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AND
TECHNOLOGY

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (INDIA)

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2. To provide a forum for the exchange, discussion and dissemination of current developments in the field of Food Science, Technology and Engineering.
3. To promote the profession of Food Science, Technology and Engineering.

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EDITORIAL

I am happy to place in your hands the first issue of Vol. 30, No. 1, 1993 of the "Journal of Food Science and Technology" with a new look and format. I hope that you will appreciate and welcome the changes with pleasure.

The following are the important changes :

* Publication of papers under the head 'Rapid Communication" on innovative research findings or impact-making industrial practices.

* Publication of critical and authoritative reviews on 'State of the Art' in Food Science, Technology and Engineering, including Methodologies for the analyses. To begin with, these are by invitation only.

* Reintroduction of 25 complimentary reprints with window cover for each paper.

* Use of bigger sized letters and the type, which is highly pleasing to eyes.

* Abbreviation of the titles of all scientific periodicals for citing in Reference Section, will be as in *Chemical Abstracts, Biological Abstracts, Annual BIOSIS List of Serials* and *Serial Sources for the BIOSIS Data Base.*

* Introduction of keywords for all papers published.

* Adoption of Harvard system for arranging references alphabetically in the Reference Section.

* Changes in the format/style to facilitate better appeal and utility to the readers.

    Authors are, therefore, requested to follow the guidelines stipulated in the "Instructions to Authors" (published in the first issue of each volume) in order to avoid delays in processing their manuscripts.

The Editor, the Editorial Board Members, Central Executive Committee Members and Staff of AFST (I) wish you all a Happy and Prosperous New Year 1993.

B.K. LONSANE
EDITOR

OUR EDITOR
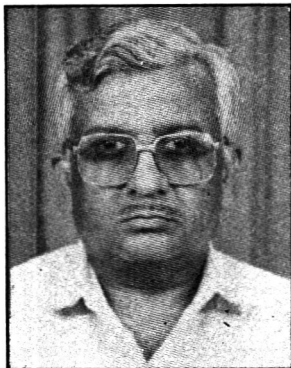
Dr. B.K. Lonsane, Scientist E-II, Fermentation Technology and Bioengineering Discipline, Central Food Technological Research Institute, Mysore, has taken over the editorship of the Journal of Food Science and Technology from July 1992. The improvements he has brought about, in terms of clarity, brevity, speediness and utility values have been witnessed by all in the July, September and November issues of 1992. His deeper involvement and tenacity in getting the things done have resulted in a totally newer and attractive look, improved print quality and enhanced utility of the journal to the readers.

It is my privilege to introduce our dynamic editor to the members, subscribers and readers of the Journal of Food Science and Technology.

Born on October 12, 1941 at Nandura, Buldana District, Maharashtra, he had his early education in Nandura and Nagpur. He obtained his M.Sc. degree with I Class in Biochemistry from Nagpur University in 1964 and was placed second in order of merit. He worked in National Environmental Engineering Research Institute, Nagpur, from August 1964 to May 1968 and then moved, on promotional transfer to National Botanical Research Institute, Lucknow. Subsequently, he moved to Regional Research Laboratory, Jorhat, in July 1964. He got his Ph.D. in Microbiology from Gauhati University in 1974, while working in RRL, Jorhat. He later joined Fermentation Technology Discipline at Central Food Technological Research Institute, Mysore in October 1977. He is continuing in CSIR for the last 28 years.

Dr. Lonsane has wide experience in various areas such as fruit processing, food fermentation, ethanol production, application and production of food enzymes, starch recovery and processing, waste management, yeast exploitation, single cell protein from petroleum and algae, sanitary microbiology and fermentation technology. His expertise on solid state fermentation is recognized internationally.

Dr. Lonsane has guided M.Sc. and Ph.D. students. He has contributed over 100 research papers in reputed National and International journals. He has to his credit a number of critical review papers published in reputed journal series. He has also contributed invited chapters in his specialized areas in books published by renowned publishers such as Marcel Dekker, Elsevier Science publishers and Springer-Verlag. Some of the technologies developed by Dr. Lonsane and his group have been released to the industries for commercial exploitation. He has also participated in preparing a number of detailed project reports for establishment of fermentation and alcohol industries.

Under exchange of Scientists' Scheme and Overseas (DBT) Associateship Programme, Dr. Lonsane visited Hungary in 1983 and France in 1991. Dr. Lonsane is also an International Editorial Board Member of 'Process Biochemistry', the prestigious journal published by Elsevier, Essex, UK.

The Association of Food Scientists and Technologists (India), Mysore is happy in entrusting the Editorship to Dr. Lonsane. On behalf of AFST (I), I wish him all the success.

A.M. NANJUNDASWAMY
PRESIDENT, AFST(I)

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

CONTENTS

INDEXED/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.

Methods for Detection and Enumeration of Foodborne Bacterial Pathogens : A Critical Evaluation

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The increasing consumer awareness has laid a greater emphasis on producing microbiologically safe foods for human consumption. A few important foodborne bacterial pathogens are predominant in foods and thus, are responsible in causing serious public health hazards. In this context, it becomes necessary to subject the foods to accurate microbiological analysis, particularly for these dominating bacterial pathogens. This review critically analyses the methods of detection and enumeration of foodborne pathogenic bacteria with specific reference to the advantages and limitations of the methods.

Keywords : Foodborne bacteria, Bacterial pathogens, Emerging pathogens, Detection, Enumeration, Methodology evaluation.

In recent years, the consumer awareness has played a predominant role in emphasising the need for microbiologically safe foods for human consumption. Serious health hazards due to the presence of pathogenic microbes in foods can lead to food poisoning outbreaks. The commonly occurring bacterial pathogens are *Bacillus cereus, Campylobacter jejuni, Clostridium botulinum, Cl. perfringens, Escherichia coli, Listeria monocytogenes, Salmonella* spp., *Shigella dysenteriae, Staphylococcus aureus, Vibrio parahaemolyticus* and *Yersinia enterocolitica.*

The reliability of the techniques for detection and enumeration of foodborne pathogenic micro-organisms has assumed critical importance. These techniques should be (a) as simple and rapid as possible; (b) accurate and reproducible; and (c) economically feasible. Traditionally, microbiological analysis involves isolation, identification and confirmation of the desired micro-organisms (Speck 1976; Pierson and Stern 1986).

With this background, an attempt has been made in this review to present methods of detection and enumeration of foodborne bacterial pathogens of common occurrence. For an easy presentation and better understanding, the bacterial species are dealt with individually.

Guidelines for microbiological analysis

An accurate microbiological assessment of the foods is possible only when the guidelines given below are followed:

a. *Sampling :* Numbers to be drawn.
b. *Handling of samples :* Exposure of samples to time and temperature during transit i.e., before examination.
c. *Sample preparation :* Maceration and dilutions.
d. *Enumeration procedure :* Composition and preparation of plating media, plating methodology, incubation time-temperature and recording of the counts.
e. *Confirmation and identification :* Isolation of representative colonies, pureefication and determination of specific morphological, cultural and biochemical characters and need for confirmation of the organism to a specific group.

Bacillus cereus

Bacillus cereus has been well established as a major cause of food poisoning outbreak (Kramer and Gilbert 1989). Consequently, studies have been aimed at developing simple and rapid procedures for the enumeration, isolation and identification of *B. cereus.*

Enumeration media: Several selective and differential enumeration media have been formulated to achieve maximum recovery of *B. cereus* from foods. An early method of Hauge (1955) involves surface plating on blood agar, incubation at 37°C for 18 h and testing of the colonies surrounded by a clear zone for lecithinase activity. Later on, a peptone-beef extract-egg yolk-agar containing lithium chloride and polymyxin B as selective agents was formulated (Donovan 1958). Typical *B. cereus* colonies were surrounded by an opaque zone after 18 h at 30°C. A further improvement in this medium was made by the addition of mannitol (mannitol-egg yolk-polymyxin-agar) which enabled in differentiating non-*B. cereus* from mannitol negative *B.cereus* organisms (Mossel et al. *1967*). Kim and Goepfert (1971) formulated an equally sensitive egg yolk containing medium (KG medium) which enabled

TABLE 1. IMPORTANT CHARACTERISTICS OF FOOD RELATED *BACILLUS* SPECIES

Characteristics	*Bacillus* species							
	1	2	3	4	5	6	7	8
Catalase production	+	+	+	+	+	v	+	+
V-P reaction	+	–	+	–	+	–	–	+
pH in V-P broth (6.0)	+	v	+	–	v	–	+	v
Anaerobic growth	+	v	+	+	+	+	–	–
Growth at 50°C	–	+	+	+	+	+	+	+
Growth at 65°C	–	–	–	–	–	–	+	–
Growth in 7% NaCl	+	v	–	–	+	–	–	+
Starch hydrolysis	+	+	+	–	+	+	–	+
Casein hydrolysis	+	v	v	+	+	–	+	+
Nitrate reduction	+	v	v	+	+	+	+	+
Acid from glucose	+	+	+	+	+	+	+	+
Gas from glucose	–	–	–	–	v	+	–	–
Mannitol fermn.	–	+	v	+	+	+	+	+

+ = Positive; – = Negative; v = Variable; V-P = Voges-Proskauer, 1 = *B. cereus*; 2 = *B. circulans*; 3 = *B. coagulans*; 4 = *B. laterosporus*; 5 = *B. licheniformis*; 6 = *B. macerans*; 7 = *B. stearothermophilus*; 8 = *B. subtilis*.

sporulation within 24 h. These media led to a good recovery of *B. cereus* from foods containing a large number of other micro-organisms. Polymyxin is used for selectivity, egg yolk to record lecithinase activity and low levels of peptone for sporulation.

Holbrook and Anderson (1980) formulated a medium, polymyxin-pyruvate-egg yolk-mannitol-bromothymol blue-agar (PEMBA) which utilised the mannitol negative and lecithin hydrolyzing nature of *B. cereus*. This medium supports the growth of even small numbers of *B. cereus* cells and spores and hence, has a wider application in enumeration of *B. cereus* from foods. PEMBA consists of (g/l) : Peptone 1, mannitol 10, sodium chloride 2, magnesium sulphate 0.1, sodium pyruvate 10, disodium hydrogen phosphate 2.5, potassium dihydrogen phosphate 0.25, bromothymol blue 0.1, agar 15 and pH 7.2±0.2. The final medium is dispensed at the rate of 95 ml in glass containers and autoclaved. Prior to pouring into the plates, 5 ml of sterile egg yolk and 10^4 units of membrane filtered polymyxin B sulphate are added to the molten medium, mixed well, poured into pre-sterilized petri plates. surface dried, inoculated with appropriate dilutions of the sample and incubated at 37°C for 36 to 48 h. *B. cereus* appears as peacock blue-coloured colonies surrounded by a zone of precipitation of egg yolk. The advantages of PEMBA are its highly selective nature. The presence of pyruvate reduces the colony size, so that the spreading character of spore-formers is restricted

and also detects lecithinase negative isolates. Colonies of *B. cereus* were easily differentiated from other *Bacillus* species when PEMBA was used to enumerate *Bacillus* in indigenous snack and lunch foods (Varadaraj et al. 1992).

A later modification of PEMBA was the substitution of bromocresol purple for bromothymol blue and designation of the medium as PEMPA (Szabo et al. 1984). The reduction in incubation period to 22 h from initial 48 h, reliable colony colouration and consistent egg yolk reaction are the advantages of this medium. A few research papers have documented the characteristics of *B. cereus* and other *Bacillus* species for a systematic identification (Deak and Timar 1988; Shinagawa 1990). The characteristics of a few important *Bacillus* species of common occurrence in food are presented in Table 1.

Clostridium botulinum

Clostridium botulinum is a metabolically diverse species of anaerobic, spore-forming, rod shaped bacteria producing one or more serologically distinct protein neurotoxins. *Cl. botulinum* types A, B, C_1, C_2, D, E and F occur widely throughout the world (Smith 1977; Huss 1980). The characteristics of *Cl. botulinum* groups are presented in Table 2. Most procedures do not directly quantitate *Cl. botulinum*, but enrichment procedures enable detection of a very small number of the organism.

Enrichment : The enrichment media which are in

TABLE 2. CHARACTERISTICS OF *CL BOTULINUM* GROUPS

Group	Characteristics
I	Type A and proteolytic strains of types B and F
II	Type E and non-proteolytic strains of types B and F
III	Types C and D (all are non-proteolytic)
IV	Type G (all are proteolytic, but non-saccharolytic)

Source : Smith (1977)

use include Robertson's cooked meat broth with or without added carbohydrate (1% w/v), cooked meat medium, peptone-glucose broth, glucose-peptone broth, fish infusion broth, reinforced clostridial medium, brain-heart infusion broth, papain broth and corn extract medium. The incubation period generally is between 5 and 10 days at 26 to 35°C (Bott et al. 1968; Kautter and Lynt 1978; Hobbs et al. 1982; Foegeding 1986). An enrichment medium designated as TPGYT, containing trypticase, peptone, glucose, yeast extract and trypsin (1 mg/ml), was developed for the detection of *Cl. botulinum* type E and toxin production (Lilly Jr. et al. 1971). The TPGYT medium and cooked meat medium are officially recommended by the U.S. Food and Drug Administration for the isolation of *Cl. botulinum* from foods (Foegeding 1986). The recovery of *Cl.botulinum* is improved through the addition of compounds such as, lysozyme, lactate plus L-alanine or bicarbonate which are known to stimulate germination of spores (Crowther and Baird-Parker 1984).

Isolation: Direct plating has not been successful, since *Cl. botulinum* is generally outnumbered by other anaerobic microorganisms. Isolation is achieved by subculturing an enrichment culture on suitable agar media. Horse blood agar and egg yolk agar, with the addition of antibiotics, are commonly used for isolation. A selective medium designated as CB1 agar was developed for the recovery of *Cl.botulinum* and to obtain isolates within 1 or 2 days (Dowell Jr and Dezfulian 1981). This medium consists of (mg/l), cycloserine 250, sulphamethoxazole 76 and trimethoprim 4 in a base of modified McClung-Toabe egg yolk agar. The confirmation of the cultures is by toxin test (mouse bio-assay). Other important characteristics include anaerobic growth on blood agar ; casein hydrolysis ; lecithinase, catalase, lipase and protease activities ; end product analysis from growth on glucose and indole production.

Clostridium perfringens

Clostridium perfringens causes a generally mild gastro-enteritis which typically follows ingestion of approximately 10^8 viable colony-forming units. The vehicle of infection is frequently the red meat or poultry (Willardsen et al. 1979; Crowther and Baird-Parker 1984). Five types of *Cl. perfringens* have been identified todate, distinguished by the major exotoxins produced.

Enumeration and isolation : Media for enumeration and confirmation of *Cl.perfringens* have evolved from a sulphate-glucose-iron-agar medium (Wilson and Blair 1924; Labbe 1983). The main principle for *Cl.perfringens* differentiation is its ability to reduce sulphite to sulphide which is precipitated as ferrous sulphide in the presence of an iron salt, thereby forming black colonies. The other principles used are lecithinase and haemolytic activities, stormy clot fermentation and abundant gas from fermentation of sugar (lactose). The media commonly used for enumeration and isolation of *Cl.perfringens* are tryptose-sulphite-cycloserine (TSC) agar, oleandomycin-polymyxin-sulphadiazine-perfringens (OPSP) medium, neomycin-blood agar and Shahidi-Ferguson-perfringens (SFP) agar (Sutton and Hobbs 1968; Harmon et al. 1971; Shahidi and Fergugon 1971; Hanford 1974).

The widely used selective enumeration media namely TSC and SFP consists of perfringens agar base. This base medium comprises of (g/l), tryptose 15, soya peptone 5, beef extract 5, yeast extract 5, sodium metabisulphite 1, ferric ammonium citrate 1, agar 15 and pH 7.6 ± 0.2. The medium is autoclaved.

TSC agar : To 100 ml. of molten base medium, are added 40 mg of membrane filtered D-cycloserine and 8 ml of sterile egg yolk emulsion (50% in saline), mixed well and poured into pre-sterilized petri plates. Surface plating of 0.1 ml sample dilutions are made followed by overlaying with egg yolk-free TSC agar. Plates are incubated at 37°C for 18 to 24 h in an anaerobic jar.

SFP agar : To 100 ml of molten base medium, are added membrane filtered kanamycin sulphate (1.2 mg), polymyxin B sulphate (3000 units) and egg yolk emulsion (10 ml). The medium is mixed well and poured into petri plates. The process of inoculation and incubation are same as in TSC.

A need to detect small numbers of *Cl. perfringens* led to the development of enrichment media such as, fluid thioglycollate medium without glucose and with 0.4 mg D-cycloserine/ml and incubated at 46°C (Debevere 1979). For a good *Cl.perfringens* enumeration, adequate anaerobiosis is essential which can be achieved by using N_2,

H_2 plus 5% CO_2 or N_2, H_2 and CO_2 in varying proportions (Harmon et al. 1971; Hanford 1974; Willardson et al. 1979).

Cultural characteristics : The cultural characteristics of *Cl. botulinum* and *Cl.perfringens* grown in cooked meat and TSC/SFP media, respectively include (i) in cooked meat medium, the anaerobes (*Cl. botulinum*) grow deeper in medium, saccharolytic clostridia produce acid and gas rapidly with no digestion of meat. Proteolytic clostridia decompose meat producing foul smelling sulphur compounds and blackening. (ii) in TSC and SFP media, *Cl.perfringens* produce large (2-4 mm diameter) black colonies within the depth of the agar, while lecithinase positive cultures produce opaque zone around the colony. A few differential characteristics of three important *Clostridium* species are presented in Table 3.

salts mixture 1.5, sodium chloride 5, neutral red 0.03, crystal violet 0.001, agar 15 and 7.1±0.2 pH. The medium is autoclaved. For achieving best results, surface inoculation of sample aliquots are carried out on the pre-poured plates of MCA and incubated at 37°C for 24-48 h. Colonies of *E.coli* appearing on the surface are pink to red in colour, shining and with a smooth margin.

Violet red bile agar (VRBA) : This medium enables a direct plate count of coliform bacteria in water, milk and milk products and other food products. VRBA comprises of (g/l) yeast extract 3, peptone 7, bile salt No.3 1.5, lactose 10, sodium chloride 5, neutral red 0.03, crystal violet 0.002, agar 15 and pH 7.4±0.2. The medium is boiled to dissolve the ingredients and no autoclaving is required. To achieve best results, pour plating of the sample

TABLE 3. DIFFERENTIAL CHARACTERISTICS OF *CLOSTRIDIUM*

Characters	Clostridium species		
	Cl.botulinum	*Cl. perfringens*	*Cl. butyricum*
Spores	S/s	S/N	S/s
Motility	+	–	+
Gelatin liquefaction	+	+	–
Hydrogen sulphide production	+	+	–
Indole production	–	–	–
Nitrate reduction	–	+	D
Acid and gas from :			
Lactose	–	+	+
Sucrose	–	+	+
Mannitol	–	–	D

+ = positive; – = negative; S = Subterminal; s = swollen; N = not swollen; **Source** : Mossel (1986)

Escherichia coli

Based on the data from epidemiological studies throughout the world, *E.coli* is judged to be a significant pathogen (Mehlman and Romero 1982). Several selective and differential media for the enumeration of *E.coli* have been described in the literature. However, two media, which have yielded maximum recovery of *E. coli* from foods are commonly used.

MacConkey agar (MCA) : Several formulations of MCA are provided considering the specificity of isolation from different sources (HiMedia 1989). MCA with bile salt (0.15%), crystal violet and sodium chloride enables detection, enumeration and isolation of coliforms and intestinal pathogens in water, dairy products, pharmaceutical preparations and others. This medium consists of (g/l), pancreatic digest of gelatin 17, tryptone 1.5, lactose 10, peptic digest of animal tissue 1.5, bile

aliquots is performed which is over-layered with VRBA and incubated at 37°C for 24 h. Colonies of *E.coli* appear as dark red surrounded by reddish zone.

Confirmatory medium for E. coli : Confirmation of *E.coli* is carried out by streaking the suspected colonies from MCA and VRBA onto pre-poured plates of eosin-methylene blue-agar (Levine). This medium consists of (g/l), peptone 10, dipotassium phosphate 2, lactose 10, eosin-Y 0.4, methylene blue 0.065, agar 15 and pH 7.1±0.2. The medium is autoclaved (HiMedia 1989). After an incubation period of 36 h at 37°C, colonies of *E.coli* appear as green with metallic sheen and the colonies of closely related *Enterobacter aerogenes* appear as pink.

Rapid techniques : The standard procedure of enumeration and isolation of *E.coli* from foods takes nearly 4 to 6 days. Research work aimed at

achieving rapidity in detecting the presence of *E.coli* in mixed populations of food and water led to the development of fluorogenic methods (Feng and Hartman 1982).

About 97% of *E.coli* produce β-glucuronidase, an extracellular enzyme that hydrolyses substrates such as, 4-methyl umbelliferyl-D-glucuronide (MUG) resulting in a fluorescent end product which could be detected within 24 h at 37°C (Hartman et al. 1986). To detect the enzyme, MUG is added to media selective for enterobacteriaceae. Feng and Hartman (1982) described MPN tests with lauryl tryptose (LT)-MUG broth and plate counts on VRB-MUG agar. The value of LT-MUG broth and VRB-MUG agar was confirmed in discriminating seafood samples with high total coliform and/or *E.coli* counts (Alvarez 1984).

Salmonella spp.

Five steps are common to most culture methods for isolation and identification of *Salmonellae* in foods, i.e., (i) pre-enrichment of the food sample in a nutritious, non-selective broth; (ii) selective enrichment in a broth that allows *Salmonellae* to grow, but suppresses the growth of competing bacteria; (iii) isolation of *Salmonella* by streaking onto selective plating agar; (iv) bio-chemical characterisation of isolates; and (v) serological confirmation of biochemically characterised isolates (Andrews 1985). The procedures leading to the detection of positive presumptive *Salmonella* isolates in food samples would give an indication about the presence or absence of *Salmonella* in a food.

Isolation : Different steps involved are schematically represented in Fig. 1. The composition of the different selective enrichment and plating media are well documented in the literature (HiMedia 1989). The differential colony characteristics of *Salmonella* on the selective plating media are important for proper isolation of positive *Salmonella* colonies. These characteristics are based on the biochemical reactions of the organism in relation to the specific substrate(s) present in the respective media which are reflected due to the pH indicators incorporated in the media. *Salmonella* colonies appear as pinkish white surrounded by bright red medium in BGA; black with metallic sheen in BSA; opaque/ transparent pale pink colonies (uncoloured) in SSA; colourless colonies with surrounding blackening in deoxycholate citrate agar (DCA); red colonies with black centre in xylose lysing deoxycholate agar (XLDA) and colourless colonies in MCA. Red slant with yellow butt and black precipitate at the junction of slope and butt are the reactions exhibited by positive *Salmonella* isolates in the slant and butt of TSIA. The reaction is reddish purple slant with the same colour butt or yellow colour butt in LIA. The positive presumptive isolates are subjected to biochemical characteristics for final confirmation.

Rapid techniques for identification : Standard cultural methods for isolation of *Salmonella* in foods are labour-intensive and generally require 4 to 5 days for presumptive identification of *Salmonellae*. Attempts at greater method brevity have resulted in the use of selective enrichment cultures as test material for short immunological tests (D'Aoust 1984). In an immuno-fluorescent antibody (FA) technique, portion of an enrichment culture is fixed, and stained on a glass slide and the reaction between somatic and/or flagellar antigens and conjugated fluorescent antibodies is visualised by fluorescence microscopy. The sensitivity of the technique is over-shadowed by a high incidence of false-positive reactions and need for well-trained analysts (Thomason 1981). In enrichment serology (ES) procedure, portions of selective enrichment cultures are post-enriched for 6 h in a non-selective M broth; flocculation from a mixture of Spicer-Edwards flagellar antisera and M broth culture is indicative of *Salmonella*. Prolonged incubation (24 h) of the M broth reportedly increases method sensitivity. The ES procedure compares favourably with conventional culture techniques and the presumptive identification of *Salmonellae* is obtained one day earlier to that of standard cultural method (Sperber and Deibel 1969; Boothroyd and Baird-Parker 1973; Hilker 1975). In enzyme-linked immunosorbent assay (ELISA), a sample of enrichment culture is placed in the well of a micro-titration plate and reacted with rabbit polyvalent flagellar (H) antisera; unbound antiserum is removed by repeated washings. A goat anti-rabbit globulin coupled to alkaline phosphatase is then added to the well and incubated for 1 h at room temperature; unbound globulin is removed by washing. In the presence of *Salmonella*, the addition of alkaline phosphatase substrate produces a visible colour reaction that can be quantitated spectrophotometrically. This technique performed well in identifying naturally contaminated food samples with *Salmonella* (Minnich et al. 1982).

Besides the short immunological assays, several

Sample (25 g)

↓

Lactose broth
(37°C/20 to 24 h)

Enterobacteriaceae
enrichment broth (EEB)

(37°C/20 to 24 h)

Tetrathionate
broth (ГГB)

(37°C/20 to
24 h)

Selenite cystine
broth (SCB)

(43°C/20 to 24 h)

Surface streaking

↓

Selective plating media

Brilliant green
agar (BGA)

Bismuth
sulphite
agar (BSA)

Salmonella
Shigella agar
(SSA)

MacConkey
agar (MCA)

(37°C/24 to 48 h)

Suspected *Salmonella* colonies isolated based on
differential characteristics

↓

Inoculated onto
slants and butts

Triple sugar iron agar
(TSIA)

Lysine iron agar
(LIA)

(37°C/24 h)

Presumptive positive isolates

↓

Identification

Biochemical
characteristics

Serotyping

Phagetyping

Fig. 1. Schematic representation of *Salmonella* isolation

non-immunological diagnostic techniques based on enrichment cultures have been proposed for the rapid isolation/identification of *Salmonella*. The lysine-iron-crystine-neutral red (LICNR) broth is a non-selective medium that provides for a 24 h

presumptive identification of *Salmonella* through development of typical colour reactions (Hargrove et al. 1971). *Salmonella* decolourises the red broth medium to yellow with a concomitant blackening of the medium. This procedure has a wider

TABLE 4.   DIFFERENTIAL CHARACTERISTICS OF ENTEROBACTERIACEAE GENERA

Characteristics	Genera				
	Escheri-chia	*Entero-bacter*	*Salmo-nella*	*Shigella*	*Proteus*
Motility	v	+	+	–	+
Indole production	+	–	–	v	v
Methyl red	+	–	+	+	+
Voges-Proskauer reaction	–	+	–	–	–
Citrate utilization	–	+	v	–	v
Urease production	–	+	–	–	+
Malonate utilization	–	v	–	–	–
KCN utilization	–	+	–	–	+
Phenylalanine deaminase production	–	–	–	–	+
Gas from :					
Glucose	+	+	+	v	v
Lactose	+	v	–	v	–
Mannitol	+	+	+	v	v
Sucrose	v	+	–	v	v
Dulcitol	v	v	v	v	–

+ = positive;   – = negative;   v = variable.

application in dairy products. The limitation for its use in other foods is the interaction with other food components.

The differential characteristics of a few important genera of enterobacteriaceae are presented in Table 4.

Staphylococcus aureus

In the prevailing conditions of hygiene and sanitation in food processing, handling, storage and distribution as well as aided by the tropical climatic conditions of our country, *Staphylococcus aureus* is an important foodborne pathogen and the thermostable enterotoxins elaborated by this organism is of serious concern to public health aspects.

