysis. The book has 23 sections and each section is dealt in a very simple and methodical manner with up-to-date information. Each topic starts with a general description of the component and then, the actual analytical procedure is described. In addition to food and contaminant analysis, it also includes explanation on various latest methods of instrumental methods of analysis.

Section 26 consists of a elaborative description of mycotoxins in food and their analysis by GLC, GC-MS, HPLC and immunoassay. Analytical methods for fumonisins, zearlenone, orchratoxin, patulin, deoxynivalenol, trichothecene, cyclopiazonic acid, alternaria toxin and ergot alkaloids are also given. Phycotoxins or algal toxins produced by planktonic algae are covered in section 27. Extraction, cleanup and methods of determination by bioassay, GLC and HPLC are given. Analysis of residual antibacterial in food of animal origin given in section 28. The residues of antibacterials in meat, milk and eggs and their possible acute toxicity, mutagenic and carcinogenic effects are mentioned. The safety regulations in the world in preventing the hazards in food are also highlighted. The analytical methodologies for these antibacterials including sample preparation, separation and detection are well documented. The importance of microbiological assays in the determination of antibacterials is also highlighted.

Section 29 deals with residues of growth promoters in edible products. This section includes prohibition of chemical substances with harmonal action in animals. It also prohibits use of thyrostatic, esterogenic, androgenic or gestagenic action. This section also includes analysis, detection and separation methods for growth promoters. Very sensitive immunological and chromatographic

methods of quantification are also given.

Residues of urea pesticides in foods are given in section 30. Extraction, cleanup, derivitization, detection and separation of urea pesticides in foods are also given.

The methods of analysis of various organochlorine insecticides in dairy products, meat, fish, poultry, human milk, cereals, leafy vegetables and fruits, fats and shortenings, sugar adjuncts, beverages, spicy food and water by GC-ECD and their detection limit are given in section 31.

Carbamate pesticide residue analysis is included in section 32. List of various carbamate pesticides, methods of chemical and biological methods of analysis is given.

The organophosphorus insecticide residues in foods are dealt with very methodically in section 33. Toxicology, regulations, extraction, cleanup, gas chromatography, HPLC, immunoassays, liquid chromatography with mass spectrometry, super critical fluid chromatography and sensor technology are described in this section.

Fungicide extraction, cleanup and multi-residue method with acetonitrile and acetone extraction are given in the 34th section.

Herbicide residues in fruits and vegetables and their various quantification methods are described in section 35. Analysis by GLC, HPLC, capillary electrophoresis, super critical chromatography, immunoassay methods for various groups of herbicides is discussed.

The toxic residues resulting from packaging materials like polymeric and monomeric, solvent, plasticizers, antioxidants, polychlorinated biphenyls and inorganic compounds are discussed in the 36th Section. The analytical methods for the above residues are given.

Determinations of chlorinated dibenzo-p-dioxens, dibenzofurans and biphenyls in food are given in section 37.

The harmful effects of PCDDs, PCDFs and PCBs on fish and others have set off a new wave of public alarm. Food regulation, analytical methods including extraction column chromatography, HPLC, mass spectrometeric technique, high resolution glc, electron impact mass spectrometry and chemical ionisation mass spectrometry is described. Exhaustive references upto 1991 are given.

The hazards of N-nitroso compounds in foods, formalin in foods, food legislation, extraction, cleanup, derivatization and determination by GLC, HPLC, mass spectrometry are given in this section.

In section 39, polycyclic aromatic hydrocarbons (PAHs) formed by incomplete combustion and high temperature pyrolysis of coal, oil and other forms of organic matter and their harmful effects like carcinogenic and mutagenicity are described. GLC and HPLC methods of determination for metal contamination in foods and their determination by anodic inverse voltamperometry adsorption inverse voltamperometry polarography, atomic absorption spectrometry, AES and enzymatic methods are given. Non-enzymatic browning in foods (Maillard reaction), and the determination of non-enzymatic browning products by chemical assays, biological and biochemical assays, GLC and HPLC methods are described.

Natural and synthetic colorants added in foods and their separation and determination are given in section 42. Paper chromatography, TLC, HPLC, electrophoresis, polarographic methods and NMR and IR methods are given in this section.

Various preservatives permitted to be used in food products and procedures for determination are given in section 43.

Synthetic food antioxidants in foods and their resolution and various analytical methods are given in section 44.

Sweeteners are of two types viz., bulk and intense sweeteners. The intense sweeteners are artificial compounds obtained by organic synthesis. HPLC methods and their respective columns for the determination of sweeteners are given in this section.

Determination of cation and anions by capillary electrophoresis, which have remarkable resolving and separation power is described.

The methods of identification of irradiated foods like DNA methods, biological and microbiological methods, chemical methods and lipid methods are described.

The last section 48 includes details about various instrumental methods like chromatography, gas chromatography, HPLC, Planar chromatography, supercritical fluid chromatography, electrophoresis, flow injection analysis, ion selective electrodes, and mass spectrometry, which are of immense help us food analysis.

The various chapters covered in this book provide up-to-date information and is of immense help to both students and scientists working in food science in general and agriculture in particular.

K.M. APPAIAH
CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE
MYSORE-570 013

FOOD CHEMISTRY - THIRD EDITION, FOOD SCIENCE AND TECHNOLOGY/SERIES/76 Edited by Owen R. Fennema, Published by Marcel Dekker Inc. Cameroon Road, Monticello N.Y. 1270 pp 1088; Price US $ 185/-.