Enumeration : Several selective and differential media have been developed making use of the important cultural and biochemical characteristics of *S. aureus* to achieve higher isolation of pathogenic strains (Varadaraj and Ranganathan 1985). The important non-tellurite-based media in use are Staphylococcal No. 110 (SM : 110), SM :110 with the addition of egg yolk and mannitol agar (Chapman 1946; Carter 1960; Ward et al. 1981). Colonies of *S. aureus* are mannitol-positive and produce a zone of precipitation. In case of tellurite-based media, colonies of *S. aureus* are black-coloured due to reduction of tellurite salt to elemental tellurium. Few important media in this category include tellurite-lithium chloride agar; modification of this medium with addition of glycine and pH indicator;

egg yolk-tellurite-glycine-pyruvate-agar (ETGPA); ETGPA with supplementation by sulphamezathine, acriflavine, polymyxin and sulphonamide; and substitution of egg yolk with pork plasma in ETGPA (Ludlam 1949; Zbovitz et al. 1955; Vogel and Johnson 1960; Baird-Parker 1962; Smith and Baird-Parker 1964; Giolitti and Cantoni 1966; Devriese 1981). Other media in use are fibrinogen medium, phenolphthalein phosphate agar with polymyxin and sodium azide (Deneke and Blobel 1962; Trinko 1971; Schleifer and Kramer 1980).

Among the various media in use, there is a distinct advantage in favour of using ETGPA and it is also the official AOAC method for the enumeration and isolation of *S.aureus* (Baer 1971). ETGPA consists of (g/l), tryptone 10, beef extract 5, yeast extract 1, glycine 12, sodium pyruvate 10, lithium chloride 5, agar 20 and pH 7.0±0.2 (HiMedia 1989). The medium is dispensed in 95 ml amounts and autoclaved. Prior to pouring the plates, 5 ml of sterile egg yolk (50% emulsion) and 0.3 ml of 3.5% membrane filtered potassium tellurite are added to the molten medium, mixed well, poured into plates and surface-dried. Sample aliquots are surface-inoculated and plates are incubated at 37°C for 24-48 h. Colonies of *S.aureus* on ETGPA are black, shining, with a surrounding halo (lecithinase positive) and a clearing zone of egg yolk. This medium enabled in recording staphylococcal counts of 10^6 to 10^8 cfu/g in samples of market *khoa* comprising of a mixed microflora (Varadaraj and Nambudripad 1982).

Staphylococcus aureus is a Gram positive cocci occurring in irregular clusters; non-sporulating; non-motile; catalase positive; producing golden yellow pigmentation in the presence of 7.5% NaCl and acid from glucose and mannitol under aerobic and anaerobic conditions; positive for production of phosphatase, acetoin, ammonia, coagulase and thermostable deoxyribonuclease (TDNase); gelatin liquefaction; and nitrate reduction.

Vibrio parahaemolyticus

The successful isolation of *Vibrio parahaemolyticus from* sea-foods depends upon the organism's sensitivity to cold stress and halophilic growth as well as survival requirement. To avoid cold stress, samples are to be examined immediately after receipt without allowing for storage under refrigeration temperature or frozen condition. Preferably, the culture media should contain NaCl (about 3%) and all diluents should also contain 2 to 3% NaCl, in view of the inactivation of *V.parahaemolyticus* in distilled water (Lee 1972; Clark 1977).

Pre-enrichment of samples in a non-selective medium has been recommended for detecting injured *V.parahaemolyticus* in refrigerated or frozen seafoods (Ray et al. 1978). Such samples are first cultured overnight at 35°C in trypticase soy broth containing 3% NaCl and then transferred to a selective medium such as, glucose-salt-teepol broth for overnight at 35°C. Following enrichment, the culture is streaked onto thiosulphate-citrate-bile salts-sucrose (TCBS) agar which comprises of (g/l), yeast extract 5, proteose peptone 10, sodium thiosulphate 10, sodium citrate 10, oxbile 8, sucrose 20, sodium chloride 10, ferric citrate 1, bromothymol blue 0.04, thymol blue 0.04, agar 15 and pH 8.6±0.2 (HiMedia 1989). The final medium is heated to dissolve the ingredients, cooled to 50°C, poured into petri plates and allowed to solidify. The medium is not autoclaved. TCBS agar inhibits most of the other bacterial species due to the presence of bile salts and the highly alkaline pH (8.6). *V.cholerae* and *V.alginolyticus* ferment sucrose, produce yellow colonies, while *V.parahaemolyticus* does not ferment sucrose in TCBS agar and hence produces a bluish or blue-green colony.

Campylobacter jejuni

In recent years, there has been a significant increase in the incidence of food poisoning outbreaks caused by *Campylobacter jejuni*. It is viewed as pathogena of "emerging public health significance"

due to its ubiquitous nature, tolerance to adverse conditions and typical characteristic of being able to survive and even multiply in refrigerated foods (Blaser 1982; Patel and Sannabhadti 1991). Isolation procedures for recovering very low numbers of *Campylobacter jejuni* from foods involves four important critical aspects.

i) Conditions for handling and storage of specimens: This is an important factor due to the fragility of the organism which is influenced by temperature and atmospheric O_2. The death of *C.jejuni* at 25°C is 8 times rapid than that at 4°C, while the survival is greater in O_2-free environment (100% N_2). Besides, addition of 0.01% sodium bisulphite to medium enhances survival. A combination of 4°C storage in O_2-free medium containing 0.01% sodium bisulphite makes the survival of *C.jejuni* longer by 10 times.

ii) Enrichment procedures : It is a vital necessity considering the occurrence of very low numbers of C. *jejuni* in foods. A few important enrichment procedures are shown in Fig. 2. These make use of one of the many enrichment media (Doyle 1986). Preston enrichment medium (Bolton and Robertson 1982) which detected 46.5% *Campylobacter* positive samples comprised of Oxoid nutrient broth No. 2 which is supplemented with lysed horse blood (5%), polymyxin B (5 IU/ml), rifampicin (10 μg/ml), trimethoprim (10 μg/ml) and actidione (100 μg/ml). Rosef (1981) enrichment medium showed 40.2% higher isolation frequency than that involving direct plating. The medium consisted of Oxoid "Lab lemco" powder, peptone, yeast extract, sodium chloride, resazurin, vancomycin (10 μg/ml), trimethoprim lactate (5 μg/ml) and polymyxin B (2.5 IU/ml). Enrichment medium of Doyle and Roman (1982 b) gave a higher isolation rate. The medium comprised of Brucella broth which is supplemented with lysed horse blood (7%), sodium succinate (0.3%), cysteine hydrochloride (0.01%), vancomycin (15 μg/ml), trimethoprim (5μg/ml), polymyxin B (20 IU/ml) and cyclohexamide (50 μg/ml).

iii) Selective plating media : Four selective plating media enabled a good recovery of this organism from enrichment culture broths (Doyle 1986). The Skirrow's medium (Skirrow 1977) comprises of blood agar base or Brucella agar which is supplemented with lysed horse blood (5-7%), Vancomycin (10 μg/ml), polymyxin B (2.5 IU/ml) and trimethoprim (5 μg/ml). The Butzler's medium (Lauwers et al. 1978) consists of Columbia agar or blood agar base which is supplemented with sheep blood (5-7%), bacitracin (25 IU/ml), novobiocin

I	II	III
(Park et al. 1981; 1983)	**(Acuff et al. 1982)**	**(Doyle and Roman 1982b)**

I (Park et al. 1981; 1983)

Sample
(10 or 25 g)

↓

Washing in
nutrient broth

↓

Filteration through
cheese cloth

↓

Centrifugation

↓

Sediment retained

↓

Enrichment culture

↓

Membrane filtration
(0.65 um)

↓

Plating onto
Skirrow's agar

* In an anaerobic incubator or under a constant flow of 5% O$_2$, 10% CO$_2$, 85% N$_2$ (5 to 7 ml/min) bubbled into broth.

II (Acuff et al. 1982)

Sample
(50 or 40 g)

↓

Dilution in enrichment
broth (1:10)

↓

Held at 4°C for 12h
followed at 42°C for 48 h

↓

Planting on selective agar

↓

Suspension in enrichment
broth under
microaerobic environment*
(48 h at 42°C)

III (Doyle and Roman 1982b)

Sample
(10 or 25 g)

↓

Inoculation

↓

Enrichment medium #

↓

Incubation at 42°C
16-18 h Microaerobic
agitation
(100 gyrations/min)

↓

Plating onto
Campy-BAP medium

enrichment medium is taken in 250 ml sidearm od capped Erlenmeyer flask, evacuated 3 times at 20 in. Hg, replaced with the microaerobic gas mixture.

Fig. 2. Enrichment procedures for the isolation of C. jejuni

(5 µg/ml), actidione (50 µg/ml), colistin (10 units/ml) and cephalothin or cephazolin (15 µg/ml). Blaser Wang's or Campy BAP medium (Blaser et al. 1979) comprises of Brucella agar or blood agar base which is supplemented with sheep blood (10%), vancomycin (10 µg/ml), trimethoprim (5 µg/ml), polymyxin B (2.5 IU/ml), amphotericin B (2 µg/ml) and cephalothin (15 µg/ml). Brucella agar- base comprises of (g/l), tryptone 10, peptone 10, yeast extract 2, dextrose 1, sodium chloride 5, sodium bisulphite 0.1, agar 15 and pH 7.0±0.2. The blood agar base consists of (g/l), proteose peptone 15, liver digest 2.5, yeast extract 5, sodium chloride 5, agar 15 and pH 7.4±0.2. Columbia agar base comprises of (g/l), special peptone 23, corn starch 1, sodium chloride 5, agar 15 and pH 7.3±0.2 (Himedia 1989).

On the plating media, after suitable incubation period, typical *Campylobacter* colonies are smooth, convex, translucent, colourless to cream-coloured, pin-point to 2-4 mm in diameter and often show confluent growth. *C.jejuni* is a motile, Gram negative, catalase and oxidase positive, obligate microaerophilic vibrio requiring small amounts of O$_2$ (3-6%) and CO$_2$ (10%). It grows best at 42°C, but not at 25°C. Growth also occurs in the presence of 1% glycine and 1% ox-gall, but not at 1.5% NaCl. It can hydrolyse hippurate, reduce nitrate and produce alkaline phosphatase.

Listeria monocytogenes

It is yet another pathogens of emerging public health significance. Recent reviews have focussed on the various methods and media to isolate and enumerate Listeria monocytogenes from various sources including foods (Donelly 1988; Cassiday and Brackett 1989).

Enrichment : Early enrichment procedures involved incubation of the sample for 24 h in a nutrient broth containing one of the selective agents such as, guanofuracin, lithium chloride, nalidixic acid, acriflavin (trypaflavin) and cyclohexamide. Plating is done on a non-selective or selective agar containing the same inhibitory agent (Gray et al. 1950). Other effective enrichment media used are : (i) tryptose phosphate broth containing 10 ppm of furacin (McBride and Girard 1960) and (ii) trypaflavin-nalidixic acid-cyclohexamide broth (TNCB) consisting of bacto tryptose broth (Difco) 26 g per 1000 ml with 10 mg trypaflavin dissolved in 5 ml water, 40 mg nalidixic acid dissolved in 4 ml of 0.05 N NaOH and 50 mg cyclohexamide dissolved in 5 ml of 40% ethanol (Northolt 1989). The advantage of a fluid medium is the semi-anaerobic condition which enhances multiplication of facultatively anaerobic Listeria spp.

Selective plating media : McBride Listeria Agar (MLA) enables isolation of L.monocytogenes from mixed cultures. The medium comprises of phenyl ethanol agar base (Difco), lithium chloride, glycine and blood (McBride and Girard 1960). In the modified MLA (MMLA), the blood is replaced with cyclohexamide to inhibit the growth of molds (Lovett et al. 1987) and this medium is presently used by United States Food and Drug Administration. In a later modification of the medium, known as ARS-modified McBride agar (ARS-MMA), nalidixic acid, moxalactam and bacitracin are added to the medium (Buchanan et al. 1987). Another commonly used medium is the trypaflavine-nalidixic acid-serum-agar (TNSA), the selectivity of which is enhanced by the addition of polymyxin B (Cassiday and Brackett 1989).

The procedure for isolating L.monocytogenes from milk and dairy products (Lovett et al. 1987), presently used by the FDA (US), involves enrichment of the sample for 48 h at 30°C in a broth consisting of trypticase soy broth, yeast extract, acriflavine HCl, nalidixic acid and cyclohexamide and this is followed by streaking onto MMLA. Besides, 1 ml of the enrichment mixture is added to 9 ml of

0.5% KOH, mixed briefly, then streaked onto MMLA. Plates are incubated at 35°C for 48 h. Presumptive L. monocytogenes colonies are viewed through a dissecting microscope, wherein the light reflects on the bottom of the petri plate at a 45°C angle. The colonies appear pearlescent blue when observed in this manner. Extending the incubation period in the enrichment broth to 7 days allows better recovery of environmentally stressed organisms (Hayes et al. 1986).

The FDA procedure was modified to isolate L.monocytogenes from raw milk and soft cheeses (Beckers et al. 1987). In this case, the sample is enriched for 24 h at 30°C in tryptose broth supplemented with trypaflavine HCl, nalidixic acid and cyclohexamide. A loopful of this mixture is then streaked onto TNSA plates, while the remaining is incubated at 4°C and plated onto TNSA after 1 and 2 weeks and 1 and 2 months. TNSA plates are incubated microaerophilically in an atmosphere of 5% O_2, 10% CO_2 and 85% N_2 at 37°C for 48 h. Results indicated that cold enrichment in broth gave a higher recovery of L.monocytogenes. In another study (Northolt 1989), enrichment in TNCB and plating on TNSA resulted in the detection of low number of L.monocytogenes in samples of milk, whey, Gouda cheese and smeared cheese.

On TNSA medium, Listeria spp. produce bluish-green colonies with characteristic unevenly glittering surface. L.monocytogenes is a motile, Gram positive, oxidase negative, catalase positive and non-sporulating rod. It has a wide range of growth temperature from 3 to 45°C with an optimum of 30-37°C. Growth is enhanced under decreased O_2 concentration and with supplementation of CO_2.

Yersinia enterocolitica

It is also known as a pathogen of emerging public health significance. The procedure to detect Yersinia enterocolitica primarily focusses on isolation and identification of virulent strains in foods. This is achieved by the enrichment treatment which promotes growth of very small numbers of the organism and also enable recovery of injured cells (Doyle 1986).

Enrichment : The alkali post-enrichment treatment is accomplished in a non-selective medium such as, phosphate-buffered saline (PBS) for 2-4 weeks at 4°C. A 4-fold increase in the recovery of Y.enterocolitica and elimination of other background contaminants by same folds are achieved by exposing

cultures grown in enrichment broth to 0.5% potassium hydroxide prior to plating (Aulisio et al. 1980). A 2-4 week cold enrichment procedure is not amenable to routine testing of foods. This led to the development of a 1-3 day enrichment, KOH post-enrichment treatment which gave effective recovery of *Y.enterocolitica* (Doyle and Hugdahl 1983). In this procedure, 1 g sample of food is enriched in PBS at 25°C for 48 and 72 h and 25 g sample for 24 and 48 h. After incubation, 0.5 ml of enrichment culture is treated with 4.5 ml of 0.25% KOH for 2 min. or 0.5% KOH for 15 sec and 0.1 ml aliquots of treated culture are plated onto the selective agar.

The two-step enrichment procedure is a promising method to recover *Y.enterocolitica* from foods (Schiemann 1982). Pre-enrichment of the sample is carried out at 4°C with either PBS for 14 days or yeast extract-rose bengal (YER) broth for 9 days. This is followed by selective enrichment in bile-oxalate-sorbose (BOS) broth for 5 days at 22°C. In a later modification, pre-enrichment is done in trypticase soy broth for 1 day at 22°C and then for 4 to 7 days at 2-4°C followed by selective enrichment in BOS at 22°C for 3-5 days (Schiemann 1983).

On the basis of selectivity and easy differentiation of colonies of *Y.enterocolitica*, cefsulodin-irgasan-novobiocin (CIN) agar has given best results for isolating this organism from food homogenates or enrichment cultures (Schiemann 1979; Devenish and Schiemann 1981). CIN agar comprises of (g/l), peptone special 20, yeast extract 2, mannitol 20, sodium pyruvate 2, sodium chloride 1, magnesium sulphate 0.01, sodium deoxycholate 0.5, neutral red 0.03, crystal violet 0.001, agar 15 and pH 7.4±0.2 (HiMedia 1989). The medium is autoclaved. Prior to pouring the plates, cefsulodin 7.5 mg, iragasan 2 mg and novobiocin 1.25 mg are mixed in 2 ml sterile distilled water and 1 ml ethyl alcohol and added to 500 ml quantity of molten CIN basal medium.

Typical *Y.enterocolitica* colonies on CIN agar are transluscent with a dark red centre "bull's eye" surrounded by transparent border and precipitation of bile. *Y.enterocolitica* is a motile, Gram negative, oxidase negative, catalase positive and non-sporulating ovoid rod, arranged singly or in short chains. It has an optimum growth temperature of 25 to 39°C and optimum pH of 7 to 8. The organism is sensitive to NaCl (5%) and acidity (pH 4.6). Acid is produced from glucose, galactose, fructose, maltose, mannose, mannitol and sucrose, but not from xylose, rhamnose, raffinose and arabinose. *Y.enterocolitica* is positive for urease and negative for phenylalanine and tryptophan deaminase.

Conclusion

A substantial progress has been made in developing · media and methods for easier and reliable enumeration, isolation and identification of important foodborne bacterial pathogens. However, selection of the methodology is of critical importance. There is a growing need for more rapid, sensitive and reliable tests to detect these pathogens as well as their toxigenic properties for enabling to overcome the public health hazards.

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Preparation of Medium Fat-Soyflour at Small Scale

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Process for partial extraction of oil using an expeller and production of flour from partially deoiled soycake has been developed. The process consists of cleaning, dehulling and splitting of soybean, steaming and flaking of soysplits, deoiling of flakes in a screw press and grinding of edible cake to flour which has less than 6% oil and about 50% proteins. The process is potential for producing oil as well as protein-rich flour on a small scale.

Keywords : Soybean, Soybean processing, Medium fat soyflour, Proximate analysis, Storage of flour, Organoleptic evaluation

Though soybean is rich in proteins and low in oil content, as compared to other oilseeds, it is mainly being utilized in India for producing oil through solvent extraction. The deoiled meal obtained after extraction is exported to foreign countries for feed purposes. Nowadays, great emphasis is being placed on the use of soybean, not only for its oil but also for its proteins for human consumption. This aim can be achieved if soybean is partially extracted for its oil, using a screw press and the protein-rich cake is converted into edible product for human consumption. As screw presses are used widely in India at village and small scale levels for extraction of oil from oilseeds other than soybean, mechanical deoiling of soybean would be a better option for extraction of oil and production of edible grade cake for further processing to obtain medium fat soyflour. Use of wheat and medium fat soyflour blend for making traditional food items can offer an unique opportunity for combating protein-calorie malnutrition prevailing among our masses. Keeping this in view, a process which consists of conditioning soy splits for partial oil extraction through an expeller and production of flour from the edible grade cake for making medium fat soyflour on small scale has been developed.

Materials and Methods

Soybean, variety 'JS-7244', grown at experimental farm of Central Institute of Agricultural Engineering, Bhopal, was used in the present experiment. Whole and sound soybeans were cleaned with a swinging type manually-operated double screen grain cleaner and dehulled manually to get clean splits. The splits were then steamed for 18 min. at atmospheric pressure which was optimum for flaking in a small capacity roller flaking machine. The flakes were expelled in a mechanical screw

press to get edible grade soycake and oil in three passes. The cake was ground into flour of desired particle size using a grinder. The process flow chart for making medium fat soyflour on small scale is shown in Fig. 1.

RAW SOYBEAN
↓
CLEANING ⟶ Impureeties
↓
DEHULLING ⟶ Hulls
↓
STEAMING
↓
FLAKING
↓
OIL EXPELLING ⟶ Oil
↓
GRINDING
↓
SOYFLOUR
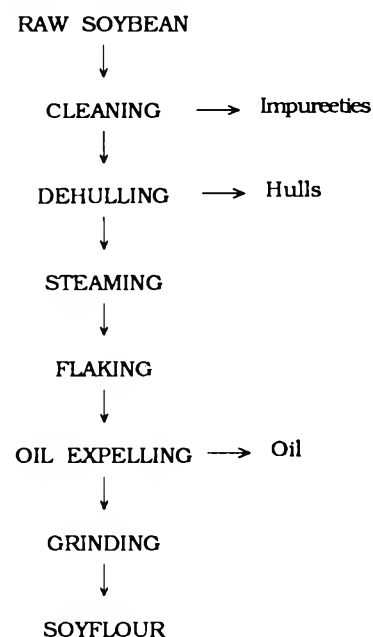
Fig. 1 Process flow chart for making medium fat-soyflour

The flour was stored in metallic containers and in 400 gauge polyethylene bags for four months. Free fatty acids (FFA), moisture and insect infestation were measured at monthly intervals. Soyflour was analysed for various components, viz. protein, fat, fibre, ash, FFA and moisture as per AOAC (1984) methods. Nitrogen solubility index, urease activity and water absorption were determined by AOCS (1975) methods. Blends of wheat flour and medium fat soyflour at 10 and 15% levels were used for preparation of traditional foods viz. *chapati, puree* etc., These products were evaluated for sensory characteristics by BSI (1975) methods.

Particle size distribution was measured according to the procedure described by Sahay (1984).

Results and Discussion

Proximate analysis : Ten kg of soybean produces 7.5 kg of edible grade medium fat soyflour. The quality of the flour is in accordance with the standards prescribed by the BSI (1975b). The proximate analysis of the flour revealed that it

TABLE 1. COMPOSITION OF MEDIUM FAT SOYFLOUR

Parameter	Value
Protein content, %	49.8
Oil content, %	5.0
Moisture content, %	6.3
Ash content, %	5.8
Fibre content, %	2.5
Water absorption, %	284.0
Average particle size, mm	0.3
Nitrogen solubility, %	17.5
Urease activity, change in pH unit	Nil

contains about 50% protein, 5.4% oil and 6.3% moisture; other constituents accounting for the remaining 38.3% of the flour (Table 1). Particle size distribution analysis as shown in Table 2 indicates that the total flour passes through 50 mesh size. Comparison of particle size of medium fat soyflour with that of wheat *atta* (ISI 1968) indicated that flour obtained has finer particles than wheat *atta*. The water absorption capacity of the medium fat soy flour is 284%, thereby indicating that its blend with wheat flour would absorb more water.

Storability of the flour : The medium fat soyflour was stored in metallic containers and polyethylene bags for a period of 4 months under room conditions. The minimum and maximum room temperatures during the period were 6.2° and 43.4°C, respectively, while the relative humidity fluctuated from 12 to 93%. The average temperature

TABLE 2. PARTICLE SIZE DISTRIBUTION OF MEDIUM FAT SOYFLOUR

Sieve No. ISS	Sieve aperture	Particle size on sieve	Wt. of particles on sieve	% of particle on sieve by wt.	% of wt. finer than sieve aperture
100	1000	1096	0.0	0.00	100.00
70	708	842	0.0	0.00	100.00
50	500	595	1.0	0.99	99.01
40	420	458	22.0	21.87	77.14
30	296	353	10.0	9.94	67.20
20	211	250	64.0	63.62	3.58
15	157	182	1.0	1.59	1.59
Pan	-	140	2.0	1.99	-

Geometric mean particle = 294μ, Geometric standard deviation = ± 0.99, Feeding rate = 10 kg/h, Rotor speed = 1800 rpm.

TABLE 3. MEAN SCORE OF SENSORY PANEL JUDGES FOR THE CHARACTERISTICS OF MEDIUM-FAT SOYFLOUR

Characteristics	Blending of traditional food with medium-fat soyflour					
	Bhajia from chickpea		*Puree* from wheat flour		*Chapatis* from wheat flour	
	10%	15%	10%	15%	10%	15%
Taste	7.78	6.89	7.89	6.67	7.11	6.22
Flavour	7.67	7.44	7.33	6.67	7.56	7.11
Feel	7.11	7.00	7.56	6.89	7.56	7.33
Colour	7.67	7.33	7.33	7.33	7.22	7.22
Appearance	7.56	7.56	7.89	7.44	7.44	7.22
General acceptability	7.56	7.45	7.33	7.00	7.89	6.78

and relative humidity were 26.9°C and 40%, respectively. Analysis of the samples indicated no appreciable variation in FFA and moisture contents. These samples were also free from insect infestation.

TABLE 4. ANALYSIS OF VARIANCE OF THE TEST PANEL SCORES FOR THE CHARACTERISTICS OF DIFFERENT MEDIUM-FAT SOYFLOUR-BASED PRODUCTS

Source of variation	Degree of freedom (df) n-1	Mean score of square					
		Taste	Flavour	Feel	Colour	Appearance	General acceptability
Products	5	3.75	1.19	0.73	0.24	0.43	1.42
Judges	8	0.59	0.74	0.80	1.98	0.94	0.96
Error	40	0.48	0.94	0.55	0.98	0.60	0.68

TABLE 5. COMPARISON OF WHEAT FLOUR WITH MEDIUM FAT SOYFLOUR AND THEIR BLEND FOR PROTEIN CONTENT AND OTHER VALUES.

Constituents/ values	Wheat flour	Medium soyflour	Wheat-medium fat soy-flour blend (10:90)	% increase in value of soywheat blend over wheat flour
Protein, %	12.1*	49.8	15.9	23.8
Fat, %	1.7	5.4	2.1	26.1
Fibre, %	1.9	2.5	2.0	12.8
Cost, Rs.	5.5**	9.5**	5.9	7.3
Approximate cost of 1 kg protein (Rs.)	45.5	19.1	42.9	(-) 5.7

* Gopalan et al. (1987)

** Bhopal based

Organoleptic evaluation : The quality characters and general acceptability of traditional food items viz. *chapati, puree* etc., blended with medium-fat soyflour were above the minimum acceptable score of five (Table 3) on Hedonic scale. This indicates that the products have been liked by the judges. Analysis of variance of the test panel scores for each characteristic of different products, made from medium-fat soyflour, revealed that the difference among the treatment is significant only for taste among the products and not for other characteristics and general acceptability (Table 4). The blend of 10% medium-fat soyflour and 90% wheat flour may be used for making acceptable traditional foods.

Cost of protein : Medium-fat soyflour is an economical source of protein as compared to wheat flour (Table 5). Mixing of wheat flour with 10% medium-fat soyflour increases the protein content of the blend to 15.9% at a nominal increase in price. The protein-rich medium-fat soyflour can be used in traditional foods to combat protein malnutrition.

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Studies on Milling, Physico-chemical Properties, Nutrient Composition and Dietary Fibre Content of Millets

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Pearl millet and small millets were studied for milling characteristics and the milled fractions were analysed for chemical composition and dietary fibre content. The milled grains were also evaluated for cooking quality. The yields of milled grains, bran and husk varied from 63.2 to 90.0%, 5.0 to 11.0% and 1.5 to 29.3% respectively. Milled grains contained about 90 and 70% of the grain protein and grain fat, respectively. The oil contents of the bran from pearl, finger and other small millets were 15, 3 and 23-27%, respectively. The total dietary fibre contents of milled grains ranged from 9-16% out of which 32-50% was soluble dietary fibre. Milled millet grains cooked soft within a short period when added to boiling water. The Brabender visco-amylograms of milled millet flour indicated a gelatinisation temperature of about 75 ± 2°C, peak viscosity of 220-560 BU, breakdown viscosity of 20-120 BU and cold paste viscosity of 340-1120 BU.

Keywords : Millets, Milling, Physico-chemical properties, Nutrient composition, Dietary fibre, Cooking time.

Pearl millet (Pennisetum americanum) and small millets, viz., finger millet (Eleusine coracana), proso millet (Panicum miliaceum), kodo millet (Paspalum scrobiculatum), foxtail millet (Setaria italica), barnyard millet (Echinochloa colona) and little millet (Panicum miliare) are classified as coarse grains and cultivated mostly in Asian and African countries. The total production of millets in the World and in India during 1990 was 30 and 10.5 million MT, respectively (FAO 1991).

Millets are small-sized grains, containing large proportions of husk and bran, require dehusking and debranning prior to consumption (Hulse et al. 1980). The nutritive value of millets is comparable to other cereals, some of them are even better with regard to average protein and mineral contents (Gopalan et al. 1989). Millets were earlier decorticated at household level by hand pounding, but are currently milled in rice milling machinery with a slight modification of the process. The millets are mostly powdered in plate mills and the whole meal is used for traditional food preparations (Desikachar 1975).

Although millets are nutritionally superior, the non-availability of refined and processed millets in ready-to-use form has limited their wider use and acceptability. Millets are, therefore, confined to traditional consumers and also to the people of lower economic strata (Desikachar 1977). Hence, there is a need to develop suitable milling systems to obtain milled millet grains at household and small-scale industrial levels with a view to facilitate their easy availability in ready-to-use form. In the present paper, the results of the studies on dehusking and debranning of millets using laboratory scale rice milling machinery, the physico-chemical properties, nutrient composition and dietary fibre content of the milled fractions of millets are reported.

Materials and Methods

The pearl millet sample was obtained from the Agricultural Research Station, Gulbarga, and the small millets, viz., finger, foxtail, proso, little, kodo and barnyard millets were procured from the University of Agricultural Sciences, Hebbal, Bangalore.

The small millets were dehulled in a centrifugal sheller (Smith 1955) and debranned in a laboratory scale McGill Mill No. 1. The yields of glumes/husk, bran and debranned millets were recorded. In case of finger millet, moist conditioning, grinding and sieving techniques were followed and the yield of the -60 mesh fraction was recorded as debranned flour (Malleshi and Desikachar 1981). The pearl millet was deglumed and debranned in the McGill Mill. Thousand kernel weight and volume of whole, dehusked and debranned millets were determined using a Numigral Grain Counter (Tecator) and Beckman Air Comparison Pyconometer (Model 930), respectively.

The hardness of whole and milled grains was measured using a Brabender hardness and structure tester; a 50 g sample was ground in the hardness

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tester with a clearance adjusted to 0.5 mm (1.5 mm in case of pearl millet and finger millet) and the torque developed during grinding was plotted by the instrument against time axis and the area covered by the peak in sq cm was taken as the hardness in Brabender units (BU).

Dehusked and debranned millets were powdered in a Udy cyclone mill and their defatted flours were analysed for their nutrient composition by standard AACC (1983) procedures. Total and soluble dietary fibre content of debranned millets and that of bran was estimated by the enzymatic method (Asp et al. 1983). The cooking time of milled grains was determined by dropping the grains into boiling water and cooking till the grains became translucent. The translucence was tested periodically by pressing between two slides. Pasting characteristics of debranned flour (10% slurry) was studied in a Brabender visco-amylograph (Hallic and Kelly 1959).

Results and Discussion

The husks of proso, foxtail, kodo, little and barnyard millets were separated effectively in a centrifugal sheller. The yield of husk from small millets ranged from 16.0 to 29.0% (Table 1), the highest being from kodo millet (29.0%). Pearl millet glumes accounted for only 1.5% of the millet and were easily removed in the McGill Mill. The

Desikachar 1985).

The thousand kernel weight and volume of the millets ranged from 2.3 to 7.1 g and 1.4-5.1 ml. respectively, while their densities ranged from 1.37 to 1.64 g/ml (table 1). Thousand kernel weight and volume of milled grains ranged from 1.9-5.8 g and 1.3-3.8 ml, respectively. As expected, the density of milled grains ranged from 1.40-1.80, which was higher than whole grains. The hardness values of whole and milled pearl millet grain at 1.5 mm clearance were 56 and 43 BU, respectively. Under same clearance, the hardness of finger millet was 46 BU. However, because of the small size of other small millets, the hardness was measured at lower clearance (0.5 mm). Hardness varied from 44-144 BU for whole grains and 53-67 BU for milled millets. Among the small millets, the hardness of whole kodo millet was highest (144 BU), whereas among the milled millets, foxtail exhibited highest hardness value (67 BU). From this, it may be inferred that the seed coat (husk and bran) of millets contribute substantially to the hardness of grains .

The nutrient composition of dehusked and debranned grains, and the dietary fibre contents of milled grains and bran are presented in Table 2. The protein contents of the dehusked

TABLE 1 : MILLING YIELD, PHYSICAL PROPERTIES AND COOKING TIME OF MILLED MILLETS

Millet	Milling yield, %			1000 kernel weight, g			1000 kernel volume, ml			Density			Hardness, B.U.		Cooking time, min
	Milled grain	Bran	Husk	W	D	M	W	D	M	W	D	M	W	M	M
Pearl	90.0	10.0	1.5	6.0	5.8	5.2	4.3	3.9	3.6	1.39	1.48	1.44	56*	53*	9
Finger	89.0	11.0	-	2.9	-	-	2.1	-	-	1.38	-	-	40*	-	6
Proso	78.8	5.0	16.2	7.1	6.1	5.8	5.1	4.3	3.8	1.39	1.42	1.46	56	60	4
Foxtail	77.2	6.0	16.8	2.6	2.2	2.1	1.9	1.8	1.5	1.37	1.22	1.40	69	67	4
Kodo	63.2	7.5	29.3	6.7	4.4	4.2	4.8	3.3	2.8	1.38	1.33	1.50	114	58	3
Little	77.0	6.1	17.0	2.3	2.0	1.9	1.4	1.2	1.3	1.64	1.66	1.46	44	59	3
Barnyard	77.5	6.0	16.5	3.3	2.6	2.7	2.4	1.9	1.5	1.37	1.37	1.80	83	62	4

W : Whole grain; D: Deglumed; M: Milled; - : Not available; * At clearance 1.5 mm, all others at 0.5 mm

debranned millets were white in colour and resembled rice, except in case of foxtail millet, which had light golden yellow colour. Bran yield was around 10% for pearl millet and 5.0 to 7.5% for small millets. Moist conditioning, grinding and sieving of finger millet yielded 89% debranned flour. From Table 1, it could be seen that the yield of milled grain was lowest for kodo millet (63%) and highest for pearl millet (90%). The milling yield data are in agreement with those reported in the literature (Kadkol et al. 1954; Malleshi and

millets varied between 8.7% (kodo millet) and 13.8% (pearl millet), whereas in case of milled grains, it varied from 5.8% (finger millet) to 12.7% (pearl millet). The fat content of deglumed millets ranged from 1.1% to 5.0%. Milled grains contained nearly 70% of total fat of the whole seeds. The calcium and phosphorus contents of milled millets varied from 2.3 mg% to 162.8 mg% and 105 mg% to 425 mg%, respectively. Milling removed nearly 50% calcium and about 65% of phosphorus of whole seeds. The bran fraction from small millets,

other than finger millet, contained 23.0-27.0% oil, whereas pearl millet bran contained 15% oil.

The total dietary fibre content of debranned millets ranged from 9.0 to 16.0%. This indicates

rice and wheat (Wisker et al. 1981). This indicates that the small millets form an important source of soluble dietary fibre. It may be mentioned here that soluble fraction of the dietary fibre has

TABLE 2.: NUTRIENT COMPOSITION AND DIETARY FIBRE CONTENT OF MILLED FRACTIONS OF MILLETS

Millet	Protein, g %		Fat, g %			Ash, g %		Calcium, mg %		Phosphorus mg %		Dietary fibre, g %					
												milled			Bran		
	D	M	D	M	B	D	M	D	M	D	M	Soluble	Insoluble	Total	Soluble	Insoluble	Total
Pearl	13.8	12.7	5.2	4.3	15.0	1.8	1.5	64.7	30.0	395.0	300.5	6.0	10.2	16.2	6.5	42.5	49.0
Finger	9.4	5.8	1.1	0.8	3.0	2.8	1.0	454.1	162.8	327.9	127.2	6.5	5.3	11.8	3.0	31.8	40.8
Proso	11.0	10.2	4.5	2.1	27.3	1.4	0.6	7.8	4.5	411.9	194.7	4.4	9.0	13.4	7.2	36.0	43.2
Foxtail	13.7	10.5	4.8	2.3	23.2	1.5	1.4	28.2	19.1	384.5	280.9	3.4	6.2	9.6	2.4	40.2	42.6
Kodo	8.7	8.3	3.8	1.1	25.2	1.1	0.3	8.1	2.3	280.4	105.5	5.2	6.4	11.6	5.7	42.5	48.2
Little	11.4	9.5	5.4	3.1	25.6	1.6	0.9	13.1	4.4	412.4	262.7	5.7	10.2	15.9	3.7	45.0	48.7
Barn-yard	11.5	11.0	5.5	4.5	24.9	1.9	1.6	20.5	5.5	458.4	425.3	6.2	8.7	14.9	5.9	31.2	37.1

D : Deglumed; M : Milled; B : Bran

that millets, even after removal of husk and major portion of bran, contained appreciable amounts of dietary fibre. The soluble dietary fibre content of milled millet was 32-50% of the total dietary fibre, which represents a fairly higher proportion as compared to the soluble fibre values reported for

Fig. 1. Visco-amylograms of millet flours.

beneficial effect in rats (Ranhotra et al. 1990). The millet bran, besides containing considerably higher proportion of oil, appears to be a good source of dietary fibre. The defatted bran contained 37-49% total dietary fibre, out of which 10-15% was soluble fraction. Investigation with regard to hyper-cholesteremic effect of millets may reveal useful information for diversified uses of their bran.
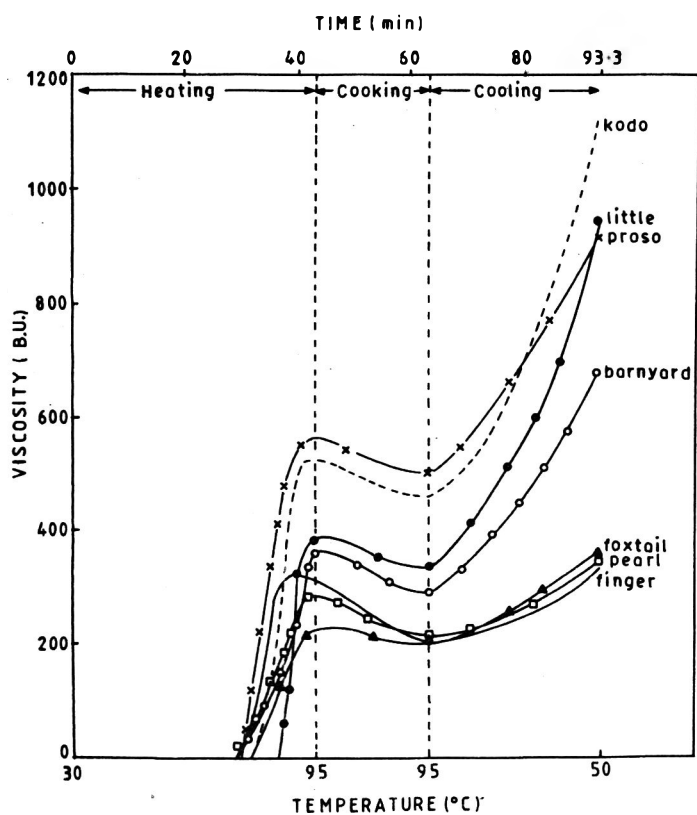
The cooking time for the small millets ranged from 3-6 minutes and for pearl millet, it was 9 minutes. Quick hydration and softening property of millets may be advantageous for the development of flaked products and quick cooking cereals. The Brabender visco-amylograms of millet flour (Fig. 1) indicated that the gelatinisation temperature of millet starch was around 73-77°C except for little millet (80°C), while the peak viscosity was highest for proso millet (560 BU) and lowest for foxtail (220 BU). The low breakdown viscosity during continuous heating phase at 95°C indicates the stability of cooked starch and suitability of millets for use in porridges. The cold paste viscosity (at 50°C) was high (1120 BU) for kodo millet and low (340 BU) for finger millet. The viscograms are generally similar to rice, maize and sorghum viscograms.

The present study clearly indicated that dehusking in centrifugal sheller and debranning in huller type machine may be adopted to get milled grains and pure bran, although there is a need to develop exclusive mills for milling of small millets. Milled grains hydrate quickly and cook to soft

texture in a short time. This shows the possibility of their use as quick cooking cereals. Milled millet can also serve as an extender to rice or wheat semolina for conventional Indian food preparations like *idli* and *dosa*. The bran from millets can be explored for its use as an extender to rice bran for oil extractions and the defatted bran as a source of dietary fibre.

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Studies on Low-Fat Soft Dough Biscuits

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Studies on reduction in fat from 20 to 7.5%, in soft dough biscuits, indicated decrease in spread from 5.5 to 5.15 cm and increase in hardness from 0.9 to 1.3 kg/biscuit. In addition, the overall quality score decreased from 48.5 to 30.0. Further reduction in fat adversely affected machinability of the dough. The adverse effects were considerably lower with bakery shortening as compared to other fat or oil. Lecithin at 0.5% was found to be most effective for improving the overall quality of low-fat biscuits. The crispness of low-fat biscuits could also be improved by incorporating flaked rice flour at 5% level.

Keywords : Low fat biscuits, Fat levels, Additives, Crispness, *Maida*, Breaking strength.

Bakery products have become popular in India, as evidenced by two-fold increase in their production in the last five years. Among these, biscuit forms the most popular item. Soft dough biscuits account for over 70% of total production in India. These types of biscuits normally contain high amounts of fat ranging from 15 to 25% (SIB 1981). As a result, fat alone contributes to about 50% of total raw material cost. It has been reported that incorporation of some of the recently developed surfactants could replace upto 8% of fat in cookies (Tsen et al. 1973). However, the information on the possibilities of reducing fat in biscuits is limited (Nisbet et al. 1986). Hence, the present studies were undertaken to find out the possibilities of reducing fat in biscuits.

Materials and Methods

Refined wheat flour *(maida)*, milled in a flour mill of 20 tonnes capacity at International School of Milling Technology at CFTRI, was used. Commercially available hydrogenated fat, refined oil and bakery shortening were employed. Different rice products like puffed rice, flaked rice, parboiled rice and raw rice were procured from local market and ground in a Kamas hammer mill using 0.8 mm sieve.

Flour characteristics : Moisture, dry gluten, total ash and Zeleny's sedimentation value of flour were determined according to AACC (1962) procedures. Sweet biscuits were prepared according to the procedure of Haridas Rao and Shurpalekar (1976). Consistency of biscuit doughs was measured as extrusion time using "Research" water absorption meter and maintaining the time at 60 ± 5 sec by

adjusting the level of water (Chandrashekar et al. 1986). Biscuits were also made using different additives, like glyceryl-mono-stearate, sodium-2-stearoyl lactylate, lecithin and potassium metabisulphite as well as differently processed rice flours. Physical characteristics such as spread, thickness and weight were measured for 5 biscuits and the average values are reported. Breaking strength of biscuits which reflects the texture (Greethead 1969) was measured in Instron Universal tester under the following conditions : cross head speed-200 mm/min, chart speed 500 mm/min and load of 5 kg. Various quality parameters like colour, crispness, taste etc. were evaluated by a panel of semi-trained judges.

Results and Discussion

Flour used had the following quality characteristics expressed on 14% moisture basis: moisture 12.69%, dry gluten 9.24%, sedimentation value 20 ml, colour grade value 3.1. Low values of gluten, sedimentation value and colour grade value indicated its suitability for biscuit making (Yamazaki and Lord 1971).

Effect of level and type of fat : The water requirement of dough increased with reduction in the level of fat (Table 1). The increase is about 1% for every 2.5% reduction. Bakery shortening and hydrogenated fat at any level required nearly same amount of water to make the dough of optimum consistency. However, when oil was used, the requirement of water was still higher. At 20% level, dough containing hydrogenated fat, bakery shortening and refined oil required 27, 27 and 26% water, respectively. Lowering the level of bakery shortening, hydrogenated fat and refined oil reduced the spread

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TABLE 1. EFFECT OF DIFFERENT LEVELS OF SHORTENING ON BISCUIT QUALITY

Level of shorten-ing, %	Water %	Spread W, cm	Thick-ness T, cm	Surface chara-cteristics scale of 10	Colour, scale of 10	Crispness, scale of 20	Taste and mouthfeel, scale of 10	Overall score, scale of 50	Breaking strength, kg
Hydrogenated fat									
20.0	27	5.40	0.50	9.5[a]	9.6[a]	19.3[a]	9.3[a]	47.7	-
12.5	30	5.36	0.54	8.9[b]	7.9[b]	16.7[b]	7.9[bc]	41.4	-
10.0	31	5.30	0.58	6.9[de]	6.4[c]	13.1[e]	6.7[d]	33.1	-
7.5	32	5.16	0.62	5.9[f]	5.5[d]	11.9[f]	5.9[ef]	29.2	1.35
Bakery shortening									
20.0	27	5.50	0.50	9.6[a]	9.8[a]	19.6[a]	9.5[a]	48.5	0.90
12.5	30	5.35	0.53	9.0[b]	8.2[b]	17.2[b]	8.1[b]	42.5	1.09
10.0	31	5.30	0.57	7.1[d]	6.6[c]	13.8[d]	6.8[d]	34.3	1.10
7.5	32	5.15	0.61	6.2[fg]	5.5[d]	12.2[f]	6.1[e]	30.0	1.30
Refined oil									
20.0	26	5.20	0.58	9.2[ab]	9.6[a]	19.1[a]	9.0[a]	46.9	-
12.5	29	5.10	0.60	7.6[c]	8.0[b]	16.0[c]	7.5[c]	39.1	-
10.0	30	5.08	0.62	6.5[cf]	6.3[c]	12.1[f]	6.1[e]	31.0	-
7.5	31	5.04	0.64	5.2[h]	5.2[d]	10.7[g]	5.4[f]	26.5	1.48
SEm (df = 52)				± 0.11	±0.13	± 0.12	± 0.11		

Means of the same column followed by different superscripts differ significantly $(p<0.05)$ according to Duncan's Multiple Range test.

of biscuits (Table 1). With reduction in fat levels, the raise of biscuits gradually increased and the spread decreased with the use of all types of fats used, while the effect was minimum with bakery shortening. Spread ratio recorded a gradual fall with reduction in fat level. Reduction in all the types of fats studied affected the crust colour which changed from golden brown to undesirable whitish colour. Surface characteristics were also affected on reduction of fat.

In agreement with the observations of Greethead (1969), reduction of fat had most significant effect over crispness of biscuit, as it became hard, irrespective of the type of fat used. The observation made by subjective evaluation in this regard, was well supported by objective measurements of the breaking strength. Biscuits made with hydrogenated fat or bakery shortening, at 12.5% level, were crisp and acceptable, while those containing lower levels yielded harder biscuits. Breaking strength for the normal biscuit containing 20% shortening was 0.9 kg and it increased by 14.4% when the shortening was lowered from 20.0 to 12.5%. Further reduction by just 2.5% increased the breaking strength by 21.4%. Burt and Thacker (1981) have also reported 37% increase in hardness, when shortening in lincoln biscuits was reduced by 30%. Type of shortening used, also had a bearing on the breaking strength. Biscuits containing 7.5% hydrogenated fat had a breaking strength of 1.35, while that made with refined oil had a breaking strength of 1.48 kg.

Use of additives : Addition of different additives slightly softened the dough, as indicated by the lower extrusion time (Table 2). Hence, the water requirement to get the dough of desired consistency reduced from 32 to 31% on incorporation of various additives. Inclusion of various additives had beneficial effect on different physical characteristics of low-fat biscuits. In general, they increased the spread, and reduced the thickness of biscuits (Table 2). Maximum spread was obtained in biscuits containing lecithin or lecithin and glyceryl-mono-stearate.

Crispness of low-fat biscuits was improved considerably with additives and the most significant effect was found with lecithin as indicated by sensory as well as objective evaluation. Breaking strength of low-fat biscuit decreased from 1.3 to 1.09 kg, with inclusion of 0.5% lecithin and the same was comparable to that containing 12.5% shortening. Addition of 0.5% glyceryl-mono-stearate also improved the crispness considerably and these biscuits recorded breaking strength of 1.11 kg. Potassium metabisulphite was found to be least effective. Adding lecithin in combination with glyceryl-mono-stearate did not have any further beneficial effect.

Use of rice products : Consistency of doughs containing 5% flaked or puffed rice flour was found to be similar. The dough containing parboiled and raw rice flour had softer consistency as indicated by lower extrusion values (Table 3). Inclusion of various processed rice flours reduced the spread of low-fat biscuits. Extent of change in spread at any particular level was similar, irrespective of the type of flour used. The thickness of biscuits was not affected when 2.5% of rice flours processed differently, were added. However, at higher levels, slight change in the above parameter was observed. Addition of 2.5% of differently processed rice flours had no significant influence over the biscuit texture. However, higher level of incorporation improved the crispness of biscuit. Addition of flaked rice flour at 5% was found to be most beneficial in improving the crispness of biscuits. This was also confirmed

TABLE 2. EFFECT OF DIFFERENT ADDITIVES ON QUALITY OF LOW-FAT BISCUITS

Additives	Extrusion* time, sec	Spread, W	Thickness, T	Breaking strength, kg	Crispness sensory score of 20
Control (7.5% Marvo)	68	5.15	0.61	1.30	12.2[a]
Glyceroyl-mono-stearate (0.5%)	56	5.15	0.60	1.11	16.1[b]
Sodium stearoyl lactylate (0.5%)	62	5.14	0.62	1.21	13.7[d]
Potassium meta bisulphite (0.05%)	42	5.20	0.62	1.22	14.2[d]
Lecithin (0.5%)	43	5.24	0.59	1.09	16.9[c]
SSL (0.25%) + GMS (0.25%)	60	5.20	0.62	1.17	14.0[d]
Lecithin (0.25%) + GMS (0.25%)	55	5.24	0.62	1.22	16.5[bc]

Means of the same column followed by different superscripts differ significantly (p<0.05) according to Duncan's Multiple Range test. SEm (df = 28) for crispness sensory score = ± 0.18. *Water = 37%

TABLE 3. EFFECT OF DIFFERENT RICE PRODUCTS ON QUALITY OF LOW-FAT BISCUITS

Level of incorporation of fat, %	Extrusion time, %	Spread W, cm	Thickness T, cm	Surface character, scale of 10	Colour, scale of 10	Crispness sensory score, scale of 20	Taste and mouthfeel, scale of 10	Total score, scale of 50	Breaking strength kg
Control									
7.5	68	5.15	0.61	6.2[a]	5.5[a]	12.2[a]	5.9[a]	29.8	1.30
Flaked rice flour									
2.5	60	5.10	0.61	7.1[d]	6.5[e]	14.2[e]	7.1[de]	34.5	-
5.0	65	5.08	0.58	7.3[e]	6.7[e]	16.8[h]	7.4[e]	37.4	1.00
7.5	69	5.08	0.58	6.8[bcd]	5.7[ab]	13.1[c]	6.1[ab]	31.7	-
Puffed rice flour									
2.5	60	5.12	0.62	7.0[cde]	6.4[de]	13.5[d]	6.9[d]	33.8	-
5.0	69	5.12	0.58	7.1[de]	6.5[e]	15.3[g]	7.2[de]	36.1	1.16
7.5	80	5.06	0.58	6.7[bcd]	5.8[abc]	12.6[b]	6.0[a]	31.1	-
Rice flour									
2.5	47	5.13	0.59	6.9[bcde]	6.5[e]	12.9[bc]	6.0[a]	32.3	-
5.0	47	5.09	0.62	7.0[cde]	6.1[cd]	15.2[g]	6.4[bc]	34.7	1.09
7.5	49	5.09	0.62	6.5[ab]	5.7[ab]	12.0[a]	5.8[a]	30.0	-
Parboiled rice flour									
2.5	41	5.13	0.64	6.6[bc]	5.8[abc]	12.7[bc]	6.2[abc]	31.3	-
5.0	42	5.11	0.60	7.0[cde]	6.0[bc]	14.6[f]	6.5[c]	34.1	1.18
7.5	58	5.09	0.60	6.5[ab]	6.0[bc]	10.9[i]	6.0[a]	29.4	-
SEm (df = 52)				± 0.12	± 0.11	± 0.13	± 0.12		

Means of the same column followed by different superscripts differ significantly (p<0.05) according to Duncan's Multiple Range test.

by the breaking strength which decreased from 1.3 to 1.0 kg. Incorporation of 7.5% of various rice flours, yielded slightly harder biscuits, which were fragile in nature.

The studies suggest the possibility of reducing the level of fat in short dough biscuits. The quality of such biscuits could be improved by incorporating either 0.5% lecithin/glyceryl monostearate or 5% flaked rice flour.

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Functional Properties of Two Pollutant Grown Green Algae

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Scenedesmus obliquus and *Klebsormidium flaccidum* (filamentous) were predominant algae collected from polluted areas. Studies on the functional properties of their proteins showed that the isoelectric point of these proteins was in acidic range. Their solubility decreased in sodium chloride and ammonium sulphate solutions, while relative viscosity decreased in the presence of urea and sodium lauryl sulphate. Foaming capacity and emulsifying activity were minimum, while emulsion stability was maximum around isoelectric point. The viscosity, foaming and emulsification properties of *Scenedesmus* protein were more than *Klebsormidium* protein at equal protein concentration (0.4%).

Keywords : Green algae-pollutant grown, *Scenedesmus obliquus*, *Klebsormidium flaccidum*, Algal protein, Functional properties, Potential applications

The yield of protein from algae is much high as compared to other agricultural crops (Venkataraman 1991). Algae form the major flora of polluted fresh water bodies in rural and urban areas, but our knowledge regarding these algae is lagging far behind. Therefore, the present studies were undertaken to focus on some of the functional properties (solubility, viscosity, foaming and emulsification) of proteins of two algae collected from polluted areas, with a view to find their suitability for incorporating in food formulations and for extending traditional foods.

Materials and Methods

Scenedesmus obliquus, isolated from the Satluj river at Nangal (India) and *Klebsormidium flaccidum* (Chlorophyta), collected from Rock Garden, Chandigarh, were mass cultured in BG 11 medium (Stanier et al. 1971) and the algal mass was sun-dried. Algal powder (10%) was homogenized in distilled water (pH 6.8) and centrifuged at 4000 rpm for 10 min at 4°C. Proteins were precipitated from the supernatant with 70-75% solid ammonium sulphate, centrifuged at 10,000 rpm at 4°C for 15 min and dialysed against distilled water for 48 h. Clear extract containing soluble protein was taken for various estimations.

Isoelectric point was determined by flocculation and nitrogen-solubility method. The flocculation was performed at different pH values with 0.2% sodium lauryl sulphate. The nitrogen content at different pH values was estimated by micro-Kjeldahl method (Mckenzie and Wallace 1954). The highest pH value above which flocculation ceased and the pH value where solubility of protein was minimum were taken as isoelectric points. Effect of pH and temperature on the protein solubility was studied by keeping the protein solution at these specific

conditions for 30 min. To study the effect of salts, known concentration of protein solution (0.4%) was treated with different concentrations (0.1 M-1.0 M) of potassium iodide, sodium chloride, ammonium sulphate, sodium citrate and ammonium nitrate solutions. The supernatant, after centrifugation was tested for protein content (Lowry et al 1951).

Viscosity measurement : Time of flow of protein solution (0.4%) was determined using Oswald's viscometer. Density was measured with the help of bicapillary pyknometer and was calculated by the following formula.

$$D = \frac{\text{Weight of protein solution}}{\text{Weight of distilled water}} \times \text{Density of distilled water}$$

Relative viscosity was calculated by the equation:

$$\eta_{rel} = \frac{\eta}{\eta_0} = \frac{t}{t_0} \cdot \frac{d}{d_0}$$

Where η, t, d are coefficients of viscosity, time of flow and density of protein solution respectively. η_0, t_0, d_0 are corresponding values of pure solvent.

Effect of urea and sodium lauryl sulphate (0.1 M-0.5 M) on viscosity was studied by treating the protein solution (0.4%) with these denaturating agents for 1 h and time of flow of each solution was noted. Foam capacity (FC) and foam stability (FS) were measured (Lawhon et al. 1972; Ahmed and Schmidt 1979). Emulsifying activity (EA) and emulsion stability (ES) were determined according to the procedure employed by Yasumatsu et al. (1972).