The book edited by Owen R. Fennema is a part of series of 77 monographs, text books and reference books published under food science and technology. Seventeen chapters in the book are written by different outstanding authors and the editor has taken care to co-ordinate the texts of all the chapters and see that the coverage is even and unwarranted duplications, different philosophies and inadvertent omissions of important materials are minimised. These efforts have culminated into a high quality book that provides a comprehensive coverage of the subject of food chemistry with adequate depth and thoughtfulness.

The book is primarily designed to serve as a textbook that is suitable for food science and technology students with background in organic chemistry and biochemistry. Secondly, it is aimed to serve as a reference source for persons involved in food research, food product development, quality assurance, food processing and the activities related to food industry.

All the chapters in the book are well organised and in proper sequence. The topics covered are:

Major constituents of food: Water and ice, carbohydrates,lipids, and amino acids/peptides/ proteins.

Minor constituents of food: Enzymes, vitamins, minerals, colorants, flavours, food additives and toxic substances.

Food dispersions : Edible animal tissues, edible plant tissues, edible fluids of animal origin and integrated concepts.

Although the book has not attempted the complete coverage of all the aspects of food chemistry, all important topics are covered adequately. Figures and tables with improved graphics have been used liberally to facilitate the understanding of the subject matter. The number of references cited are adequate to permit easy access to additional information on the subject.

Introductory chapter (chapter 1) defines food chemistry, the role of food chemists and deals with how to establish an analytical approach to the chemistry of food formulation, processing and storage stability.

Chapter 2 on water and ice, explains their structures and deals widely with water-solute interactions at macroscopic and molecular levels, with ions and ionic groups etc. It clearly defines water activity, relative vapour pressure, as well as moisture sorption isotherms, their temperature dependence and their roles in food processing. The chapter details out of a nine key concepts, underlying the molecular mobility approach to the food stability.

Chapters 3 to 13 on major and minor food constituents, give detailed information on the classification and chemical and physical aspects of each of the constituents. In chapter 5, for example, the basics related to the nomenclature, classification, physical and chemical aspects and chemistry of fats and oils processing are nicely elaborated. Additionally, the behaviour of food lipids during frying, their safety on hydrogenation and irradiation as well as nutritional and health aspects are explained in a crisp manner.

Chapter 8 on vitamins, for example, while giving in-depth information on each vitamin, gives in a tabular form the summary of vitamin stability as well as the stability of added vitamins in enriched cereal grain products and breakfast cereals. Nutritional aspects of vitamins such as dietary recommendations, and bio-availability as well as general causes of variations/losses of vitamins in foods after various processing treatments are also fully elaborated.

Chapters 14, 15 and 16 on characteristics of milk, edible muscle tissues and edible plant tissues. cover comprehensively the chemical composition, structure and composition of the constituents, biochemical and physiological changes during the handling, processing and storage operations.

Thus, in every chapter, the authors have given precise and in-depth information on the subject matter, in a manner, that it would serve as a text book for the students of food science and technology. The first and second editions of the book published earlier are popular amongst the food scientists. Thorough revision of earlier editions and addition of new chapters have made this third edition more useful and as the edition claims, 'more than 60% are new'. The breadth of information covered within 1088 pages of this book would have wide appeal to the students of food science and technology.

C.L. Nagarsekar
Nagarsekar Associates,
Mumbai-400016

INDIAN FOOD INDUSTRY

A Publication of Association of Food Scientists and Technologists (India)

Contents of November-December 1996 Issue

Phone : 0821- 515557;
E Mail: afst@nicfos. ernet.in

FAX : 0821 - 518670
Cable : FOODSEARCH

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (INDIA)

CFTRI Campus, Mysore-570 013

Publications of AFST (I)

		INDIA		FOREIGN	
		Price (Rs.)	Postage (Rs.)	Price (US$)	Postage (US $)
1.	Symposium on the status & prospects of the confectionery industry in India 1979	30	18	12	8
2.	Symposium on by-products from food industries: Utilization and disposal 1980	30	12	12	6
3.	2nd ICFoST-1981: Sensory analysis, Contaminants and Nutrition	40	18	15	7
4.	Symposium on recent developments in food packaging 1986	65	18	35	8
5.	Prof. V. Subrahmanyan Commemoration Issue 1980 (Part of Journal of Food Science & Technology) contains write-up on various aspects of Food Science & Technology	30	18	12	8
6.	Production & processing of meat & poultry products	100	18	45*	-
7.	IFCON-88, 2nd International Food Convention & Exhibition: Food Technology Overview	100	18	45*	-
8.	IFCON-88, 2nd International Food Convention & Exhibition: Abstracts of papers	100	18	45*	-
9.	Trends in Food Science & Technology: IFCON-88 Proceedings, 1988	300*	-	105* or £57*	-
10.	Collective Index (Volumes 1-25) Journal of Food Science & Technology	200*	-	30* or £15*	-
11.	Pollution management in food industries 1989	200	12	30* or £15*	
12.	Developments in milling and baking technology	125*	-	30*	-
13.	Trends in Food Science & Technology: IFCON-93 Proceedings, 1993	1250*	-	200*	-
14.	IFCON-93 Poster Sessions Abstracts	125	-	-	-
15.	IFCON-93 Souvenir	125	-	-	-

Payment may be made in the form of Demand Draft drawn in favour of Secretary, AFST (I), Mysore * Including Postage

Reg. No. 24918/64

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.