Results and Discussion

The isoelectric points of *Scenedesmus* and *Klebsormidium* proteins were around 3.6 and 4.0 respectively, as found by flocculation and nitrogen solubility method. These values are comparable to those for algal proteins (Devi and Venkataraman

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TABLE 1. EFFECT OF pH, PROTEIN CONCENTRATION, SODIUM LAURYL SULPHATE AND UREA ON RELATIVE VISCOSITY OF *SCENEDESMUS* AND *KLEBSORMIDIUM* PROTEIN.

Attribute	Relative viscosity	
	Scenedesmus	*Klebsormidium*
pH Value		
2	1.24	1.27
4	1.15	1.13
6	1.18	1.23
8	1.20	1.24
10	1.30	1.32
12	1.35	1.41
Protein concentration, %		
0.1	1.16	1.08
0.2	1.26	1.15
0.3	1.28	1.17
0.4	1.36	1.27
0.5	1.40	1.32
Sodium lauryl sulphate (M)		
0	1.36	1.27
0.1	1.03	1.02
0.2	1.10	1.08
0.3	1.18	1.13
0.4	1.22	1.14
0.5	1.28	1.20
Urea (M)		
0	1.36	1.27
1.0	1.22	1.18
2.0	1.20	1.16
3.0	1.15	1.16
4.0	1.13	1.13
5.0	1.11	1.10

All results are mean of three replicates

1984) and some of the oilseed proteins (Blaicher et al. 1983; Zhou et al. 1990). Since the isoelectric points are not the same, the binding properties of different proteins in a given system will also be different. Data of protein solubility as a function of pH indicated that solubility of protein from both the algae was high at extreme pH values and least at pH 4 (Fig. 1a, b). At this pH, which is near the isoelectric point, there is no net charge on protein molecules. Therefore, solubility is minimum at this pH. The protein-solvent interaction favours solubility at extreme pH values. The solubility of both the proteins increased at 35°C and 80°C and decreased with increasing concentrations of sodium chloride, ammonium nitrate, ammonium sulphate and sodium citrate (Fig. 1), and was minimum with ammonium sulphate. The lower solubility with these salts may be due to salting-out effect at these concentrations.
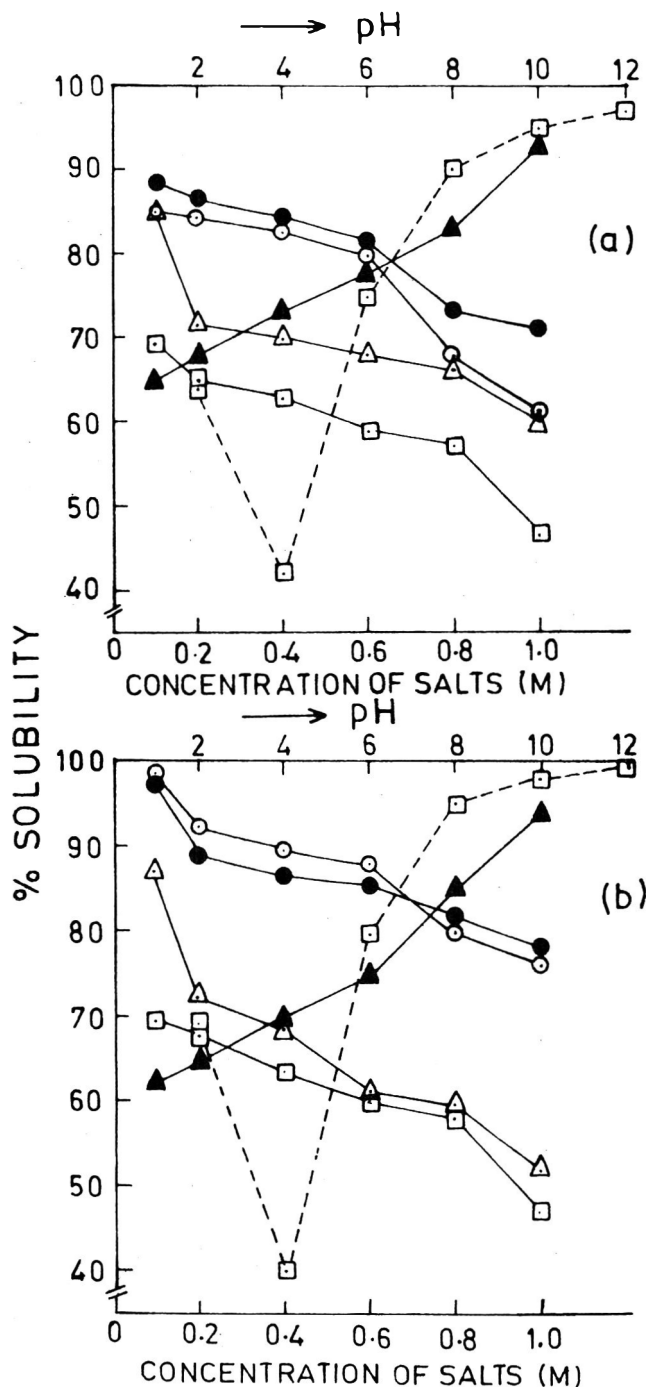
Fig. 1. Effect of different anions : (— ●—●— sodium chloride, —O—O— ammonium nitrate, —△—△— sodium citrate, —▲—▲— potassium iodide, —□—□— ammonium sulphate and pH --□-□ ---) on the solubility of (a) *Scenedesmus* (b) *Klebsormidium* proteins.

However, solubility of both the proteins increased with the addition of different concentrations of potassium iodide, probably due to salting-in effect. These algal proteins differ from legume proteins where solubility increases in the presence of sodium chloride (Shen 1976).

Relative viscosity of both the proteins was high

TABLE 2. EFFECT OF pH, AND SODIUM CHLORIDE ON FOAM CAPACITY, FOAM STABILITY, EMULSIFYING ACTIVITY AND EMULSION STABILITY OF *SCENEDESMUS* AND *KLEBSORMIDIUM* PROTEIN.

	Foam capacity		Foam stability at 120 min		Emulsifying activity		Emulsion stability	
pH	Scenedesmus	Klebsormidium	Scenedesmus	Klebsormidium	Scenedesmus	Klebsormidium	Scenedesmus	Klebsormidium
2	90.0	75.0	31.5	28.3	48	46	40	33.6
4	60.0	50.0	25.0	Foam collapsed	46	45	61	59.1
6	80.0	65.0	30.0	25.0	54	50	54	57.5
8	125.0	100.0	44.4	32.5	57	52	56	52.0
10	160.0	140.0	52.0	41.6	60	58	58	54.0
Sodium Chloride M								
0.1	110.0	110.0	40.0	25.5	55.0	50.5	47.0	48.5
0.4	180.0	125.0	30.0	35.0	51.0	45.9	49.0	52.0
0.6	112.0	108.0	25.0	22.1	53.0	47.0	48.0	50.0
0.8	110.0	100.0	23.0	20.0	54.0	48.9	47.5	51.0
1.0	98.0	90.0	Foam collapsed	Foam collapsed	54.5	47.7	47.8	50.5

at pH 2 and 12 and minimum at pH 4.0 (Table 1). It increased slightly with increase in concentration of protein and sodium lauryl sulphate; but decreased with the use of urea. Lower viscosity in the presence of urea and sodium lauryl sulphate, in relation to control, indicated a gradual change due to unfolding or swelling of protein molecules. Only few studies are reported on polluted or fresh water algae (Venkataraman 1980), although oilseed proteins are widely characterised for these properties (Mahajan 1990; Junichiro et al. 1989).

Foam capacity (FC) of both the proteins was high at extreme acidic and alkaline pH and minimum at pH 4 (Table 2). Foam stability was minimum at pH 4 and foam from *Scenedesmus* protein was more stable than that from *Klebsormidium*. The EA *vs* pH pattern showed least emulsification at pH 4 and it resembled solubility *vs* pH profile, indicating direct correlation between nitrogen solubility and EA. Similar types of changes have been observed with soy and rapeseed proteins. ES showed maximum value at isoelectric point in both the proteins (Table 2). In sodium chloride solution, FC increased upto 0.4 M concentraton and decreased thereafter, showing a minimum value at 1.0 M concentration. *Scenedesmus* protein showed high FC values. Salts probably affect foaming by enhancing solubility at lower concentrations and *vice versa* (Kinsella 1976). The foam collapsed after 2 h in both the proteins, on addition of 1 M sodium chloride. EA was reduced to 51% and 45.1% in *Scenedesmus* and

Klebsormidium proteins, respectively, upto 0.4 M concentration of sodium chloride (Table 2), while ES was maximum at this salt concentration. The beneficial effect of low concentration of salt has been reported by several workers (Volkert and Klein 1979; Dev and Mukherjee 1986) and this possibly is due to the salting-in effect of NaCl.

No significant differences exist in various functional properties of these two pollutant grown algae and some of the fresh water algae reported by Devi and Venkataraman (1984). Although no toxicological properties are studied in the present investigation, these two algal proteins have the potential to find use in meat, ice cream, bakery and other food products due to comparatively good foaming and emulsification properties. The increase in the solubility of these proteins with increase in temperature is a useful criterion for acceptance in food. The relatively high solubility of these proteins in the acidic range may present opportunities for use in carbonated beverages.

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Respiration and Ethylene Evolution of Certain Fruits and Vegetables in Response to Carbon dioxide in Controlled Atmosphere Storage

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Respiration was depressed by 10-30% CO_2 in ripening bananas, pink tomatoes and pickling cucumbers; increased by 20-30% in carrot roots and unaffected by CO_2 exposure in guava, orange and onion bulb. Changes in respiration seldom coincided with changes in C_2H_4 evolution. Evolution of C_2H_4 from guavas and tomatoes was substantially reduced by all levels of CO_2. However, 30% CO_2, accelerated C_2H_4 evolution in bananas, carrot roots, cucumbers, onions and potatoes which may have been due to an early injury response.

Keywords : Fruits, Vegetables, Respiration, Ethylene evolution, Elevated CO_2 atmosphere, Short time exposure to elevated CO_2.

Atmosphere enriched with CO_2 is generally assumed to reduce respiration of harvested fruits and vegetables (Kader 1986, 1987; Wills et al. 1989). This generalised concept has primarily evolved from results of studies that examined the combined influence of elevated CO_2 and reduced O_2, which may not have been solely reflected the independent action of CO_2 on respiration. The influence of CO_2 on ethylene evolution is also reported to vary among different commodities (Kader et al. 1989; Groschel et al. 1966). However, only a few studies have examined the effects of elevated CO_2 on ethylene evolution in non-limiting O_2 atmospheres. The present study was conducted to examine short-term responses of elevated CO_2 on respiration and ethylene production by certain fruits and vegetables in a non-restrictive level of O_2 (20 ± 2%).

Materials and Methods

Source and preparation : Bananas, guavas (Californian Feijoas), yellow onion bulbs, naval oranges, russet potatoes, pink tomatoes and pickling cucumbers were selected on the basis of uniform size, shape, colour and free from defects. Individual fruits or vegetables were used for exposure to air or elevated CO_2 atmosphere. Each commodity was separated into uniform lots consisting of 8-10 fruits or vegetables, and each lot was weighed into air-tight containers equipped with inlet and outlet ports. Guavas, cucumbers or the other commodities were held in 0.95, 8.0 or 3.8 *l* containers, respectively. Three containers (replicates) of each commodity were prepared for exposure to the atmosphere treatments.

Treatment and assay : Atmospheres consisting of air, 10, 20 or 30% CO_2 with 20 ± 2% O_2 and N_2 balance were created and delivered through the containers held at 14 ± 1°C by the system described by Shaw and Kattan (1971). The containers were continuously purged with air or CO_2 enriched atmospheres at a flow rate of 3.6 *l*/h for at least 24 h until a stabilized desirable atmospheric composition was achieved. Subsequently, the container ports were sealed and initial as well as timed interval samples of the air space were assayed for O_2 and ethylene contents. The amount of time required to measure the rates of O_2 depletion and ethylene accumulation was dependent on the commodity. A minimum amount of time in the sealed container (0.5 to 4 h) was used to determine the rates of changes in O_2 and ethylene to avoid responses caused by the changing atmospheres.

Gas samples were obtained with N_2 flushed syringes. Oxygen consumption (respiration) was assayed by Fisher-Hamilton gas partitioner equipped with a thermal conductivity detector, molecular sieve (13x), using DEHS columns and spectra Physics 4290 integrator. Ethylene evolution was assayed by a Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector, chromosorb 101 80/120 mesh-packed column and HP integrator. Injector, column and detector temperatures were 150, 100 and 200°C, respectively. The pH of the commodities was measured using a pH meter. Surface colour was determined by a Gardener XL-10 Colour Difference

Meter (CDM) standardized with red coloured (L=26.0; a = 28.0; and b = 13.3) plate for tomatoes and white plate (L=92.87; a=-0.84; b=-0.56) for bananas.

Statistical Analysis : The data represent the mean of three replicates per treatment with each replicate consisting of 10 fruits or vegetables. Data were statistically evaluated by analysis of variance, and the means were separated by Duncan's multiple range test procedure of the Statistical Analysis System (SAS 985) programme.

Results and Discussion

Data on the effect of short-time exposure of fruits and vegetables to CO_2 on the pH and colour values are presented in Table 1. Large differences in responses to elevated CO_2 atmospheres on

TABLE 1.   EFFECT OF SHORT-TIME EXPOSURE TO CO_2 ON pH AND COLOUR OF FRUITS AND VEGETABLES

Fruits/ Vegetables	Ambient	10% CO_2	20% CO_2	30% CO_2
Cucumber	5.4[c]	5.8[b]	6.0[a]	6.2[a]
Onion	5.5[b]	5.6[a]	5.7[a]	5.7[a]
Potato	5.8[c]	5.8[b]	5.8[b]	6.0[a]
Tomato	4.2[b]	4.4[a]	4.4[a]	4.4[a]
Banana	4.9[b]	5.0[ab]	5.0[a]	5.0[ab]
Orange	3.3[a]	3.4[a]	3.3[a]	3.1[a]
Guava	3.1[a]	3.3[a]	3.1[a]	3.1[a]
Colour				
Tomato (a-value)	23.2[a]	17.2[b]	17.6[b]	14.6[b]
Banana (b-value)	34.8[a]	34.5[ab]	33.6[b]	33.6[b]

Means in each row with different superscripts differ significantly.

respiration and C_2H_4 evolution were observed between the different commodities. Respiration was reduced in ripening bananas, tomatoes and pickling cucumbers by CO_2 (Fig. 1 and 2). Concentrations of 10 to 30% CO_2 were similarly effective in reducing the respiration of these three commodities. Respiration of cucumbers, bananas and tomatoes was reduced by about 25, 30 and 45%, respectively, in response to the short-term exposure to the CO_2-enriched atmospheres. Reduced C_2H_4 evolution from ripening tomatoes in CO_2 enriched atmospheres was also reported earlier (Buescher 1979). The lowest level of CO_2 (10%) also reduced C_2H_4 production in cucumbers. However, cucumbers exposed to 30% CO_2 and bananas exposed to 20 or 30% CO_2 experienced increased C_2H_4 production.

Respiration of guavas, onion bulbs and oranges was unaffected by elevated CO_2 (Fig. 2, 3, 4). However, C_2H_4 evolution from guava was substantially reduced by increasing concentrations

of CO_2 (Fig 2). In contrast, C_2H_4 evolution from oranges was slightly reduced by 20 and 30% CO_2 (Fig. 4), while these concentrations of CO_2 caused a significant increase in C_2H_4 production by onions (Fig. 3).
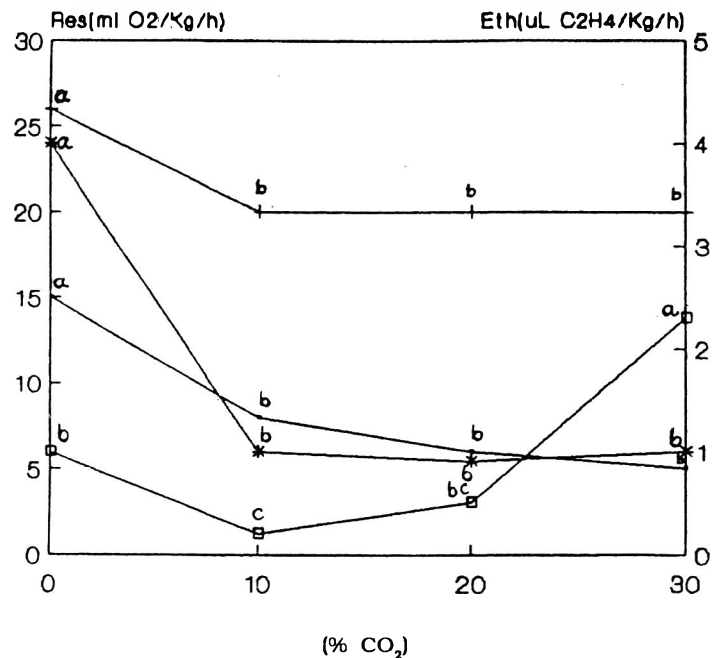

Fig. 1.   Respiration and ethylene evolution of tomato and cucumber.

——— Res. tomato   —+— Res. cucumber

—*— Eth. tomato   —☐— Eth. cucumber

Res - Respiration, Eth - ethylene
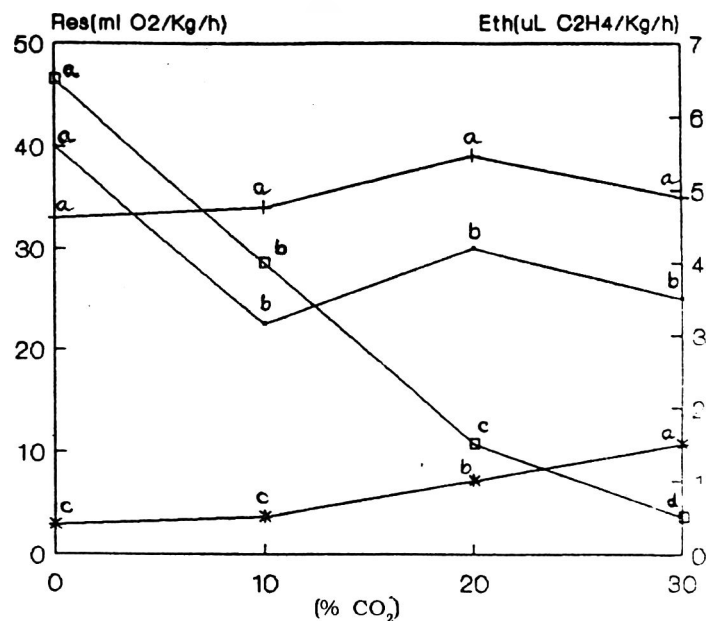

Fig. 2.   Respiration and ethylene evolution of banana and guava

——— Res. banana   —+— Res. guava

—*— Eth. banana   —☐— Eth. guava

Res - Respiration, Eth - ethylene

Although 10% CO_2 had no influence on respiration or C_2H_4 evolution by potatoes, respiration was accelerated by 20 and 30% CO_2 , and C_2H_4 production was enhanced by 30% CO_2 (Fig. 3). Respiration of carrots exhibited a different pattern in response to CO_2 than did the other commodities (Fig. 4). Exposure to 10% CO_2 reduced respiration,
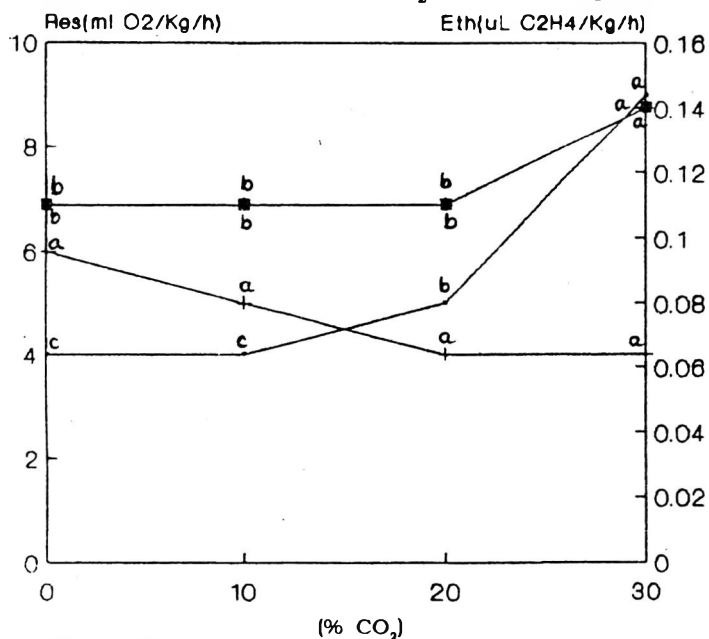

Fig. 3. Respiration and ethylene evolution of potato and onion

———·— Res. potato   —+— Res. onion
—*— Eth. potato   —■— Eth. onion

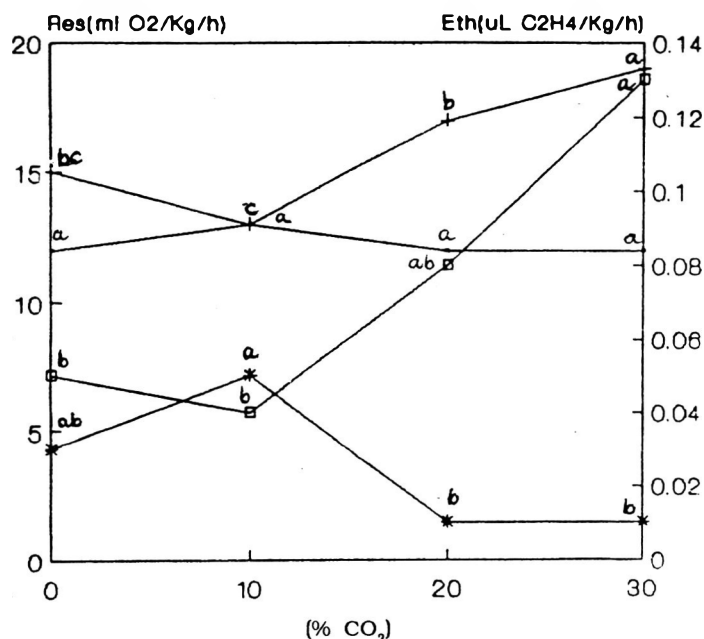Res - Respiration, eth - ethylene

Fig. 4. Respiration and ethylene evolution of orange and carrot

———·— Res. orange   —+— Res. carrot
—*— Eth. orange   —□— Eth. carrot

Res - Respiration, Eth - ethylene

and 30% CO_2 enhanced it. Also, the 30% CO_2 treatment increased C_2H_4 evolution from carrot roots.

These results revealed that respiration and C_2H_4 evolution may be decreased or increased or unaffected by CO_2 depending on the fruit/vegetable and CO_2 concentration. Further, changes in respiration induced by CO_2 were not necessarily accompanied by parallel changes in C_2H_4 production. Only ripening tomatoes provided the expected results by having both respiration and C_2H_4 evolution suppressed by CO_2. However, in ripening bananas and cucumbers, 30% CO_2 maintained a suppression of respiration, and ethylene production increased. The response of guavas to CO_2 was particularly surprising since large reductions in C_2H_4 evolution occurred and respiration was unaffected.

Certain fruits and vegetables are injured by exposure to elevated CO_2 atmospheres (Lougheed 1987, Bufler 1984). Although physical injury symptoms were not visually observed in any of the commodities treated by CO_2 in this study, the detection of accelerated C_2H_4 production in bananas, carrots, cucumbers, onions and potatoes caused by exposure to 30% CO_2 may have been due to an early response to physiological injury. Bufler (1984) reported that C_2H_4-induced ACC (1-aminocyclopropane-1-Carboxylic acid) synthase in apples was suppressed by CO_2. Since C_2H_4 from non-wounded and wounded plant tissues appears to originate through a common pathway involving ACC synthase (Yu and Yang 1980), CO_2 should have suppressed C_2H_4 production even though it caused injury. Assuming that increased C_2H_4 production caused by CO_2 was an injury response, then our data support previous observations that CO_2 does not suppress wound-induced C_2H_4 production (Buescher 1979).

In summary, relatively short time exposure of fruits and vegetables to elevated CO_2 (10-30%) and 20 ± 2% O_2 atmospheres reduced respiration and/ or ethylene evolution of some, but not all the fruits and vegetables.

Acknowledgement

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Biochemical Effects of Diallyl Disulphide in Ethanol Fed Rats

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Rats fed with ethanol and high lipid diet (HLD) exhibited higher levels of triacylglycerols, total cholesterol and total lipids in the serum and liver, as well as lower serum albumin and higher levels of α and γ globulins. When different doses of diallyl disulphide (DADS) were fed along with ethanol to HLD fed rats, all the above lipid levels were reduced. The optimum dose of DADS for producing the hypolipidemic effects appears to be 0.66 mg/100 g body weight, when there was also an improvement in the serum albumin and α globulin levels.

Keywords : Ethanol-fed rats, Diallyl disulphide, High lipid diet, Triacylglycerols, Total cholesterol, Hypolipidemic effect.

Increasing consumption of ethanol and the consequent incidence of liver disease is viewed with great concern in all parts of the world. Efforts to relieve fatty liver in alcoholics usually fail because of continuing alcohol abuse. Alcoholic fatty liver could not be prevented by a high protein diet, lipotropic factors like choline or methionine, anti-oxidants or drugs like chlofibrate or anaebolic steroids (Lieber 1973, Lieber et al. 1965, Lieber and Rubin 1968). It was reported in a preliminary communication that garlic oil could reduce the triacylglycerols and total cholesterol in ethanol-fed rats (Bobboi et al. 1984, Shoetan 1984). Garlic oil chiefly contains diallyl disulphide and smaller quantities of diallyl trisulphide as well as diallyl polysulphides (Raghavan et al. 1982). The present investigation was undertaken to study the biochemical effects of diallyl disulphide, the active principle of garlic oil.

Materials and Methods

Male albino rats, 6-8 months old and weighing 200-250 g, were divided into eight groups of six each. Rats of groups 1-7 were fed *ad libitum*, a high lipid diet (HLD) containing 20% whole milk powder, 50% whole wheat flour, 30% hydrogenated vegetable oil, 0.12 mg% thiamine, 0.2 mg % riboflavin and 0.3 mg% niacin. This diet had a calorific value of 5.7 Cals/g. Control rats of group 1 consumed an average of 6-7 g of this diet per day. Group 2 rats were fed intragastrically, 3 ml of 30% ethanol (v/v) per 100 g body weight daily, which correspond to the consumption of 40% of the calorie intake of a normal rat. This may correspond to the consumption of about 400 ml of whisky or rum by an average adult per day. Rats of groups 3-6 were fed the same dose of ethanol mixed with

various doses (0.66 mg, 1.0 mg, 1.5 mg and 2.0 mg/100 g body weight, respectively) of diallyl disulphide (DADS, Fluka).

Control rats of group 7 were fed daily with 3 ml of 52% (w/v) glucose solution, which is isocaloric with the above dose of ethanol, but without any DADS. Group 8 rats were fed *ad libitum* stock laboratory diet (Gold Mohur).

After 30 days, all the rats were sacrificed by decapitation, 4 after the last dose of ethanol or isocaloric glucose feeding. Lipids in the liver were extracted using chloroform : methanol (1:1 v/v) mixture (Enteman 1957), Triacylglycerols (Wybenga et al. 1974), total cholesterol (Abell et al. 1958) and total lipids (Chowdhary 1989) were estimated both in the serum and in the extracts of liver. Serum proteins were separated electrophoretically on Whatman No 3 filter paper (Varley 1975) and each fraction was extracted and estimated colorimetrically.

About 5 μ thick sections were cut from the liver and stained with hematoxylin and eosin. To determine the interference of DADS with the intestinal absorption of ethanol, one group of rats was fasted for 8 h and fed intragastrically, 2 ml of 30% (v/v) ethanol/100 g body weight. Another group of rats was fed similarly with the same dose of ethanol mixed with 1 mg DADS/100 g body weight. Blood was collected by transretinal bleeding at 30, 60, 120, 150, 180 and 240 min and ethanol level was estimated (Bonnichsen 1963). Statistical analysis of results was carried out according to students 't' test (Ramachandran 1962).

Results and Discussion

Group 1 rats which were fed with HLD and group 2 rats fed with HLD and ethanol, exhibited significantly (p<0.01) higher levels of triacylglycerols, total cholesterol and total lipids in the serum and

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liver (Table 1) as compared to control rats of group 7. Alcohol fed rats of group 2 had serum and liver groups 3 to 6, all the above lipid levels were significantly reduced (p<0.01). Serum albumin levels

TABLE 1. BIOCHEMICAL EFFECTS OF DIALLYL DISULPHIDE (DADS) IN RATS FED WITH ETHANOL AND HIGH LIPID DIET (HLD)

	HLD-fed rats	HLD, and ethanol-fed rats	HLD, ethanol and 0.66 mg DADS-fed rats	HLD, ethanol and 1 mg DADS-fed rats	HLD, ethanol and 1.5 mg DADS-fed rats	HLD, ethanol and 2 mg DADS-fed rats	HLD and isocaloric glucose-fed rats
Serum triacylglycerols, mg/dl	148 ± 22	176 ± 26	86 ± 5.8*	74 ± 7.0*	75 ± 8.0*	56 ± 7.0 *	46 ± 5.2*
Liver triacylglycerols, mg/g	19.1 ± 3.2	23 ± 2.9	14.2 ± 0.9*	13.2 ± 1.1*	14.3 ± 1.3*	12.1 ± 1.6*	12.4 ± 0.9
Serum total cholesterol, mg/dl	152 ± 7.3	186 ± 8.7	86 ± 9.1*	81 ± 7.2*	82 ± 5.2*	76 ± 3.5*	76 ± 3.6
Liver total cholesterol, mg/g	26 ± 2.1	32 ± 2.1	9.6 ± 0.7*	12 ± 1.1*	13 ± 1.2*	10 ± 0.9*	10.5 ± 0.9
Serum total lipids, mg/dl	562 ± 33	667 ± 32	412 ± 23	440 ± 27*	416 ± 23*	396 ± 19*	300 ± 10
Liver total lipids, mg/g	670 ± 90	760 ± 95	422 ± 42*	410 ± 40*	396 ± 52*	352 ± 26*	480 ± 10
Serum albumin, g/l	46 ± 3.2	32 ± 2.8	38 ± 2.9*	33 ± 2.6*	28 ± 2.3	27 ± 2.5	52 ± 3.6
Serum α-globulins, %	19.8 ± 2.4	31.9 ± 1.9	25.3 ± 1.9*	23.7 ± 1.5*	32 ± 2.0	23.8 ± 1.5*	17.8 ± 1.3
Serum β-globulins, %	16.8 ± 2.3	13.1 ± 1.7	15.8 ± 1.9	21.2 ± 1.9	20 ± 1.8*	22.7 ± 2.0*	13.6 ± 1.1
Serum γ-globulins, %	17.7 ± 2.3	23 ± 2.5	20.6 ± 2.1	22.1 ± 2.1	20 ± 1.9	26.6 ± 2.2	16.6 ± 1.2

* p<0.01 compared to the rats fed with ethanol and HLD. All results are expressed as Mean ± SD. Number of animals in each group is 6.

cholesterol levels significantly (p<0.05) higher than those of HLD fed rats of group 1, but not triacylglycerol levels (p<0.05). When DADS was mixed with ethanol and fed to HLD-fed rats of

were significantly (p<0.01) decreased in alcohol fed rats of group 2. Group 3 rats which were fed with 0.66 mg DADS/100 g body weight, showed significantly higher serum albumin levels as
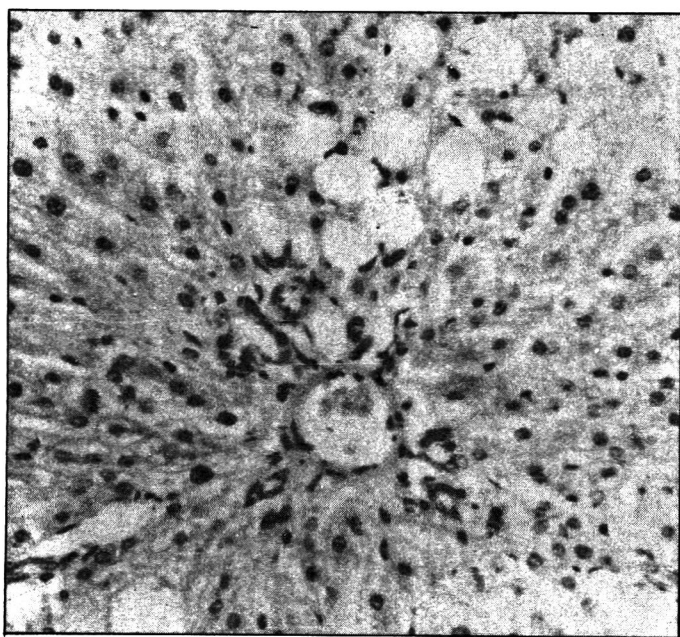
Fig. 1. Photomicrograph showing a portal tract with macrovasicular fatty change in the peripheral hepatocytes of group 2 rats fed daily with 3 ml of 30% (v/v) ethanol/100 g body weight for 30 days and a high lipid diet ad libitum. (H & E x 200)
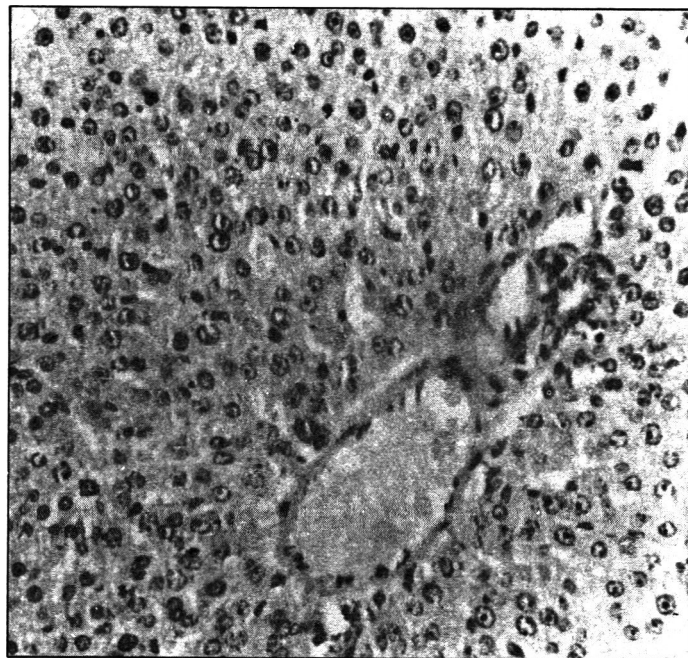
Fig. 2. Photomicrograph showing portal tract and columns of hepatocytes with no evidence of fat in the rats fed daily with 3 ml of 30% ethanol (v/v) and 0.66 mg diallyl disulphide/100g body weight for 30 days. (H & E x 200)

Fig. 3. Blood ethanol levels in rats fed with 2 ml of 30% (v/v) ethanol with or without 1 mg diallyl disulphide/ 100 g body weight.

Legend:
o—o Rats Fed 2ml. 30% (v/v) Ethanol containing 1 mg DADs per 100 g Body Wt.
●—● Rats Fed 2ml. 30% (v/v) Ethanol per 100 g Body Wt.

compared to ethanol-fed rats of group 2 (p<0.01), but higher doses of DADS did not increase the serum albumin levels. α and γ-globulins levels were significantly increased in alcohol-fed rats of group 2 as compared to group 1 rats (p<0.01), but they were reduced in rats of groups 3, 4 and 6 which were fed DADS along with ethanol. Group 8 rats, which were fed with only stock laboratory diet and no ethanol or isocaloric g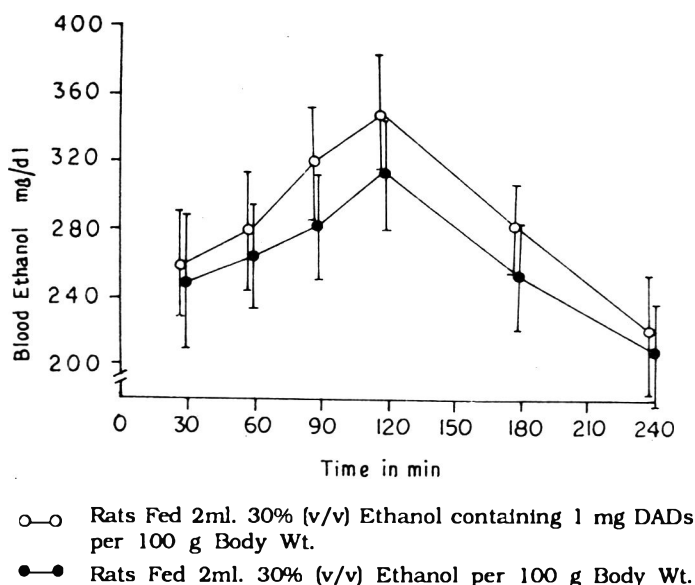lucose solution, exhibited similar results of all the above estimations similar to the control rats of group 7. Isocaloric glucose-fed rats consumed only 2-2.5 g HLD/day. Thus, the lower consumption of lipids might be responsible for the normal lipid levels in the serum and liver of these rats.

Alcohol feeding is known to increase the biosynthesis of fatty acids and cholesterol and decrease their oxidation (Lieber and Rubin 1968), thereby resulting in their accumulation in the liver. Diallyl disulphide undergoes exchange reactions with thiol groups of enzymes and proteins (Black 1962, Joselyn 1972) as follows.

$C_3H_5\text{-S-S-}C_3H_5 + X\text{-SH} \rightarrow C_3H_5\text{-S-S-X} + C_3H_5\text{-SH}$

Where X-SH represents an endogenous thiol group protein or enzyme. Diallyl disulphide and CoA disulphide are known to inhibit HMG CoA reductase reaction *in vitro*, possibly by such exchange reactions (Gilbert and Stewart 1981; Linn 1989). Thus, DADS may inhibit fatty acid and cholesterol biosynthesis, producing hypolipidemic effects. The optimum dose of DADS to be used for hypolipidemic effects in ethanol-fed rats, which were also fed with HLD, is 0.66m g/100 g body weight daily. Group 3 rats,

which were fed with HLD and ethanol showed focal macro and microvesicular fatty change (Fig. 1). Group 3-6 rats, which were fed with ethanol, HLD and DADS, showed focal necrosis, Kupfer cell prominence and lymphocyte infiltration of sinusoids (Fig 2). No evidence of fatty change was observed in the liver of these rats.

The blood levels of ethanol in rats fed with ethanol alone or ethanol mixed with DADS were not significantly different (p<0.05) till 4 h after feeding (Fig.3). Thus, DADS has no effect on ethanol absorption from the intestines.

Thus, it may be concluded that diallyl disulphide possesses hypolipidemic effects in rats fed with ethanol and high lipids diet and the optimum dose appears to be 0.66 mg/100 g body weight.

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In vitro Digestibility of Protein and Starch of Energy Food and Its Bulk Reduction

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Energy food had an apparent viscosity of 35,200 centipoise units (cpu) at 25% slurry concentration and this decreased to 260 cpu when it was blended with 10% malted wheat and cooked. Toasting of various ingredients such as wheat or maize, Bengalgram or peanut cake, did not have any marked effect on reducing apparent viscosity of energy food. *In vitro* protein and starch digestibility of energy food were 80 and 98% respectively.

Keywords : Energy food, Bulk reduction, *In vitro* digestibility of protein and starch, Viscosity profile, Malt flour, Germinated wheat.

To combat malnutrition in children, a nutritious food supplement, popularly referred to as energy food, is being produced on a large scale by government agencies and supplied to social welfare departments in India. Energy food is a ready-to-eat supplementary food for children and is convenient for use in feeding programmes due to its ease in preparation. The major ingredients of energy food are wheat or maize, Bengalgram, soya or peanut cake and jaggery, which are blended after heat treatment. The dry energy food, when mixed with water or milk prior to feeding, becomes viscous and bulky. As a result, children are unable to consume the desired quantities due to limited stomach capacity. Consequently, a daily supplement of 40 g of energy food is fed, at present, to weaned infants as a 15% slurry for providing a calorie density of 0.6 cal/g, which is far below the recommended level of 1.1 cal/g for a pre-school child aged 2-3 years (Svanberg 1987).

Addition of a small quantity of malted barley flour is known to reduce considerably the viscosity of weaning foods due to its high amylase content (Rajalakshmi 1974; Hellstrom et al. 1981). The possibility of using malted wheat for reducing the viscosity of energy food and the effect of toasting the main ingredients of energy food on the viscosity and digestibility are reported in this paper.

Materials and Methods

All the ingredients of the energy food were procured from the local energy food factory. It consisted of the blend of wheat 56%, extruded soya 8%, defatted peanut 7%, toasted Bengalgram 5%, jaggery 35%, ferrous sulphate 0.4% and calcium

carbonate 1%, along with vitamin premix (Prasannappa et al. 1976; Prasannappa and Jagannath 1985).

Wheat procured from local market was tested for its germination and two-day germinated-wheat was used to prepare malt on semi-pilot scale (Suhasini et al. 1992). Toasting adopted at energy food factory for wheat was a HTST treatment at 200 to 230°C for 1 to $1^1/_2$ min in a rotary electric heater. Bengalgram and peanut were toasted at 80 to 85°C for 20 to 25 min. The toasted products were ground to pass through a 60 mesh BSS sieve.

Analytical methods : The amylases of germinated wheat malt were extracted in acetate buffer for the assay of amylase activities (Bernfeld 1955). For apparent viscosity measurement, 20 to 30% slurry of the ingredient was suspended in cold water, cooked on a boiling water bath for 20 min, boiled over an open flame for a few minutes with continuous stirring, cooled to about 40°C and the apparent viscosity was measured in a Brookfield viscometer (RVT model) using appropriate spindles at 50 rpm.

In vitro protein digestibility was determined according to Walter and Mark (1964), with the following modifications : slurried samples containing 100 mg protein were treated with 12.5 mg of pepsin (1:2500) in 50 ml of 0.1 N HCl at 37°C for 3 h. After neutralization with 0.5 N NaOH, 6 mg of pancreatin (USP x 3) dissolved in 25 ml of phosphate buffer (pH 8.0) was added and the digestion continued for 24 h at 37°C. The volume was then made upto 100 ml and a 50 ml aliquot was treated with 10% TCA overnight to precipitate the proteins. The suspensions were centrifuged at 6000 rpm. The undigested material was assayed

for protein by micro-Kjeldahl method and protein digestibility was calculated.

For determining *in vitro* starch digestibility, the slurry of the food material (2%) was cooked on a boiling water bath for 15 min. Thirty ml of 0.2 M glycine -HCl buffer (pH 2.0) containing 10 mg of pepsin (1:2500) was added to 50 ml slurry, incubated at 37°C for 2 h, neutralized with 0.2 N NaOH and the volume was made up to 100 ml. Five ml of 0.5 M phosphate buffer containing 15 mg of pancreatin (USP x 3) and 15 mg amyloglucosidase (3000 units/g) were added to 10 ml aliquot and incubated for 2 h at 37°C. The reaction was stopped at desired intervals by heating for 5 min in boiling water bath. Aliquots of 0.5 ml of the samples were mixed with 2 ml of dinitrosalicylic acid reagent for determining reducing sugars (Ganesh Kumar and Venkataraman 1976). Glucose was used as a standard, while starch equivalent was calculated using the conversion factor of 0.9.

Results and Discussion

The viscosity profile of germinated and ungerminated wheat at different slurry concentrations and germination period indicated that the germinated samples had very low viscosity as compared to control at corresponding slurry concentrations (Fig. 1). The consistencies of slurries from germinated samples were free-flowing at 25% concentration. The amylase activities of wheat on progressive germination at 24 h intervals, expressed as mg of maltose released per g of sample, were 1280, 2682, 3652 and 3852, respectively. Enzyme activities increased rapidly on first two days and slowed down on further germination. Although the amylolytic activity of 24 h-germinated sample was high and its slurry viscosity was low, wheat malt from 2 day-germinated sample was preferred as the development of amylolytic activity is dependent on variety and even a poor malting variety on 2 day-germination, could possess sufficient activity. Germination for longer periods resulted in higher loss of dry matter and very often, the wheat suffered mould attack. Losses of 8 and 21-34% dry matter have been found to occur in wheat upon germination upto 48 and 72-96 h, respectively.

The energy food had a cooked paste apparent viscosity of 35,200 cpu at a 25% slurry concentration. Addition of malt at various levels exerted a profound influence on bulk reduction (Fig. 2). Addition of 5% malt brought down the apparent viscosity to about 1000 cpu which was further reduced to 400 cpu by increasing the malt
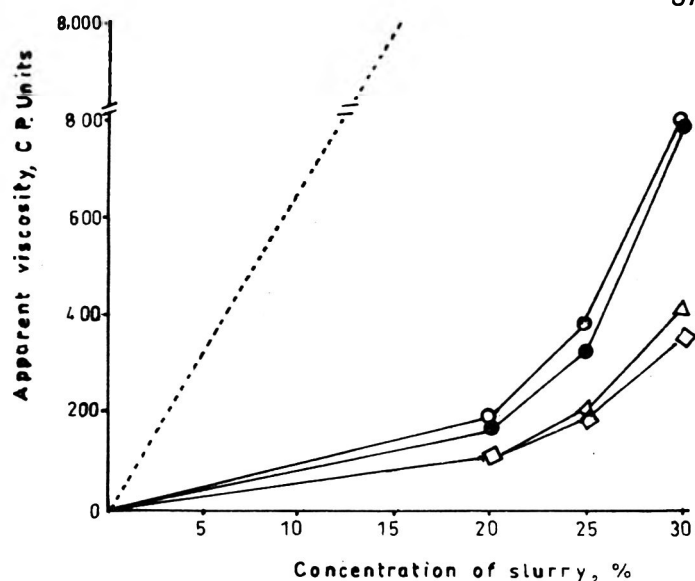

Fig. 1. Apparent viscosity profile of wheat germinated for different intervals at different concentrations of slurry. - - - - - ungerminated wheat; O—O wheat germinated for 24 h; ●——● wheat germinated for 48 h; ☐——☐ wheat germinated for 72 h; Δ——Δ wheat germinated for 96 h.

to 7.5%. However, increasing the malt to 10% had very minor effect on viscosity.

The effect of addition of malt flour on bulk reduction properties of the weaning food prior to cooking and after cooking showed that the addition of 10% malt prior to cooking and heating the slurry reduced the viscosity from 35,200 to 260 cpu at

TABLE 1. APPARENT VISCOSITY PROFILE OF ENERGY FOOD WITH ADDITION OF MALT BEFORE AND DURING COOKING.

Food material	Apparent viscosity (cp units) at slurry concentration [*]		
	25%	30%	40%
Energy food	35,200	70,400	80,000
Energy food with 10% wheat malt prior to cooking	260	750	4,000
Energy food with 10% wheat malt added after cooking	780	1,400	6,400

[*] Values are averages of duplicate determinations.

25% slurry concentration (Table 1). During cooking, enzyme activity is accelerated in the initial period upto about 55°C and culminated into a higher hydrolysis of starch. However, when the malt flour is added to the hot paste slurry (above 80°C), the enzyme activity diminished due to high temperature of the slurry, thereby resulting in a moderate reduction of apparent viscosity as compared to a high reduction when malt flour is added prior to cooking. Hence, blending the weaning food with malt before cooking will be advisable for effectively reducing the bulk density.
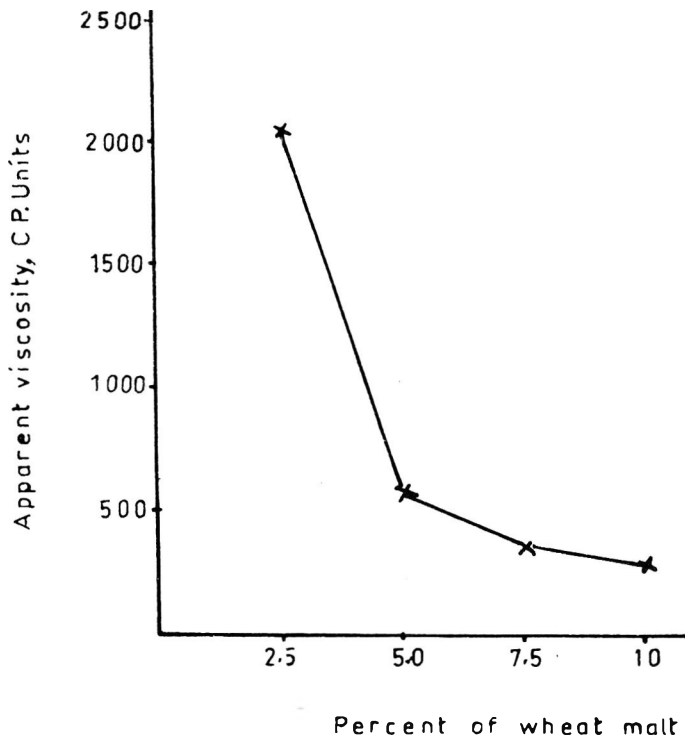
Fig. 2. Minimum level of wheat malt required for effective thinning of energy food gruel.

Fig. 3. Effect of toasting on the apparent viscosity profile of the ingredients with and without addition of 10% wheat malt at 25% hot paste slurry ▨▨▨ raw, ▥▥▥ toasted; ▨▨▨ raw + 10% malted wheat; ▤▤▤ toasted + 10% malted wheat.

Viscosities of raw as well as toasted wheat, maize and Bengalgram were high (80,000 cpu) at 25% slurry concentration, thereby resulting in a dough-like consistency (Fig. 3). The addition of 10% malt reduced the viscosity significantly in all the cases. The viscosity was about 700 cpu and the slurry was free-flowing. Malt had almost the same effect on raw as well as toasted material, except in the case of toasted wheat which showed a slightly higher viscosity. The viscosities of protein-rich materials like soya and peanut cake flours were considerably lower (28,800 cpu) than cereal flours and addition of malt had beneficial effect on peanut flour, but not on soya flour.

The data on *in vitro* protein and carbohydrate digestibilities of raw and toasted materials and the energy food are presented in Table 2. Toasting had beneficial effect on carbohydrate digestibility, the highest increase in digestibility value having been obtained with toasted wheat. Most of the carbohydrates were found to be digested within the first 30 min of digestion and later, the rate slowed down. In contrast, no marked differences were observed in protein digestibility with regard to raw and toasted ingredients of energy food except in case of wheat. The peanut cake flour and soya flour showed around 90% digestibility whereas maize and wheat showed somewhat lower digestibility.
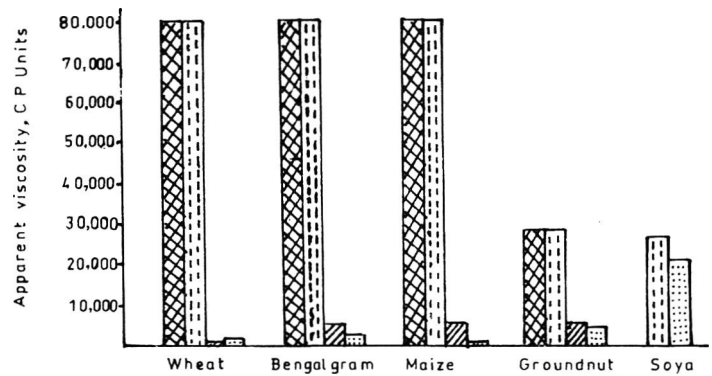
Carbohydrates in the energy food were almost completely digested, as computed from the digestibility values of its ingredients. It can be concluded that while toasting of the ingredients of energy food had no effect on reducing the viscosity, it had definitely a beneficial effect on the digestibilities of carbohydrates and proteins.

The process adopted for preparation of energy food is HTST treatment for wheat and maize and toasting of other ingredients. By this process, the cereal ingredients get gelatinized and also get partially sterilized, thereby rendering them to a ready-to-eat form. But, when energy food is recommended for infants, it is desirable that the

TABLE 2. EFFECT OF TOASTING ON THE *IN VITRO* PROTEIN AND CARBOHYDRATE DIGESTIBILITIES OF THE INGREDIENTS OF ENERGY FOOD[+]

Sample	Protein Digestibility %	Digestibility of starch[*] %			
		30 min	60 min	90 min	120 min
Raw wheat	66	58	58	67	67
Toasted wheat	78	79	79	100	100
Raw maize	62	22	63	63	63
Toasted maize	62	83	94	94	94
Raw Bengal gram	86	61	65	71	75
Toasted Bengal gram	87	69	71	78	79
Raw ground-nut flour	87	40	48	49	64
Toasted groundnut flour	91	63	87	87	92
Extruded soya	91	-	-	-	-
Energy Food	80	77	79	97	98

+ Values are averages of duplicate determinations.

* Calculated from values for the content of starch as reported by Kamath and Belavady (1980).

food is fully cooked and should have softer consistency. The present studies show that addition of 10% malted wheat and cooking the energy food not only helps in complete gelatinization of starchy ingredients, but also reduces the bulk density, thereby increasing the nutrient contents in respect of calories and protein and rendering it suitable for feeding malnourished children.

Acknowledgement

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Studies of Some Assam Rice Varieties for Cooking, Organoleptic and Visco-elastic Properties

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Studies of cooking, organoleptic and visco-elastic properties of three varieties of Assam rice revealed comparatively higher cooking time, water-uptake and elongation ratio for brown and milled rice. 'Jaha', a scented variety, scored highest in aroma but was inferior in terms of non-cohesiveness, integrity, alkali spreading and clearing. 'Monoharsali' and 'Prosadbhog' gave higher peak viscosity and 'Jaha' lowest gelatinization time. Correlation co-efficients between amylose, fat and protein with water-uptake and elongation ratio were estimated.

Keywords: : Assam rice varieties, Cooking characteristics, Organoleptic evaluation, Visco-elastic properties.

New rice varieties need evaluation for their suitability for specific end uses in commercial applications and consumer preferences (Mundy 1989). Rice varieties have specific milling, cooking, eating and processing characteristics and an agronomically superior long-grained cultivar may be unacceptable for traditional long-grain cooking and processing (Webb 1985). Therefore, various cooking and visco-elastic properties need to be studied for assessing the quality of rice (Del Mundo 1979). Assam grows various *indica* and *japonica* type rice varieties. The cooking, organoleptic and visco-elastic properties of these varieties are not studied so far and therefore, this work was undertaken.

Paddy samples of the rice varieties were collected from Rice Research Station, Assam Agricultural University, Jorhat. Cooking time, water-uptake-ratio, elongation ratio and organoleptic evaluation were done according to methods of Sidhu et al. (1975). Splitting and curling of cooked rice in water was done following the method of Keneaster. The extent of alkali spreading and clearing was scored by the 7-point scale of Little et al. (1958). A higher strength of 3% KOH was used. Rice samples were ground in Falling Number Mill and amylograph curves were developed using 50 g rice powder in a Brabender amylograph set to 1.5°C rise in temperature/min.

The cooking characteristics of brown and milled rice (4% polishing) are shown in Table 1. Significant variation in cooking time, water uptake ratio and elongation ratio of brown and milled rice were noted. Brown rice took longer time for cooking than milled rice. Slender grained fine variety 'Jaha' had taken lowest time for cooking among brown

TABLE 1. COOKING CHARACTERISTICS OF BROWN AND MILLED RICE

Variety	Cooking time, min	Water-uptake ratio, g water/g rice	Elongation ratio
Brown rice			
'Jaha'	20.5	2.21	1.10
'Monoharsali'	23.5	2.25	1.06
'Prosadbhog'	21.5	2.37	1.14
SEM	± 0.2520	± 0.0716	± 0.0354
C.D. at 0.05	1.0735	0.3050	0.1508
C.D. at 0.01	2.0210	0.5742	0.2839
Milled rice (4% polished)			
'Jaha'	20.0	3.09	1.22
Monoharsali'	22.5	3.29	1.35
'Prosadbhog'	21.0	3.26	1.25
S.EM	± 0.1791	± 0.0340	± 0.0207
C.D. at 0.05	0.8396	0.1444	0.0882
C.D. at 0.01	1.5807	0.2719	0.1660

rice varieties (20.5 min) and milled rice samples (20.0 min). Significant variation in the water-uptake ratio and elongation ratio of milled rice was noted. However, highest water uptake and elongation ratio were obtained for 'Monoharsali' milled rice. The findings are similar to those reported by Sidhu et al. (1975) and Batcher et al. (1956). 'Jaha' displayed extensive splitting and curling, whereas 'Monoharsali' and 'Prosadbhog' did not show any splitting or curling.

Organoleptic scores for appearance, aroma, non-cohesiveness and integrity of cooked rice are summarized in Table 2. 'Monoharsali' had highest overall score for appearance, non-cohesiveness and integrity of kernels. However, 'Jaha' had the highest score for aroma (3.42 ± 0.75), but its score for appearance (1.50 ± 0.50), non-cohesiveness (1.92 ± 0.76) and integrity (2.08 ± 1.64) were the lowest among the tested varieties.

TABLE 2. ORGANOLEPTIC QUALITY SCORES OF COOKED RICE.

Variety	Mean score ± S.D.			
	Appearance	Aroma	Non-cohe-siveness	Integrity
'Jaha'	1.50±0.50	3.42±0.75	1.92±0.76	2.08±1.64
'Monoharsali'	2.58±0.64	2.08±0.85	2.83±0.55	2.75±0.59
'Prosadbhog'	2.17±0.69	2.00±0.71	2.50±0.76	2.33±0.64
* Scores : 1 = Poor, 2 = Fair, 3 = Good and 4 = Excellent				

The alkali score values showed that, excepting for 'Jaha', the other varieties were found to be resistant to 1.4% alkali even when kept for 24 h. When the kernels of different varieties were treated with 3% KOH solution, these were completely gelatinized, though some resistance was displayed by 'Monoharsali' rice, the translucent, short-grained variety. It was found that, the varieties were fairly resistant to alkali reactions except for 'Jaha'. It is difficult to correlate the alkali test elaborated by several investigators with the cooking test when number of varieties tested are fewer.

Correlation co-efficients for water-uptake and elongation ratio developed (Saikia and Bains 1990) with amylose, fat and protein values of rice are presented in Table 3. Amylose content was inversely and significantly correlated with the values for fat

TABLE 3. CORRELATION COEFFICIENTS BETWEEN VARIOUS PARAMETERS OF QUALITY OF RICE.

Parameter	r^m
Amylose Vs. fat	– 0.89
Protein	– 0.033
Water-uptake ratio	0.85
Elongation ratio	0.81
Fat Vs. protein	0.08
Water-uptake ratio	– 0.90
Elongation ratio	– 0.80
Protein Vs. water uptake ratio	– 0.22

content (r = – 0.89), positively correlated with elongation ratio (r = + 0.81) and more positively correlated with water-uptake ratio (r = + 0.85). Correlation between fat with water-uptake ratio, protein, elongation ratio and protein with water-uptake ratio were found to be negative.

The effects of variety and milling on amylograph curve characteristics and peak viscosity, temperature at peak viscosity and gelatinization time are presented in Table 4. Increase in peak viscosity varied with degree of milling depending on the variety of rice. In general, gelatinization time and temperature, at peak viscosity, decreased with degree of milling. Highest gelatinization time (10.8

TABLE 4. EFFECT OF VARIETY AND MILLING ON THE AMYLOGRAPH CHARACTERISTICS

Variety	Polish removed		
	Nil (Brown rice)	4%	8%
Peak viscosity (B.U.)			
'Jaha'	298	405	470
'Monoharsali'	630	725	940
'Prosadbhog'	540	745	860
Gelatinization time (min)			
'Jaha'	6.5	6.5	6.5
Monoharsali'	10.8	10.8	12.0
'Prosadbhog'	14.5	8.8	8.5
Temperature at peak viscosity (°C)			
Jaha'	72.8	66.0	68.3
'Monoharsali'	66.0	69.0	67.5
'Prosadbhog'	76.5	69.0	68.3

min) was exhibited by 'Monoharsali' rice polished at 4% level against the lowest value of 6.5 min for 'Jaha'. Temperature at peak viscosity was higher (69°C) for 4% polished 'Monoharsali' and 'Prosadbhog' varieties. Low peak viscosity and gelatinization time of 'Jaha' were found to be associated with its poor cooking and lowest amylose content. The results of amylograph characteristics are in agreement with the findings of Halick and Kelly (1959); Halick et al. (1960); Juliano et al. (1969) and Juliano and Perez (1976).

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Effect of Harvesting Time and Handling Period on Quality of Apple

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Fruits from 4 promising cultivars ('Hardeman', 'Red Spur Delicious', 'Topred' and 'Vance Delicious') were picked on 3 dates between 110 and 125 days after full bloom and their physical and organoleptic characteristics were studied upto 21 days of handling period under ambient conditions. Harvesting dates had no significant effect on the diameter of fruits of 'Hardeman', 'Red Spur Delicious' and 'Topred'. There was significant increase in organoleptic acceptability of fruits of all cultivars. Decrease in firmness and physiological weight loss (PWL) was found in late harvesting. During handling, organoleptic acceptability was at par upto 14 days in all the cultivars excepting 'Vance Delicious'.

Maturity for harvesting apple has been based on ground colour, size, ease of picking, days after full bloom (DAFB), firmness, starch test, seed colour, soluble solids concentration, acidity, internal ethylene content and respiration rate for apple fruits, without any consideration to its effect on fruit quality during post-harvest handling (Haller and Smith 1950; Louton and Hamer 1983; Krishna Prakash et al. 1985; Adhikari et al. 1988; Ingle and D'Souza 1989; Knee and Smith 1989). Harvesting period, generally, spans for 15 days and subsequent grading, packing and transportation takes approximately 20 days for marketing of fruits. This period may adversely affect the fruit quality and thus their marketing. The present investigation was carried out with a view to define optimum picking and handling time required for ensuring good dessert quality of fruits.

Physiologically mature, apples of 4 cultivars viz. 'Hardeman', 'Red Spur Delicious', 'Topred' and 'Vance Delicious' were harvested on 5th (H_1, 110 DAFB), 12th (H_2, 117 DAFB) and 19th (H_3, 125 DAFB) August, 1991 from the orchard of Regional Horticultural Research Station, Mashobra, located at latitude 31.1°N, longitude 77.1°E and 2286 m above mean sea level in the Shimla Hills of Himachal Pradesh. The fruits of each cultivar were collected randomly from 5 trees, labelled and analysed on the same day and after 7, 14 and 21 days of ambient storage (mean max. temp. 21.4°C and min. temp. 13.5°C).

The diameter was measured, using Vernier calipers and weighed by Harvard trip balance. The fruit firmness was measured, using a Magness Taylor (MT) puncture tester with a plunger of 5/16 inch (0.8 cm) diam. Physiological weight loss (PWL) was recorded by taking percentage of reduced weight of each marked fruit. The fruits were evaluated organoleptically for overall acceptability on a 5 point grading scale by a panel of 5 judges. Organoleptic mean scores, 5 = excellent, 4 = very good, 3 = good, 2 = fair and 1 = poor. Statistical analysis of the data was done according to the randomized block and split plot designs (Panse and Sukhatme 1967).

The diameter of fruits of all the cultivars increased in late harvesting (Table 1). The increase was significant only in 'Vance Delicious', where H_1 differed significantly from H_3. The weight of the fruit had also increased significantly in late harvesting in all the cultivars excepting 'Hardeman' (Table 1). Adhikari et al. (1988) have also recorded similar increases in diameter and weight of the fruits.

Fruit firmness decreased progressively with late harvesting and subsequently in handling (Table 2). Decrease in firmness became significant at H_2 and H_3 stages in 'Red Spur Delicious' and 'Vance Delicious' and at H_3 stage in 'Hardeman' and 'Topred'. Such a phenomenon has also been reported previously (Adhikari et al. 1988; Ingle and D'Souza 1989). Statistically, there was no significant difference in firmness of fruits upto 7 days of handling period in 'Red Spur Delicious' and 'Topred'. From 7 to 14 days of storage, firmness of fruits remained non-significant in 'Hardeman' and 'Vance Delicious'. After 14 days of storage, fruits of all cultivars were significantly softer. It might be due to increase in soluble pectin and decrease in insoluble pectin (Bartley and Knee 1982). Harvesting

TABLE 1. EFFECT OF HARVESTING TIME ON PHYSICAL CHARACTERS OF FRUITS

Harvesting dates	CULTIVARS							
	'Hardeman'		'Red Spur Delicious'		'Topred'		'Vance Delicious'	
	Diam, cm	Weight, g	Diam, cm	Weight, g	Diam, cm	Weight, g	Diam, cm	Weight, g
5.8.91 (H₁) (110 DAFB)	6.2	107.6	6.3	102.2	6.5	112.7	6.2	108.6
12.8.91(H₂) (117 DAFB)	6.5	117.1	6.3	110.0	6.7	134.2	6.4	111.9
19.8.91 (H₃) (125 DAFB)	6.5	128.1	6.5	133.3	6.8	139.5	6.6	128.2
CD (p<0.05)	NS	NS	NS	26.3	NS	18.2	0.4	15.8

Diam = Diameter, DAFB = Days after full bloom, NS = Non-significant.

TABLE 2. EFFECT OF HARVESTING TIME AND HANDLING PERIOD ON FRUIT CHARACTERISTICS

Particulars	CULTIVARS											
	'Hardeman'			'Red Spur Delicious'			'Topred'			'Vance Delicious'		
	FN	PWL	OA	FN	PWL	OA	FN	PWL	OA	FN	PWL	OA
Harvesting time												
H₁	19.8ᵃ	4.1ᵃ	3.5ᵃ	20.2ᵃ	3.0ᵃ	3.1ᵃ	20.2ᵃ	3.1ᵃ	3.6ᵃ	20.3ᵃ	3.7ᵃ	3.1ᵃ
H₂	19.2ᵃ	4.0ᵃ	3.8ᵇ	18.8ᵇ	3.0ᵃ	3.8ᵇ	19.1ᵃᵇ	2.6ᵇ	3.9ᵇ	18.0ᵇ	3.2ᵇ	3.7ᵇ
H₃	18.3ᵇ	3.5ᵇ	4.4ᶜ	18.3ᵇ	3.0ᵃ	4.3ᶜ	18.1ᵇ	2.6ᵇ	4.4ᶜ	17.5ᵇ	2.8ᶜ	4.5ᶜ
Handling Period days												
0	21.0ᵃ	-	4.2ᵃ	21.8ᵃ	-	3.8ᵃ	21.4ᵃ	-	4.2ᵃ	20.8ᵃ	-	4.2ᵃ
7	19.5ᵇ	1.8ᵃ	4.3ᵃ	20.2ᵃ	1.6ᵃ	4.0ᵃ	20.1ᵃ	1.4ᵃ	4.2ᵃ	19.6ᵇ	2.0ᵃ	4.1ᵃᵇ
14	18.9ᵇ	4.3ᵇ	4.1ᵃ	18.8ᵇ	3.2ᵇ	4.0ᵃ	18.3ᵇ	3.0ᵇ	4.2ᵃ	18.6ᵇ	3.3ᵇ	3.7ᵇ
21	16.9ᶜ	5.5ᶜ	3.1ᵇ	15.7ᶜ	4.3ᶜ	3.2ᵇ	16.7ᶜ	3.9ᶜ	3.3ᶜ	15.4ᶜ	4.5ᶜ	3.1ᶜ
Interaction between harvesting time and handling period	*,**	*,**	NS	*,**	*,**	NS	*,**	*,**	NS	*,**	*,**

NSMean with same superscript in each column do not differ significantly (p<0.05).

* Interaction between harvesting time and handling period, *,** Significant at p<0.05 or 0.01 respectively.
FN = Firmness lb/in², OA = Organoleptic acceptability (score out of 5), PWL = Physiological weight loss, %, NS = non - significant. H₁, H₂, H₃ = Harvesting timings as given in Table 1.

dates had a significant effect on fruit firmness of 'Hardeman' and 'Red Spur Delicious' during handling period. Physiological weight loss was more in early harvested fruits. Harvesting dates for PWL were non-significant in 'Red Spur Delicious' but highly significant in 'Vance Delicious' (Table 2). In 'Hardeman' H₃ stage and in 'Topred' H₁ stage were found significant. PWL increased significantly during handling period for all the cultivars due to evapo-transpiration and respiration (Siddiqui et al. 1991).

Organoleptic acceptability (OA) scores increased significantly with harvesting date (Table 2). OA increased probably due to increase in soluble solids concentration. Knee and Smith[4] have also reported an increase in acceptability with later harvest. During handling period, mean OA scores remained non-significant upto 14 days in 'Hardeman', 'Red Spur Delicious' and 'Topred'. After 14 days, mean scores for OA differed significantly in all the cultivars.

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Effect of Different Harvesting Periods on Shelf-Life and Quality of Kinnow Fruits

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The Kinnow mandarin fruits were harvested at different periods and stored at ambient temperature (15-20°C) to evaluate their shelf-life and quality. Delaying harvesting beyond second week of January was found to result in greater loss of fresh weights than the fruits harvested earlier. While the fruits harvested in the last week of December and middle of January showed 11.60 and 13.10% losses during 25 days of storage, the corresponding losses in fruits harvested in 3rd and 4th week of January were 20.60 and 25%, respectively. Total soluble solids and sugars gradually increased during storage irrespective of harvesting dates and storage. Ascorbic acid and juice contents decreased sharply during storage in fruits harvested after 2nd week of January.

Kinnow mandarin (Citrus nobilis x Citrus deliciosa) has become popular among fruit growers of low hills and valleys of Himachal Pradesh. The vigorous growth characteristics and high yielding potential of the tree, coupled with good fruit quality, endear it to both grower and consumer. For storage and long distance transport, the fruit is generally harvested on a date when quality criteria can best be satisfied. Fruits harvested late in season or too early, do not keep well during transport. Appearance of fruit, sugar, acid and ascorbic acid contents are the key components that contribute to the quality of citrus fruit (Ryall and Pentzer 1974). Effect of growth regulators, ripening retardants, fungicides and/or waxing on some of these parameters have been reported earlier for Kinnow fruits (Bhullar et al. 1981; Jawanda et al. 1978; Nagar 1991); but knowledge about the effect of different harvesting periods on the shelf-life and quality of Kinnow mandarin is lacking. Therefore, the present study was undertaken to evaluate the effect of harvesting dates on the shelf-life and quality of Kinnow mandarins.

Fully matured and well-ripened Kinnow mandarin fruits with well developed colour, 7.0-7.5 cm diam and 155-160 g weight were harvested in 1988-89 season on December 30, January 7, 15, 23 and 31 from 8 year old trees of horticulture orchard of H.F. Krishi Vishva Vidyalaya. Sixty fruits from each harvesting date were collected, 3 fruits were analysed immediately and the rest were used in storage studies. The fruits were washed, air dried, packed separately in perforated wooden boxes (2x1x1 ft.) lined with shredded paper and stored at ambient temperature (15-20°C. RH, 50%). The data on weight loss, juice content and chemical

changes were recorded at 5, 10, 15, 20 and 25 day intervals. Total soluble solids (TSS) were measured by hand refractometer. The fruits were analysed for titratable acidity, reducing sugars and ascorbic acid as per the methods described by Ranganna (1986). All the data were analysed statistically using randomised block design (RBD) and means compared using least significant differences (LSD) at 5% level for interactions (Gomez and Gomez 1984).

Delaying harvesting beyond 2nd week of January increased loss of fresh weight (Table 1). The fruits harvested in the last week of December and 2nd week of January showed 11.60 and 13.10% weight losses, respectively, after 25 days of storage. The corresponding losses for the fruits harvested in the 3rd and 4th week of January were 20.6 and 25.0%, respectively. Generally, severe losses are reported when citrus fruits are stored at ambient temperatures, due to transpiration from fruit surfaces, thereby leading to shrivelling and poor marketability (Sadashivan et al. 1972).

The chemical changes in fruits related to different harvesting periods during storage are shown in Table 1. Total soluble solids and reducing sugars gradually increased irrespective of harvesting periods and storage. A similar increase was also observed earlier in Kinnow mandarin following various treatments (Jawarda et al. 1978., Nagar 1991). The increase was probably due to the hydrolysis of polysaccharides and concentration of juices as a result of dehydration. No significant effect of different harvesting periods was observed on TSS or reducing sugars. The titratable acidity decreased irrespective of harvesting periods. Like other citrus fruits (Echeverria and Valich 1989; Echeverria and Ismail 1987), the decrease in

TABLE 1. EFFECT OF DIFFERENT HARVESTING PERIODS ON THE QUALITY ATTRIBUTES OF KINNOW MANDARIN FRUIT

Harvest date	Days after storage					
	0	5	10	15	20	25
Weight loss (%)						
30 December	-	2.4	5.4	7.8	10.5	11.6
7 January	-	2.7	5.7	8.0	11.5	12.4
15 January	-	2.5	5.9	9.1	12.5	13.1
23 January	-	2.9	7.6	11.3	14.3	20.6
31 January	-	4.0	9.8	12.8	16.7	24.9
LSD at 5% (txs)						
SEm						
Reducing Sugar (%)						
30 December	2.6	3.1	3.4	3.5	3.6	3.7
7 January	2.7	3.1	3.6	3.7	3.7	3.8
15 January	2.9	3.2	3.4	3.7	3.8	3.9
23 January	2.9	2.9	3.0	3.2	3.3	3.4
31 January	2.9	3.0	3.1	3.2	3.3	3.4
LSD at 5% (txs)						
SEm						
Ascorbic Acid (mg/100 mg F.wt.)						
30 December	31.5	28.1	26.7	23.3	21.8	19.7
7 January	31.8	26.4	24.5	22.5	21.9	20.0
15 January	32.0	27.1	26.2	23.3	22.7	20.2
23 January	31.4	27.6	24.7	22.4	20.2	19.2
31 January	31.2	26.3	24.5	21.9	19.2	18.1
LSD at 5% (txs)						
SEm						
T.S.S. (°Brix)						
30 December	13.6	13.6	14.8	15.2	15.7	16.1
7 January	13.5	13.6	14.7	15.2	15.5	16.1
15 January	13.8	14.1	14.4	15.4	15.7	16.0
23 January	14.0	14.8	15.2	15.8	16.0	16.1
31 January	14.4	14.8	15.4	16.0	16.1	16.2
LSD at 5% (txs)						
SEm						
Titratable acidity (%)						
30 December	1.6	1.6	1.5	1.4	1.4	1.4
7 January	1.6	1.5	1.5	1.4	1.4	1.4
15 January	1.5	1.5	1.4	1.4	1.4	1.4
23 January	1.5	1.5	1.4	1.4	1.3	1.3
31 January	1.5	1.4	1.4	1.4	1.3	1.3
LSD at 5% (txs)						
SEm						
Juice (%)						
30 December	34.2	34.0	32.4	30.7	29.7	28.1
7 January	34.5	34.0	32.4	30.6	28.8	27.5
15 January	34.9	32.0	30.0	29.4	28.0	27.1
23 January	34.4	32.5	29.1	27.9	26.6	24.7
31 January	33.8	32.1	28.5	26.8	25.2	22.8

LSD at 5% (txs) and SEm ranged between 0.33-2.16, and ± 0.11 - 0.76, respectively.

titratable acidity might be due to the utilization of acids in respiratory process.

A sharp and significant decrease in ascorbic acid content was evident during storage which further decreased during third (38.8%) and fourth (42.0%) week of January (Table 1). The loss might be attributed to the oxidation reaction mediated by ascorbic acid oxidase (Mapson 1970). The better retention of extractable juice before 2nd week of January indicated a slower rate of dehydration.

The study showed that Kinnow mandarin fruits harvested beyond 2nd week of January deteriorated faster during storage than the fruits harvested earlier. The changes in chemical composition in fruits during storage are interrelated and play an important role in determining fruit quality (Meredith et al. 1989). Thus, it could be recommended that the Kinnow mandarin fruits should be harvested upto middle of January when these are to be transported to long distances.

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Internal Atmosphere of Some Fruits and Vegetables

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The volume of interstitial gases or the internal atmosphere of 8 types of fruits and 7 different vegetables was determined. Internal volume of gas varied from 1.2 ml/100 g in grapes to 43.8 ml/100 g in pineapple. The oxygen content of the internal air of the fruits and vegetables held under ambient conditions ranged between 14.0 and 19.2%.

Keywords: Fruits and vegetables, Internal atmosphere, Interstitial gas volume, O_2 content of internal gases, Hypobaric treatment, Simple gas sampling method.

The internal atmosphere of fruits and vegetables comprises the interstitial gases, as well as the gases dissolved in the cytoplasm of the cells. The volume of the dissolved gases is negligible under ambient conditions as their solubility is low. The volume and composition of interstitial gases play a vital role in the storage of fruits and vegetables under modified (MA) or controlled atmosphere (CA) storage (Williams and Patterson, 1962; Ben-Yehoshua et al; 1963; Peleg, 1985).

Different methods have been employed for the determination of the internal gases of different fruits and vegetables (Bayer and Morgan, 1940; Blanpied, 1971; Cameron and Yang, 1982; Banks, 1983, 1988; Calbo and Sommer 1987). Under CA storage, composition of internal atmosphere of fruits and vegetables changes gradually due to respiration and exchange of gases with external atmosphere. In certain fruits like apples, better extension of storage life is achieved by initially storing them under very low O_2 (1.0-1.5%) or very high CO_2 level, later shifting to normal CA conditions (3.0% O_2 + 3 or 5% CO_2) (Sharples and Munoz, 1974; Lidster et al, 1981; Little et al, (1982). However, immature fruits of some cultivars of apples are susceptible to CO_2 injury (Lau et al, 1977). Monitoring the internal atmosphere and rapidly establishing the desired internal atmosphere (low O_2 and C_2H_4, and high CO_2, all within tolerable limits) offers possibilities to extend the storage life of fruits and vegetables either under CA or MA storage. Keeping this in view, investigations were undertaken on the internal atmosphere of some fruits and vegetables. The objectives of this study are (i) to fix the duration of evacuation (hypobaric treatment) of gases from intact fruit or vegetable for subsequently filling the void space with the desired mixture of gases (CO_2, O_2 and N_2) and (ii) to standardize a simple and rapid method for gas

sampling to determine the composition of gases inside the tissue by the use of cut pieces.

Fresh fruits and vegetables used in this study, were obtained from local market (within 24 h, after harvest). Their weight and volume were determined and the specific gravity was calculated.

Determination of internal atmosphere : Earlier workers have employed different methods for the determination of internal gases (Bayer and Morgan, 1970; Blanpied, 1971; Cameron and Yang, 1982; Banks, 1982, 1988; Calbo and Sommer 1987). The method employed in this study was based on the principle used by Bayer and Morgan (1940).

A simple apparatus was assembled, consisting of an evacuation chamber (20 1 vacuum desiccator) connected to a vacuum pump provided with a vacuum gauge. Water was filled upto 3/4th level in the desiccator and the air dissolved in it was removed by applying vacuum. The sample was kept submerged in water and covered with the gas collection flask, fitted with a stop-cock. Interstitial gases were extracted from the sample by the application of vacuum of 0.9 ± 0.1 kg/cm^2 (26 ± 1") to the desiccator. The pressure gradient caused the gases to escape from the tissue and collect in the collection flask by displacing the water. When the extraction is complete (i.e. when there is no further evolution of gas bubbles from sample), the vacuum is released before opening the desiccator lid. A graduated measuring cylinder, filled with water was inverted (keeping the thumb on its mouth until it goes below the level of water in the desiccator), over the stop-cock of the collection flask and the stop-cock is opened to allow the passage of extracted gases from the collection flask into the measuring cylinder (displacing the water inside the measuring cylinder). The collected gas was measured, corrected for water vapour and expressed as ml/100 g fresh material. The percentage of O_2 in the collected samples was measured using a gas phase oxygen analyser (range 0.1 to 100%,

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TABLE 1. INTERNAL ATMOSPHERE OF SOME FRUITS AND VEGETABLES

Commodity	Extraction time, min	Weight increase, %	Initial specific gravity	Internal gases ml/100g fresh weight	Oxygen content, %
FRUITS					
Apple-whole	15	0.4	0.814	17.3	ND
Banana-whole, green	10	5.3	0.967	13.0	ND
Grape-berries	40	0.1	1.078	1.2	16.2
Grape-berries with stalk	40	Nil	1.039	2.2	ND
Lime-whole	40	1.1	0.948	9.3	ND
Mango-whole green	240	16.4	0.968	23.8	16.5
Mango-cut	15	20.3	0.990	19.4	ND
Mosambi-whole	20	0.2	0.886	11.7	15.9
Mosambi-cut	20	32.3	0.882	23.1	ND
Mandarin-whole	15	0.1	0.889	13.5	16.7
Mandarin-cut	15	51.6	0.784	24.4	ND
Pineapple-core	40	ND	0.987	49.5	ND
Pineapple-pulp	40	ND	0.987	43.8	ND
Pineapple-peel	40	ND	0.987	30.8	ND
VEGETABLES					
Ashgourd-cut (skin + flesh + spongy tissues)	15	27.1	0.915	18.8	ND
Ashgourd-spongy tissues	15	20.2	ND	30.4	ND
Ashgourd-skin (3/16" thick)	15	3.8	1.073	3.5	ND
Beetroot-cut	20	27.1	1.027	11.0	19.2
Carrot-cut	15	23.0	1.023	11.8	14.0
Cucumber-cut (skin + Seed)	15	15.7	0.961	10.4	15.8
Mangalore cucumber-cut	15	31.6	0.879*	20.0	ND
Mangalore cucumber-cut (seed & spongy tissue)	15	12.2	0.879*	37.7	ND
Potato-whole	60	1.1	1.041	1.7	ND
Potato-cut slice	60	27.6	1.032	3.9	ND
Radish-cut	20	22.0	0.096	7.4	15.6

Each value represents mean of 3 independent determinations. Standard errors ranged between 3 and 10% of mean value

* Specific gravity of whole fruit. ND - Not Determined.

model 2608 of Orbisphere Labs, Geneva, Switzerland). All measurements were made at 28 ± 1°C, using three independent samples for each type of fruit or vegetable.

The internal volume of gases ranged between 1.18 ml/100 g for grapes and 43.8 ml/100 g in pineapple (Table 1). The time taken for the complete evacuation of gases from whole fruit or vegetable varied due to the histological characteristics of the commodity, its size, shape and the resistance of skin and tissue to diffusion of gases. In case of mango, while a 15 min evacuation was adequate to remove all gases from cut samples, it required about 4 h when whole fruit was used. Volume of internal gases of seven common vegetables, cucumber (gherkin type and yellow, elliptical-type), potato, ash gourd, radish, carrot and beet-root ranged between 1.74 and 20.0 ml/100 g. Further, cucumber (both types) and ash gourd showed marked differences in the volume of internal gases present in different parts. The increase in weight of the sample (after subjecting to vacuum and then releasing), due to absorption of water, was very much less in whole fruit or vegetable than in cut pieces, due to skin resistance for moisture pick-up. The concentration of O_2 in the internal gases from different fruits and vegetables ranged between 14.0 and 19.2%.

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Studies on the Changes in the Volatile Aroma Composition of *Alphonso* Mango Pulp as Affected by Aroma Recovery Process

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Aroma distillates of fresh mango pulp, obtained by vacuum distillation and by using aroma recovery unit, were analysed by GC-MS. A total of fourteen components were identified in vacuum distillate, of which ten were carbonyls, three alcohols and one hydrocarbon β-myrcene. Concentration of 2-methyl propan-1-al, 2-methyl propan-1-ol and myrcene were lower in aroma concentrate obtained by using aroma recovery unit.

Keywords: Alphonso mango pulp, Aroma components, Vacuum distillation, Aroma recovery unit, Volatile aroma composition.

Ever since fruit aroma recovery process was developed by Milliville (1944) for apple juice, considerable developments have taken place in the design of fruit aroma recovery equipment to suit specific requirements (Bomben et al. 1973; Sarosi 1977; Horesji 1977; Huor et al. 1980; Sulc 1984). Despite these developments, the compositional changes are known to occur in the aroma concentrate due to highly complex nature of aroma compounds in different fruit juices (Thijssen 1970). In this communication, difference in composition of mango aroma concentrate prepared in pilot plant model (Holstein and Kappert, GmbH, 4600 Dortmund, Germany) aroma recovery unit (involving fractional distillation) and volatiles recovered by rotary flash evaporation are reported.

Fully ripe 'Alphonso' mangoes were purchased from local market, washed under running tap water, the stem portion/any surface black specks removed and pulped in APV pulper fitted with 0.6 mm diam sieve. Mango pulp (500 g) was concentrated to 50% volume in rotary flash evaporator (Superfit, Model PBU-6 Continental Instruments, Bombay) at 62°C and 178 mm pressure with condenser cooled by chilled water (8°C). Volatiles were collected in two traps connected between the condenser and vacuum pump and cooled with ice-common salt mixture and with solid carbondioxide, respectively. Distillates (250 ml) and contents of the traps were combined and referred as the total aroma volatiles of mango pulp.

In case of use of aroma recovery unit, mango pulp (50 kg) was diluted with water to 200 kg, heated to 98°C, flashed in a vapour separator and the vapours passed through a distillation column, cooled to 8°C to obtain 2.9 l of aroma concentrate. Aroma concentrate (29 ml) from aroma recovery

TABLE 1. RELATIVE PERCENTAGE OF VOLATILE COMPONENTS IN VACUUM DISTILLATE AND AROMA CONCENTRATE OBTAINED IN AROMA RECOVERY UNIT

Compound identified	Vacuum distillate		Aroma concentrate	
	Retention time min	Relative per- centage	Retention time min	Relative per- centage
2-Methyl propan-1-al*	7.64	2.0	-	-
2-Methyl propan-1-ol	17.26	0.4	-	-
2-Pentanone*	20.78	7.8	20.76	7.1
β-Myrcene	22.0	0.6	-	-
2-Methyl-2-pentanal *	23.86	0.9	23.82	1.0
1-Penten-3-ol*	23.94	2.3	23.90	2.0
2-Ethyl Hexen-1-ol*	24.94	22.5	24.92	22.9
Trans-pent-2-en-1-al*	25.86	1.3	25.84	1.5
Hexan-3-one*	26.84	24.6	26.80	24.5
2, 5-Dimethyl - 4-hydroxy 2H-furan-3-one	27.02	1.2	26.98	1.10
Cyclopentanone*	27.78	1.0	27.74	1.0
3-Methyl-butan-2-one*	28.60	19.7	28.56	22.4
2,5-Dimethyl-4-methoxy 2H-furan-3-one	29.26	0.6	29.22	0.6
Pent-2-enal*	29.54	0.6	29.54	0.6

* Compounds are detected for the first time

unit was diluted to 250 ml with distilled water as well as 250 ml of aroma condensate from rotary flash evaporator were individually saturated with sodium chloride and extracted four times with 20 ml fractions of chilled methylene chloride. Combined extracts were dried over anhydrous sodium sulphate and concentrated by distillation (0.25 m Vigreux column) to 0.4 ml.
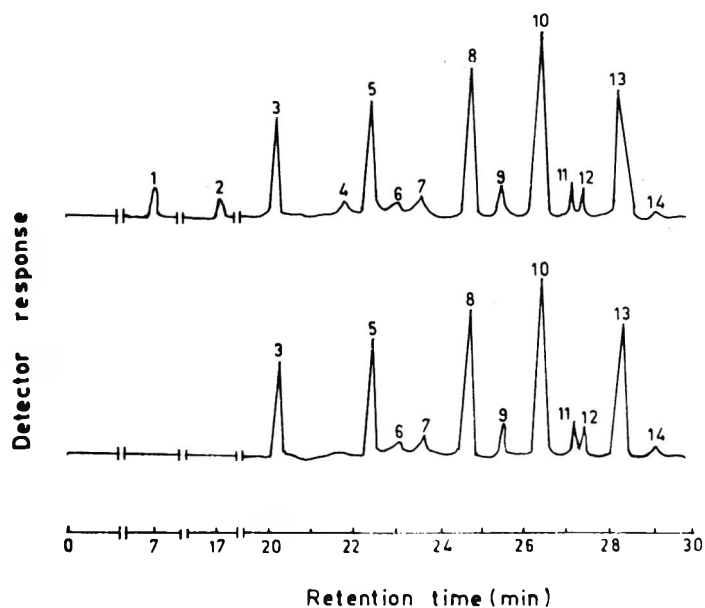
* *Corresponding Author*

Fig. 1. Ion exchange chromatogram of 'Alphonso mango' pulp

(a) Vacuum distillate

(b) Aroma concentrate

A Hewlett Packard 5995B Gas chromatograph - mass spectrophotometer fitted with fused silica capillary column (12 m long x 0.2 mm i.diam x 0.2 μm film thickness, coated with methyl silicone fluid) was used. The other analytical parameters employed include : carrier gas He; flow rate, 1 ml per min; splitless injection mode; sample size, 1 μl; temperature of injector 250°C, transfer line 280°C, column temperature programme 60 (10)-6°/min-150(10)°C; EI, 70 eV; mass range 40 to 800 amu at 2 scans per sec. The identities of 14 components of aroma were based on comparison with standard reference spectra and in some cases by mass fragmentation pattern. The data showed that ten components were carbonyls, three alcohols and one hydrocarbon β- myrcene (Table 1). The components

like 2-methyl-propan-1-ol, β-myrcene, 2.5-dimethyl-4-hydroxy 2H furanone and 2, 5-dimethyl-4-methoxy 2H furan-3-one have been reported as the constituents of 'Alphonso' mango pulp (Hunter et al. 1974; Macleod 1985). The remaining components have been reported for the first time in 'Alphonso mango'.

Some differences were observed between the chromatograms of the vacuum distillate and that of aroma concentrate of aroma recovery unit (Fig. 1). 2-methyl propan-1-al, 2-methyl propan-1-ol and myrcene were absent in the aroma concentrate probably due to losses of more volatile substances. They do not significantly contribute to the typical flavour of 'Alphonso' mango. The concentrations of 2-pentanone and 2.5 -dimethyl-4-hydroxy 2H furanone were also lower in the concentrates prepared in the aroma recovery unit.

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Microbial Quality of Whole Egg Powder

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The applications of good manufacturing practices in the preparation of freeze-dried and foam-mat-dried whole egg powders and packing in three package systems resulted in obtaining a microbiologically safe and good quality product which could be stored for considerably longer periods of time.

Egg and egg products are prone to microbial contamination at various stages of processing, particularly with a higher incidence of *Salmonellae* (Prost and Riemann 1967; Troller 1976). Processing parameters such as freezing or drying may not render these products free from *Salmonellae*. To minimise the problem of *Salmonellae* contamination in spray-dried egg powder, use of fresh and clean eggs has been recommended (Solowey et al. 1946). A reduction in the viable bacterial count and a complete destruction of coliforms, *Salmonellae* and *Staphylococci* were achieved by heating liquid eggs to 60°C for 3 min (Gibbons et al. 1946). With this background, an attempt was made to prepare microbiologically safe and acceptable quality of egg powder following the principles of good manufacturing practices.

Eggs of 'White Leghorn' birds procured from the local market were washed with water, followed with Tween 80 to remove the adhering extraneous materials. The cleaned eggs were soaked in 2% bleaching powder solution for 30 min, washed with water and surface dried at ambient temperature. The eggs were then broken by hand and inspected visually for any spoilage. The white and yolk portions were homogenized and the mixture was filtered through muslin cloth. Desugaring of egg melange was carried out, as described in an earlier study (Satyanarayana Rao and Murali 1985), using 600 mg. of Baker's wet yeast per 100 g of melange, followed by pasteurization at 64°C for 3 min in a water bath and immediate cooling to 4°C. The freeze dried (FD) and foam-mat-dried (FMD) egg powders were prepared according to the methods described in earlier studies (Satyanarayana Rao et al. 1987; Satyanarayana Rao and Murali 1989). Spray-dried (SD) egg powder was procured from a commercial firm (M/s. Foods & Inns, Bombay).

The three types of egg powders were packed in (i) aluminium foil-polyethylene laminate (a laminate consisting of 60 g brown casing (BC)

paper, 0.02 mm aluminium foil polyethylene of 150 gauge (ii) high density polyethylene (HDPE), 300 gauge and (iii) cans (with and without air). The packed egg powders were stored at 55, 42, 37, 19-27 (ambient temperature) and 4°C (control) temperatures for one year and analysed at an interval of 6 months.

The above stored samples including the sample prior to packing were analyzed for the counts of viable total bacteria (standard plate count), yeasts, moulds and coliforms and for the presence of *Salmonella* (APHA 1966). For the purpose of resuscitation of injured bacteria, 10^{-1} dilutions of the sample prepared in 0.1% peptone water were allowed to stand for 1 h before plating the appropriate dilutions . Microbial colonies appearing on the incubated plates were counted and expressed as colony-forming units per g (cfu/g) product. Moisture content of the sample was also determined (AOAC 1960).

The microbial profile of packed freeze-dried whole egg powder during storage at different temperatures over a period of one year is given in Table 1. *Salmonellae* and coliforms were absent in all the samples. With reference to the microbial counts, spray-dried and foam-mat-dried egg powder samples revealed no significant counts. The moisture contents of SD, FD and FMD egg powders were 2.15, 1.86 and 1.70%, respectively.

The initial counts of total viable bacteria (SPC) in raw liquid egg was in the range of 10.9 x 10^2 to 12.5 x 10^3 cfu/g and that of coliforms were 1.5 x 10^2 cfu/g. Pasteurization brought about a drastic reduction of these counts. *Salmonellae* were absent in the liquid egg itself, hence were not detected in the egg powder. The differences observed in the microflora of FD, SD and FMD egg powders were very low and may be due to the processing parameters. The microbial counts of the egg powders recorded in the present study were on a lower side as compared to that in an earlier study (Goresline 1951). This may be the result of improved hygienic

TABLE 1. MICROBIAL COUNTS OF FREEZE DRIED WHOLE EGG POWDER PACKED IN DIFFERENT PACKAGING MATERIALS DURING STORAGE

Storage Period (days) Initial	Storage temp. (°C)	CANS N$_2$ pack SPC	CANS N$_2$ pack Y/M	CANS Air pack SPC	CANS Air pack Y/M	PEP Laminate N$_2$ pack SPC	PEP Laminate N$_2$ pack Y/M	PEP Laminate Air pack SPC	PEP Laminate Air pack Y/M	HDPE SPC	HDPE Y/M
	-	6×10^1	Nil	-	-	-	-	-	-	-	-
90	55	9×10^1	6×10^1	1.5×10^2	6×10^1	2×10^2	8×10^1	2×10^2	-	8×10^1	Nil
180	42	5×10^1	5×10^1	2.2×10^2	Nil	5×10^1	8×10^1	7×10^1	Nil	5×10^1	Nil
365	42	4×10^1	Nil	4×10^1	5×10^1	5×10^1	Nil	Nil	Nil	Nil	Nil
180	37	Nil	Nil	Nil	3×10^1	5×10^1	Nil	1×10^2	1.10^1	-	-
365	37	6×10^1	1×10^1	Nil	Nil	1×10^2	Nil	5×10^1	1×10^1	-	-
180	19-27	1×10^1	Nil	5×10^1	1.10^1	2.5×10^2	Nil	5×10^1	Nil	Nil	Nil
365	19-27	6×10^1	Nil	1×10^1	1×10^1	1.6×10^2	Nil	3×10^1	Nil	Nil	Nil
180	4	4×10^1	Nil	4×10^1	Nil	1.2×10^2	Nil	2×10^1	Nil	-	-
365	4	Nil	Nil	4×10^1	Nil	Nil	Nil	5×10^1	Nil	-	-

SPC = Standard plate count ; Y/M = Yeast & Moulds; N$_2$ - Nitrogen pack; HDPE = High density polyethylene

practices followed in selection and handling of raw materials. Besides, the quantity of egg liquid handled and processed on a pilot plant scale will give less scope for microbial contamination from external sources. Moreover, the time gap between breaking of eggs and dehydration was less. A similar observation was made in a comparative study between experimentally prepared and commercial samples (Bartram 1943).

The total viable bacterial count of FD egg powder was slightly higher as compared to that of SD and FMD egg powders. This may be due to rapid cooling (Gibbons and Fulton 1943). As the storage period increased, there was a decrease in the microbial counts, a factor attributed to the storage temperature and period (Goresline 1951; Gibbons and Fulton 1943; Iyengar et al. 1969 a; Iyengar et al. 1969 b). The type of packaging used did not influence the microbial counts to any great extent (Table 1). However, the type of storage atmosphere did influence the survival of microorganisms.

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Prevalence of *Shigella Dysentriae* Group A Type in Fresh Water Fishes and Seafoods

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Antibiotic sensitivity pattern of seven *Shigella dysentriae* group A strains, isolated from 185 fresh water fish and seafood samples, showed that chloramphenicol, norflox, ampicillin, gentamicin, streptomycin, doxacillin, furadentin and ledermycin were effective in controlling *in vitro* growth. All the strains were resistant to septran, but only three to nalidixic acid.

Keywords: Fishes, Seafoods, Fresh water fishes, *Shigella dysentriae*, *Shigella* sp., Antibiotic sensitivity pattern.

Shigella, a causative agent of bacterial enteritis of human and sub-human primates, is excreted in stools of infected persons. The contamination of lakes, rivers, ponds and seas with excreta of these patients leads to contamination of fish and seafoods with *Shigella*. It is, therefore, not surprising that *Shigella* has been isolated from fishes collected from sea, estuary and freshwater especially the shellfish, prawn and mussels (Dartevelle and Desmet 1975; Lam and Hwee 1978: Cantoni et al. 1980; Skovgaard 1984; Saxena 1985). In Singapore, about 10% samples of shellfish and prawns were found contaminated with *Shigella* (Lam and Hwee 1978). In India, about 2.9% fresh water fish were reported to be contaminated with *Shigella*, in contrast to

fresh water molluscs were swabbed using sterile swabs. These were immediately transferred to Cary and Blair transport medium vials, stored under ice and brought to laboratory for isolation of *Shigella*. Swabs were resuscitated in 0.1% peptone water for 6-8 h at 37°C. The isolation, biochemical characterization and identification of *Shigella* was done as per standard methods (Speck 1976; Cruickshank et al. 1975). All the *Shigella* isolates were tested for antibiotic sensitivity by the paper disc method of WHO (1961).

A total of 7 isolates of *Shigella dysentriae* were isolated from 185 samples of seafoods. A total of 4, 2 and 1 samples of fresh water fishes, marine fishes and marine prawns were positive for *Shigella*,

TABLE 1. ANTIBIOTIC SENSITIVITY PATTERN OF *SHIGELLA DYSENTRIAE* GROUP A STRAINS ISOLATED FROM FRESH WATER FISH AND SEAFOODS

Source of isolation	Antibiotics tested									
	CH	NI	DO	ST	SP	LE	NO	NF	AM	GM
	Degree of inhibition									
Fresh water fish	+++	++	+++	++	-	++	+++	++	+++	+++
Fresh water fish	+++	++	+++	++	-	++	+++	++	++	+++
Fresh water fish	+++	++	+++	++	-	+	+++	++	+++	+++
Fresh water fish	+++	++	+++	++	-	+	++	+	++	+++
Marine fish	+++	-	+++	+++	-	++	+++	+	+++	+++
Marine fish	+++	-	+++	+++	-	+	+++	+	+++	++
Marine prawn	+++	-	+++	++	-	++	+++	+	++	++

- = No inhibition, + = Zone of inhibition less than 2 mm, ++ = Zone of inhibition between 2 and 5 mm,
+++ = Zone of inhibition more than 5 mm, CH = Chloramphenicol, NI = Nalidixic acid, DO = Doxacillin, ST = Streptomycin,
SP = Septran, LE = Ledermycin, NO = Norflox, NF = Furadentin, AM = Ampicillin, GM = Gentamycin.

about 10% of fishes from composite sewage receiving ponds (Saxena 1985).

In the present studies, the slime from gills or surfaces of 96 freshwater fishes, 37 marine fishes, 13 fresh water prawns, 13 marine prawns and 26

respectively. However, *Shigella* was absent in 26 and 13 samples of fresh water molluscs and prawns, respectively. All the seven isolates of *Shigella* were able to ferment only dulcitol, but not lactose, mannitol, sucrose and xylose. These were, therefore, classified into group A type *Sh. dysentriae* as the other species of *Shigella* ferment mannitol.

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The results of antibiotic sensitivity pattern (Table 1) show that all the isolates were resistant to septran, but only three to nalidixic acid. In contrast, all the isolates were found to be sensitive to chloramphenicol, norflox, ampicillin, gentamicin, streptomycin, doxacillin, furadentin and ledermycin.

These enteropathogens like *Shigella* mostly spread by transmission from person to person and affect children of low socio-economic group primarily on account of their poor personal hygiene. Several Seafood-borne outbreaks have been recorded recently (Cantoni et al. 1980; Spreekens and Von 1985; Tauxe et al. 1987). The isolation of these pathogens from the samples is probably due to the contamination of natural water sources (rivers, ponds, lakes and ocean) with human excreta. *Shigella* appears to survive longer in water as indicated by their isolation in the present study. Therefore, the fish processing industry should take this into consideration to give sufficient thermal processing to kill *Sh. dysentriae*. The fish consumers should be cautioned about it, while handling and consuming raw fish. It is also important to develop decontamination techniques to safeguard public health.

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Lipid Composition of Some Seeds of Central India

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Total lipid contents of *Acacia catechu*, *Lepidium sativum*, *Nigella sativa* and *Swetenia mohaganii* seeds were 5.7, 13.8, 31.8 and 59.4%, dry weight on respectively. Neutral lipids were found to be predominant, while the glycolipids and phospholipids were present in lower quantities. Glycolipids consisted of monogalactosyl diglyceride, digalactosyldiglyceride, acylatedsterylgalactoside and sterylgalactoside, while the phospholipids comprised of phosphatidyl choline, phosphatidylethanolamine, phosphatidylinositol, candiolipin and phosphatidylglycerol. Lysophosphatidylethanolamine and lysophosphatidylcholine were present in small amounts.

Keywords: Seeds, Total lipid content, Glycolipids, Phospholipids, *Acacia catechu*, *Lepidium sativum*, *Nigella sativa*, *Swetenia mohaganii*.

Acacia catechu (Leguminosae), *Lepidium sativum* (Cruciferae), *Nigella sativa* (Ranunculaceae) and *Swetenia mohaganii* (Meliaceae) seeds are found in abundance in Central India. Some work on physico-chemical properties including fatty acid analysis of these seed oils has been reported (Kapoor et al. 1979). This communication reports, for the first time, the lipid composition of these seeds.

The seeds, purchased from local dealers, were crushed to powder form and were extracted by chloroform : methanol (2:1, v/v) to obtain the total

separated into neutral lipids (NL), glycolipids (GL) and phospholipids (PL) by silicic acid column chromatography using chloroform, acetone and methanol. The GL fraction was separated into major individual components by preparative TLC using the solvent system chloroform : methanol : ammonia (28%) (70:20:2 v/v). Visualization of spots was done by periodate-benzidine reagent. The R_f values were compared with those of authentic standards and the spots detected by different staining reagents (Kulkarni et al. 1991). Sugars were identified by

TABLE 1. MAJOR LIPID CLASSES OF SEEDS AND THEIR FATTY ACID COMPOSITION

Seed	Lipid classes	Weight[a] %	Fatty acids (wt. %)[b]						
			14:0	16:0	18:0	18:1	18:2	18:3	20:0
Acacia catechu	TL	5.7	0.2	28.4	2.7	38.9	35.7	1.0	-
	NL	86.3	0.2	26.7	2.5	37.8	35.9	4.0	-
	GL	7.8	0.3	29.5	2.0	26.2	36.2	1.3	-
	PL	6.2	0.2	25.4	2.2	29.7	28.6	1.5	-
Lepidium sativum	TL	13.8	0.3	7.1	2.0	22.8	12.6	34.5	13.3
	NL	86.7	0.4	8.1	3.0	24.9	13.7	33.8	13.0
	GL	7.7	0.4	26.7	4.5	31.5	18.7	12.0	1.0
	PL	5.8	0.2	27.5	3.2	31.8	15.8	18.2	1.5
Nigella sativa	TL	31.8	0.2	12.4	1.8	24.5	57.6	0.6	0.2
	NL	87.2	0.2	12.8	1.7	23.7	55.4	0.8	0.2
	GL	6.7	0.2	14.6	1.0	30.7	48.6	0.4	0.1
	PL	6.3	0.2	15.2	0.8	32.5	45.6	0.3	0.1
Swetenia mohaganii	TL	59.4	0.1	14.3	11.2	28.9	29.3	15.6	-
	NL	87.1	0.2	14.8	12.3	27.6	28.3	14.8	-
	GL	6.7	0.2	12.5	15.2	29.7	27.8	10.7	-
	PL	5.8	0.2	12.7	13.4	30.5	28.5	10.7	-

TL-Total lipids, NL-Neutral lipids, GL-Glycolipids, PL-Phospholipids; a: On wt. of seed, dry basis; b: Means of triplicate analysis; - : means acids could not be detected.

lipids (TL) (Folch et al 1957). Standard lipids and fatty acid methyl esters were procured from Analabs, USA, while the chromatographic grade solvents were double distilled before use. The TL were

the method of Malkin and Poole (1953). The PL was fractionated using chloroform : methanol : ammonia (25%) (65:15:4 v/v) and chloroform : methanol : acetic acid : water (170:25:25:6 v/v) in two directions respectively. The spots were detected by specific

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TABLE 2.	GLYCOLIPIDS OF SEEDS AND THEIR FATTY ACID COMPOSITON						
Seed	Glyco-lipid component	Weight[a] %	Fatty acids wt. %[a]				
			16:0	18:0	18:1	18:2	18:3
Acacia	MGDG	23.5	28.7	1.8	27.5	38.3	1.4
catechu	DGDG	40.5	26.5	1.7	27.5	36.4	1.8
	ASG	25.7	22.5	1.5	26.9	37.0	2.0
Lepidium	MGDG	24.0	27.2	4.8	30.7	22.7	10.5
sativum	DGDG	38.7	25.4	6.8	28.5	24.6	10.8
	ASG	28.8	26.5	5.7	29.2	23.8	0.4
Nigella	MGDG	21.3	10.8	8.3	36.5	40.5	2.4
sativa	DGDG	39.5	10.0	10.3	34.5	38.5	1.2
	ASG	30.0	10.6	9.5	35.7	39.8	-
Swetenia	MGDG	22.8	23.8	31.5	24.8	14.9	-
mohaganii	DGDG	41.7	22.7	29.5	26.3	16.1	-
	ASG	25.5	21.8	30.6	28.4	15.2	-

MGDG-monogalactosyldiglyceride, DGDG-diagalactosyldiglyceride
ASG - acylated sterylgalactoside
SG - sterylgalactoside (10.3, 8.5, 9.2, 10.0%, respectively for the seeds).
a means of triplicate analysis. - = could not be detected.

TABLE 3.	PHOSPHOLIPIDS OF SEEDS AND THEIR FATTY ACID COMPOSITION.						
Seed	Phospho-lipid component	Weight[a] %	Fatty acids wt. %[a]				
			16:0	18:0	18:1	18:2	18:3
Acacia	PC	36.0	29.2	20.1	25.1	20.2	2.1
catechu	PE	25.8	19.8	10.7	47.8	17.9	2.8
	PI	21.8	13.9	20.1	43.0	22.0	-
	CL	9.0	17.1	17.3	44.7	19.8	-
	PG	7.4	25.4	19.5	28.7	18.2	-
Lepidium	PC	33.5	19.4	18.8	29.1	30.1	-
sativum	PE	29.7	14.5	16.2	34.9	30.1	-
	PI	26.0	10.9	16.1	34.1	37.9	-
	CL	6.8	15.1	11.9	36.8	29.8	4.3
	PG	4.0	14.2	12.1	34.7	30.5	3.2
Nigella	PC	32.8	40.1	16.9	29.8	11.1	1.1
sativa	PE	28.5	30.3	11.2	46.3	11.7	-
	PI	25.4	20.1	21.8	40.3	11.7	-
	CL	7.2	22.0	20.9	40.8	13.1	2.1
	PG	6.1	21.4	20.0	40.3	12.8	2.5
Swetenia	PC	31.5	28.3	25.7	34.0	3.1	1.1
mohaganii	PE	28.9	22.1	20.9	40.3	5.2	1.0
	PI	28.2	22.3	20.7	35.4	14.8	2.0
	CL	5.8	28.3	12.5	47.2	2.1	2.0
	PG	5.6	25.7	20.5	41.3	1.8	3.6

PC -phosphatidylcholine, PE-phosphatidylethanolamine, PI - phosphatidylinositol, CL-Cardiolipin, PG-phosphatidylglycerol.
a : means of triplicate analysis. - = could not be detected.

staining reagents (Kulkarni et al. 1991) and confirmed by comparing with R_f values of authentic standards. Phosphorus was estimated by the procedure of Harris and Popat (1954). The hydrolytic products, glycerol and inositol were estimated by the method of Malkin and Poole (1953). The fatty acid methyl esters of the lipid classes were prepared according to Kulkarni et al (1992) and analysed by gas-liquid chromatography (Perkin-Elmer) using flame ionization detector at 280°C and a stainless steel column containing 15% EGSS-X on chromosorb W (40-60 mesh). The conditions of GLC were:chart speed, 60 cm/h; injection port and column temperature: 300 and 200°C; nitrogen flow rate, 60 ml/min; and sample size, 0.2 µl. Identification of fatty acids was done by comparing the retention data with authentic standards and quantitation using the areas of the peaks.

Total seed lipids consisted of NL as predominant fraction with minor amounts of GL and PL. The major fatty acids present in them were 16:0, 18:0, 18:1, 18:2 and 18:3 acids (Table 1). The GL and PL fractions did not contain 14:1 and 16:1 acids which were present in traces in TL. The fatty acid composition of NL and TL was nearly similar. 18:2 and 18:3 acids constituted the major fatty acids of TL fractions of *Nigella sativa* and *Lepidium sativum* seeds.

The GL fraction contained MGDG, DGDG, ASG

and SG as major components (Table 2). The five major components of the PL were PC, PE, PI, CL and PG, while lysoPC and lysoPE were present in minor quantities (Table 3). These findings were supported by the observations on *Saraca indica*, Ritha, Kenaf and Myrobalan seeds (Kulkarni et al. 1992; Moharir et al. 1990; Kulkarni et al. 1991).

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Chemical and Microbial Changes in Full-Fat Soyflour During Storage in Different Packaging Materials

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The changes in total bacteria, lipolytic bacteria, yeast and mould counts, moisture and free fatty acids of full-fat soyflour (FFSF), when stored at 38°C and 90% RH in jute cotton bags, metal containers (tins), low density polyethylene and laminated aluminium foil packs, have been studied. Moisture impermeable metal containers and laminated aluminium foil packs gave maximum protection to FFSF under above conditions, while jute and cotton bags were found to be unsuitable.

Keywords : Full-fat soyflour, Packing materials, Storage, Chemical changes, Microbial profile, Soybeans.

Increased use of soybean in human diet is of importance in view of the prevalent protein-calorie malnutrition. Among various soy products, the full-fat soyflour (FFSF) holds promise. Being rich in fat, it is highly prone to spoilage leading to deterioration in quality during storage under tropical conditions.

The FFSF is produced from blanched and dried soysplits (Ganchi et al. 1984). The process eliminates the heat labile anti-nutritional factors like trypsin inhibitors, lipoxygenase, protease inhibitors etc. The spoilage in FFSF, is therefore, not likely to occur as a result of the inherent enzymes, but it may occur due to microbial contamination of FFSF during grinding, handling and packaging. The present investigation is, therefore, an attempt to study the changes in chemical and microbial qualities of FFSF during storage at 38°C and 90% RH in jute and cotton bags, metal containers (tins), low density polyethylene (LDPE-100μ), polyester (12μ)/polyurethane/aluminium foil (9μ)/polyure-thane/LDPE (37.5μ) laminated packs with a view to identify suitable packaging materials. The FFSF prepared from blanched and dried soysplits ('JS-7244') was packed in 500 g lots in all the above packages. The jute and cotton bags were thread-stitched, while the LDPF and aluminium foil packs were heat-sealed. The metal tins were closed with air-tight lids. The LDPE packs were stored at 38±1°C and 90 ± 2 % RH in a controlled humidity cabinet and also under ambient conditions as a control. Initially and after every 15 days, the samples were analysed for total bacterial plate counts (Speck 1976), lipolytic counts (Collins and Lyne 1976), yeast and mould counts (ISI 1969), moisture contents (AOAC 1975) and free fatty acids (AACC 1969).

The freshly prepared FFSF had 40% protein, 19.4% fat and 21.3% total carbohydrates. The total viable bacterial counts, lipolytic counts and yeast

and mould counts were 5.0x10³, 1.33 x 10³ and 1.23 x 10² per g, respectively, while the coliforms and *Salmonella* were not detected. The changes in FFA and moisture contents (wb) of packed FFSF during storage are presented in Fig. 1 and 2.
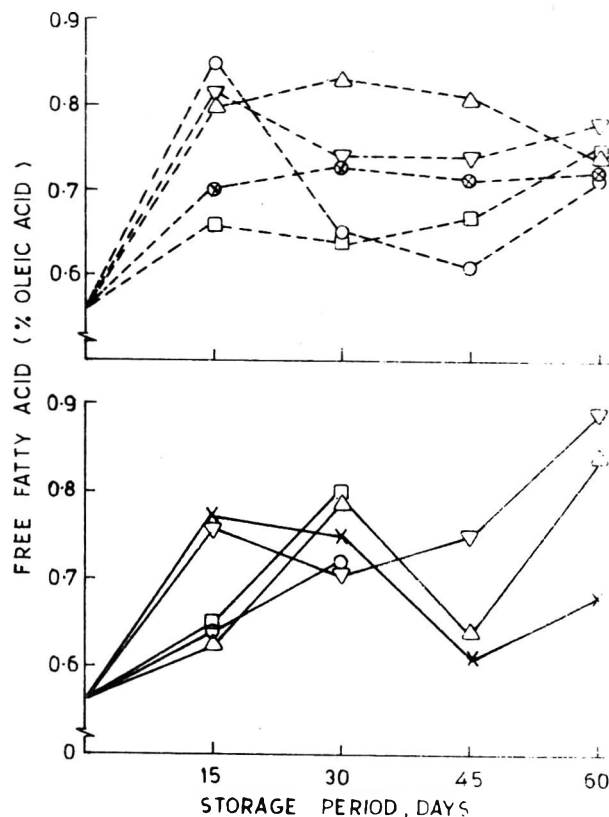

Fig. 1. Changes in free fatty acid values of full fat soyflour packed in different packaging materials and stored at accelerated (38°C/90% RH) and ambient conditions of storage.

Accelerated environment (38°C/90%RH)	Ambient (Control) (27.4°C/28.8% RH)

O : Jute, □ : Cotton, Δ : Metal container, ∇: LDPE, × : Laminated Al Foil.

Initially, the FFA increased in all the five packages under both the storage conditions which was followed by fluctuating trends. The FFA values,
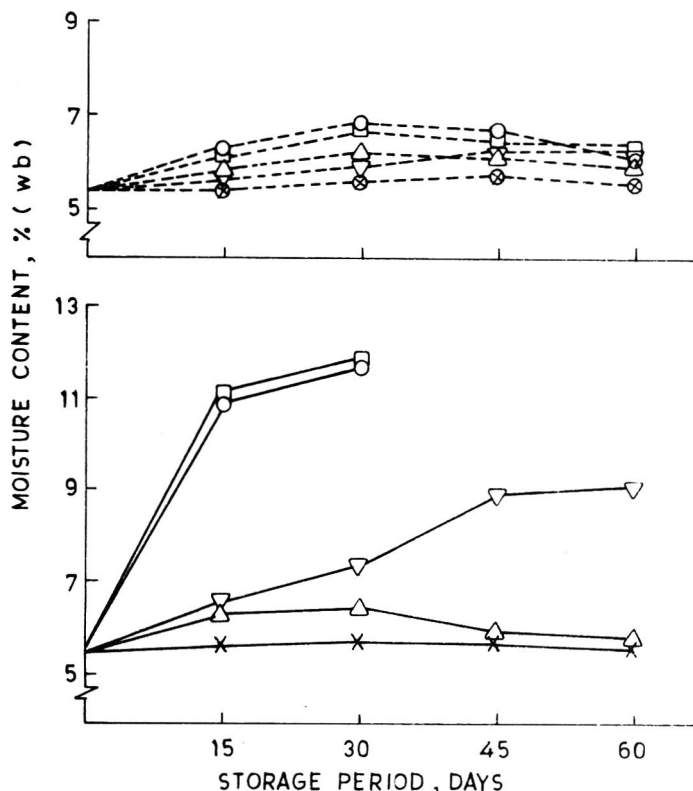
* Corresponding Author

Fig. 2. Changes in moisture contents of full-fat soyflour packed in different packaging materials and stored at accelerated (38°C/90% RH) and ambient conditions of storage.

Accelerated environment (38°C/90%RH)	Ambient (Control) (27.4°C/28.8% RH)

O : Jute, □ : Cotton, △ : Metal container, ▽: LDPE, × : Laminated Al Foil.

at this stage were 6.30×10^6 and $4.17 \times 10^5/g$, respectively. These counts were also higher than the acceptable limits (ISI 1975) of $5.0 \times 10^4/g$. The total bacterial counts in these samples were around $5.75 \times 10^3/g$. Under ambient conditions, the FFSF had a total bacterial counts of 1.66×10^4 and $9.31 \times 10^4/g$ in jute and cotton bags, respectively. The lipolytic counts were quite low with a maximum value of $7.08 \times 10^3/g$ in case of cotton bags after 60 days storage. The total bacterial counts in LDPE stored flour at 38°C and 90% RH were 2.69×10^4 and $5.13 \times 10^4/g$ after 45 and 60 days storage, respectively. The counts were, however, quite low in the samples stored at ambient conditions, the maximum value of total bacterial count being $2.46 \times 10^4/g$. The maximum total bacterial counts in tins and laminated aluminium foils were 1.77×10^4 and $2.07 \times 10^4/g$, respectively under accelerated conditions of storage. The tins and laminated foil packages protected the flour for a period of over 60 days. Since these packages protected the material from moisture ingress, these may protect the FFSF for longer periods as well.

however, remained below acceptable limits (Mustaka and Griffin 1964) in all the packages under both environments. The changes in moisture of FFSF in various packages during storage are shown in Fig. 2. In jute and cotton bags, the moisture contents were 10.91 % and 11.10 %, respectively and crossed the acceptable limits of 9.0% set by Bureau of Indian Standards on 15th day. In LDPE packages, this limit was crossed after 45 days of storage. Under ambient conditions, however, the moisture remained below 7 % in all the packages upto 60 days.

The microbial analysis revealed that the growth of the yeasts and moulds were so heavy in jute and cotton bags that the outer surfaces of the bags became greenish due to fungal spores on 30th day. The counts in FFSF packed in jute and cotton bags

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Efficacy of Tricalcium Phosphate on The Storage Quality of Sorghum Flour

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The efficacy of tricalcium phosphate (2%) as pre-storage treatment in whole and dehulled sorghum flour has been studied. Treatment with tricalcium phosphate controlled insect infestation and associated changes in fat acidity, alcoholic acidity, and lipase activity. A positive correlation between fat acidity and lipase activity was observed. Dehulling prior to milling contributed to better storage quality.

Keywords: Sorghum flour, Storage quality, Tricalcium phosphate treatment, Whole and dehulled flours, Insect infestation, Lipase, Acidity.

In India, sorghum is extensively consumed in the form of *roti* which is prepared from sorghum flour. Dehulling of the grain followed by milling would, therefore, produce an attractive flour which could be used for making *roti*, thereby improving its baking quality and consumer acceptance. Sorghum flour is highly susceptible to storage deterioration which is accelerated by moisture, temperature, insect pests, fungi and bacteria. Of these, insect infestation is the most serious threat to storage quality. Although a variety of non-toxic grain protectants are available for use as pre-storage treatments, these are not of much help in flour. Under these circumstances, tricalcium phosphate is ideal, harmless to humans, and may actually be beneficial in view of the widely prevalent calcium deficiency in developing countries. Moreover, it can be mixed with flour directly.

Sorghum (whole grain - variety 'S.P.V. 462') was procured from National Research Centre for Sorghum, Hyderabad. A total of 26.5 kg of the grain

60 mesh sieve. Both whole and dehulled sorghum flours were treated with tricalcium phosphate (2%) and stored in duplicates in polyethylene bags (62.5µ) for a period of 3 months from February to April 1990. Control samples without treatment were also stored in polyethylene bags under identical conditions. The keeping quality of stored sorghum flour was assessed by the changes in moisture content, insect count, uric acid, alcoholic acidity, fat acidity and lipase activity.

Wide fluctuations were observed in the moisture content of all stored samples, which may have been due to the prevalent humidity and temperature conditions (Sinha and Watters 1985). The initial moisture content (8%) in all samples recorded a significant ($P < 0.05$) decrease (7.8%) in treated whole flour and (7.9%) in treated dehulled flour. Untreated flour samples, however, recorded an increase to 8.03% (whole) and 8.17% (dehulled) at the end of the storage period.

The insect counts and associated uric acid

TABLE 1. CHANGES IN INSECT COUNT, TOTAL URIC ACID CONTENT AND ALCOHOLIC ACIDITY IN STORED WHOLE AND DEHULLED SORGHUM FLOUR

Parameters (Mean values)	Initial value	Whole flour				Dehulled flour			
		Period of storage, days				Initial	Period of storage, days		
		45		90			45		90
		A	B	A	B	A	B	A	B
Insect count, insects/100 g	0	2	0	7	1	1	1	7	1
Total uric acid, mg/100 g	1.0	2.1	1.8	3.9	2.8	1.6	1.5	3.8	3.1
Alcoholic acidity ml of H_2SO_4 in 90% alcohol by wt.	0.1	0.3	0.2	0.4	0.3	0.2	0.1	0.3	0.2

A : Untreated, B : Treated. Standard error was ± 0.03 - 0.4

was dehulled in dehulling machine NuHull 2000. The dehulled grains (4.5 kg) and whole grain (4.2 kg) were then ground into flour and passed through

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contents of control samples were higher than those of treated samples (Table 1). A lower insect count and uric acid content in treated flours have been attributed to the arrest of breeding development of insects by tricalcium phosphate. Similar results
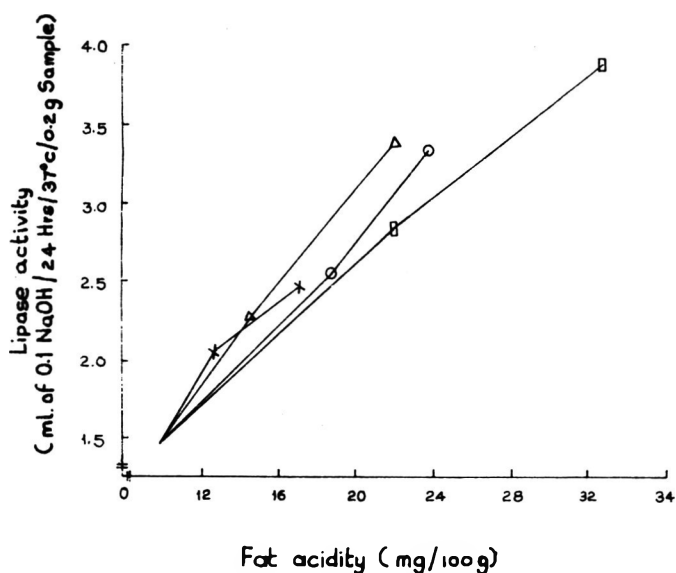
Fig. 1. Relation between fat acidity and lipase activity of Untreated (▯—▯) and treated (0-0) whole flour and unteated (Δ-Δ) and treated (x--x) dehulled flour)

have been reported in wheat flour (Calderon and Carmi 1978). The activation of enzymes due to insect infestation results in the degradation of protein and amino acids, contributing to what is referred to as alcoholic acidity. The alcoholic acidity increased significantly (P<0.05) in all samples. The treatment with tricalcium phosphate and lower moisture contents in whole and dehulled flour samples seem to lead to lower acidity values (Table 1). Whole flours were found to register a higher alcoholic acidity than dehulled flours owing to the breakdown of phytin-rich bran in the former.

Correlation coefficients were calculated between lipase activity and fat acidity in stored flour (Fig. 1). Significant positive correlations were observed in whole flour (r = 0.9856) and dehulled flour (r=0.9913), wherein increases in fat acidity were thought to reflect the enzyme activity (Clayton and Morrison 1972). The increase in fat acidity may be attributed to lipolysis or oxidative degradation of unsaturated glycerides (Premavalli et al. 1973). Similar increases have been documented in stored jowar and wheat flour (Prasad and Mandokhot

1982; Chitra 1985). Whole flours were found to record (P<0.05) higher fat acidity values when compared to dehulled flours. This could be attributed to the presence of lipase-rich bran in the former (Galliard and Tait 1988). Dehulling results in the removal of the lipase-rich bran, thereby contributing to a lowered lipase activity and associated lesser fat acidity values.

Treated whole and dehulled flour samples registered lesser increases in lipase activity and fat acidity values in comparison to control. The toxic action of tricalcium phosphate seems to have controlled insect development and therefore, an associated lesser insect lipase contribution to lipid deterioration. Lower moisture content could yet be another reason for the lesser values in treated samples. The parameters used to assess the efficacy of tricalcium phosphate (2%) as a pre-storage treatment indicated a positive role by controlling insect activity. Dehulling contributed to lesser lipid deterioration as reflected by the lower fat acidity values. From the results obtained, it can be concluded that the treatment with tricalcium phosphate proved beneficial in the storage of sorghum flour.

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Potential of Storage Insect Pests to Breed in Traditional Products of Rice

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Breeding potential of *Tribolium castaneum*, *Rhyzopertha dominica* and *Sitophilus oryzae* in salted *parmal*, puffed rice and beaten rice showed that mortality of adult insects was negligible during 3 weeks in all products except for full mortality of *S. oryzae* on beaten rice in 2 weeks. Larval development of *T. castaneum* was significantly slower in salted *parmal* and *phulian* as compared to that in raw rice. The larvae of *R. dominica* took significantly longer time to develop in salted *parmal*. *T. castaneum* produced lowest progeny in salted *parmal* and sweetened *phulian*, whereas *R. dominica* produced maximum progeny in plain and sweetened *phulian* causing 27.9 and 19.5% loss in weight respectively during 3 months. The rice weevil, *S. oryzae*, could not breed in any of the traditional products of rice tested in this study.

Traditional products such as puffed rice (plain or sweetened *phulian*), expanded rice (salted *parmal*) and beaten rice *(chiwra)* are manufactured on cottage scale in various States of India from parboiled coarse varieties of paddy. The dried parboiled paddy is processed into *phulian* which is sweetened at will later on. For making salted *parmal*, the dried parboiled paddy is milled and brown rice (unpolished rice) is used for its preparation (Chinnaswamy and Bhattacharya 1983). Beaten rice is also prepared from parboiled rice, which is flattened while it is wet, either mechanically or manually (Ali and Bhattacharya 1976). These products are consumed by millions all over India as a breakfast item or in social/religious functions. During a market survey at Ludhiana (Punjab), a few samples of these products were found infested with the rust red flour beetle, *Tribolium castaneum* (Herbst) and lesser grain borer, *Rhyzopertha dominica* (F.). There are no reports on the infestations of such products by storage insects and hence the present study was conducted to determine the feeding and breeding ability of *T. castaneum*, *R. Dominica* and the rice weevil, *Sitophilus oryzae* (L.) on the traditional products of rice.

Fresh samples of polished rice, salted *parmal*, plain/sweetened *phulian* and *chiwra* were procured locally, cleaned to remove extraneous matter, thermally disinfested by keeping for 1 h in oven at 60°C and conditioned in desiccators (70% relative humidity) for 2-3 weeks to bring the moisture content to about 12%. To determine the feeding and breeding ability of three insect species, 30 g of each sample were taken in 100 ml glass jars. Polished rice was taken as control. The experiment was replicated four times and 20 unsexed adults (10

days old) were introduced in each replicate. Mortality counts were recorded at weekly intervals for three weeks, after which the insects were discarded. The samples were incubated at 30°C and the emerging F_1 progeny was scored every other day till complete emergence. Productivity (progeny/adult - day) of the test insects in each product was calculated according to Kazmaier and Fuller (1959). To estimate the loss of weight caused by insect pests in various products, 40 g of each product was taken in glass jars, inoculated with 20 insects per replicate and incubated at 30°C for three months. The total progeny emerged and the amount of material lost due to feeding and breeding were calculated and the data were statistically analysed by ANOVA employing two-tailed test.

There was no significant adult mortality of *T. castaneum* in any of the products. Progeny emergence and productivity was significantly low in salted *parmal* and sweetened *phulian* compared to the values obtained for raw milled rice (Table 1). The developmental period was prolonged in plain and sweetened *phulian* (35.5 and 40 days, respectively) and in salted *parmal* (55 days) compared to that in milled or beaten rice. There was negligible mortality of *R. dominica* in the products. Larval development was prolonged significantly only in salted *parmal* (43 days). The insect produced significantly more progeny in *phulian* (plain/ sweetened) and lowest in salted *parmal* compared to that in raw milled rice. Complete mortality of *S. oryzae* adults occurred in beaten rice within 2 weeks, while they survived for 3 weeks in other products. None of the products supported any progeny of the rice weevil.

Results of the weight loss experiment (Table 2) revealed that during 3-month infestation period,

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TABLE 1.   ADULT SURVIVAL, ADULT EMERGENCE AND PRODUCTIVITY (PROGENY/ADULT - DAY) OF *T. CASTANEUM* AND *R. DOMINICA*   IN MILLED RICE AND DIFFERENT TRADITIONAL PRODUCTS OF PARBOILED BROWN RICE.

Test product	*T. castaneum*			*R. dominica*		
	Adult mortality after 21 days, %	Days of first emergence of F_1 progeny	Productivity	Adult mortality after 21 days, %	Days of first emergence of F_1 progeny	Productivity
White rice	1.3	30.0[a]	0.05[b]	7.5	33.8[a]	0.12[b]
Salted *parmal*	0	55.0[c]	0.01[a]	8.8	43.0[b]	0.04[a]
Phulian (plain)	0	35.5[b]	0.04[ab]	1.3	31.8[a]	0.58[d]
Phulian (Sweetened)	1.3	40.0[b]	0.01[a]	6.3	30.8[a]	0.45[c]
Beaten rice	0	28.3[a]	0.04[ab]	3.8	31.8[a]	0.08[ab]
C.D. at 5% df = 19	-	4.6	0.04	-	3.1	0.05

All values are means of four replicates, each infested with 20 unsexed adults. Values followed by the same letter(s) do not differ significantly (p>0.05).

plain or sweetened *phulian* supported 340.7 and 272.3 progeny of *R. dominica,* respectively, thereby causing weight loss of 27.9 and 19.53%. The low progeny development of *R. dominica* in white rice, salted *parmal* and beaten rice reflected in low weight losses. *T. castaneum* could not cause significant weight loss in any of the products during the 3-month infestation period. Poor progeny development of *T. castaneum* in salted *parmal* could be due to the hard external texture that might have

TABLE 2.   PER CENT WEIGHT LOSS CAUSED BY *T.CASTANEUM* AND *R. DOMINICA* IN VARIOUS RICE PRODUCTS DURING 3 MONTHS OF INFESTATION.

Test product	*T. castaneum*		*R. dominica*	
	Total number of adults emerged	weight loss, %	Total number of adults emerged	weight loss, %
White rice	25.0	1.50[c]	43.7	2.18[a]
Parmal (salted)	13.7	1.01[ab]	20.3	2.75[a]
Phulian (plain)	32.0	1.19[bc]	340.7	27.09[d]
Phulian (sweetened)	20.0	0.97[ab]	272.3	19.53[c]
Beaten rice	29.7	0.79[a]	66.3	8.03[b]

All values are averages of three replicates. In each replicate, 40 g of the test material was infested with 20 unsexed adult insects. Means followed by the same letter do not differ significantly (p> 0.05)

offered resistance to feeding by first instar larvae. Hard textured parboiled rice is also not fed upon by insects successfully (McGaughey 1974). The flattened product (beaten rice) probably did not provide enough space for the developing larvae of *R. dominica,* besides offering resistance to boring by the first instar larvae, thereby reducing the progeny emergence. It was observed that only *R. dominica* could breed very well in plain or sweetened *phulian,* causing heavy losses in weights of the products as the adults and the developing larvae produced a lot of powdery frass.

It is reported that the parboiled rice, because of its comparatively harder texture than that of raw milled rice, is not suitable for growth of the insect pests (McGaughey 1974; Singh 1980). The presently studied products are made from parboiled paddy. However, these lose their characteristic compact texture because of processing and thereby, become more suitable for growth of *R. dominica* and *T. castaneum.* Plain *phulian* and beaten rice (for *T. castaneum)* and both the *phulian* products (for *R. dominica),* gave equivalent or higher values of productivity (progeny/adult per day) than those in the raw milled rice (white rice). These products are made from brown rice (intact rice bran layer), which is known to be nutritionally better for several storage insect pests than the polished white rice having most of the bran removed during milling. It does explain the trend of the results obtained.

Though not stored for longer durations, these products may suffer heavy losses, besides degradation of the quality/acceptability, if accidentally infested with these storage pests. Therefore, better storage conditions, coupled with the use of insect proof packaging, may be desirable to preclude their infestation.

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Effect of Heat and UV on Trypsin and Chymotrypsin Inhibitor Activities in Redgram (*Cajanus cajan*, L.)

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Redgram *(Cajanus cajan,* L) seeds were analysed for trypsin and chymotrypsin inhibitor activity and the effects of heat treatment as well as UV exposure on the activities of these anti-nutritional factors. Heat treatment and UV exposure decreased the activities of both trypsin and chymotrypsin inhibitors. Proteinase inhibitors of redgram were found to be heat labile.

Redgram *(Cajanus cajan,* L.), also called as pigeonpea, is a valuable source of protein, minerals, vitamins and occupies a very important place in human nutrition in many developing countries (Singh 1988). The nutritive value and protein digestibility of legumes are improved by processing or cooking, thereby leading to destruction of heat labile anti-nutritional factors (Lienev 1976). Trypsin and chymotrypsin inhibitors have been studied in detail (Stoilova 1990). Effects of heat and germination on anti-nutritional factors in pulses have been reported (Liener 1980). However, little information is available on this aspect in redgram, one of the most important pulse crops of India. In this communication, the effects of UV and heat on trypsin and chymotrypsin inhibitors of redgram are reported.

Redgram grains were procured from Pulse Research Station, Gulbarga. Trypsin (Type III, E.C.3.4.21.4) and chymotrypsin (Type II, E.C.3.4.21.1) from bovine pancreas were from Sigma Chemical Company, U.S.A. Casein was from Wilson Lab., Bombay. The UV radiation was done in the UV instrument (Tempo Instruments and Equipments Pvt. Ltd., Bombay).

Inhibitor extract was prepared by homogenizing 2 g of sample in 30 ml of 0.1 M phosphate buffer (pH 7.6). Then, it was centrifuged at 12,000 x **g** for 20 min. The supernatant was dialysed against 0.05 M sodium phosphate buffer (pH 7.6) for 6 h. The dialysed extract was used for assay of trypsin and chymotrypsin inhibitors. The extracts of raw redgram seeds, overnight soaked seeds and the defatted meal were subjected to heat treatment (heating in boiling water bath at 100°C) and UV radiation for various time intervals, cooled and assayed for residual inhibitory activity against

trypsin and chymotrypsin. The assay of trypsin and chymotrypsin inhibitor activities was carried out by casein digestion method (Sumathi and Pattabiraman 1977). This consists of taking 10 μg of enzyme, 0.8 ml of inhibitor extract and 0.1 ml of 0.1 M sodium phosphate buffer (pH 7.6), and this mixture was pre-incubated for 10 min at 37°C. The reaction was initiated by adding 1 ml of 1% casein and stopped exactly after 20 min by adding 3 ml of 5% TCA. The solution was centrifuged at 3,000 x **g** for 20 min. Clear supernatant (0.5 ml) was analysed by the method of Lowry et al. (1957). One unit of inhibitor activity is the amount of inhibitor that suppressed one unit of proteolytic activity.

From the data presented in Table 1, it is observed that the inhibitory activity decreases as the time of exposure increases. Higher reduction is observed when the soaked seeds are exposed to UV radiation. It is further observed that trypsin and chymotrypsin inhibitor activities decrease with increase in the time of heating the defatted meal, the raw seeds and the soaked seeds. Liener (1980) has reported that proteinase inhibitors are thermo-labile. Batra et al (1986) have reported that heat treatment decreased trypsin inhibitor activity in lentil and pigeonpea. Pre-soaking followed by dry heat treatment resulted in partial inactivation of the trypsin inhibitor activity (Singh 1988). This may be due to the fact that high moisture content favours rapid cooking, thereby bringing about increase in heat susceptibility causing inactivation. Heat treatment is known to improve nutritional quality of plant proteins because of total or partial inactivation of proteinase inhibitor (Rackis and Gumbman 1981). Heat treatment alters nutritive value and products derived from them. The proteins in raw soybeans have low nutritive value and various heat treatments cause an improvement of this value and loss of trypsin inhibitor activity (Kakade et al. 1974).

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TABLE 1. EFFECT OF UV RADIATION AND HEAT TREATMENT ON TRYPSIN AND CHYMOTRYPSIN INHIBITOR ACTIVITIES IN REDGRAM

Attributes	Meal	Trypsin inhibitor units TIU/g sample		Meal	Chymotrypsin inhibitor units CIU/g sample	
		Seeds	Soaked seeds		Seeds	Soaked seeds
Radiation exposure time, min		**UV Radiation**				
30	144.6	64.8	80.7	211.8	96.1	45.7
60	130.6	41.8	20.2	183.7	85.0	19.7
90	125.8	33.4	Nil	110.6	70.8	Nil
120	84.6	28.3	Nil	32.5	20.2	Nil
150	10.2	Nil	Nil	8.7	Nil	Nil
Mean	108.1	53.7	42.4	127.5	81.6	47.2
SD	± 0.3	± 0.3	± 0.1	± 0.2	± 0.2	± 0.2
Heating time, min		**Heat Treatment**				
1	125.5	127.8	98.8	198.8	200.0	165.8
2	101.8	102.0	35.6	165.5	171.8	125.8
3	89.7	93.7	15.2	125.8	130.7	63.7
4	65.2	70.0	Nil	97.1	100.8	20.8
5	35.1	40.5	Nil	63.1	81.0	Nil
Mean	95.2	97.9	53.1	144.7	150.3	51.4
SD	± 0.8	± 1.2	± 0.6	± 0.4	± 1.4	± 0.6

Initial trypsin and chymotrypsin inhibitor activities in all the materials studied were 153.9 and 218.0 units/g sample, respectively.

In conclusion, it may be stated that trypsin and chymotrypsin inhibitor activity in redgram seeds are decreased by UV and heat treatment. The trypsin and chymotrypsin inhibitor activities of soaked redgram seeds are eliminated completely on exposure to UV for 90 min. Similarly, the trypsin and chymotrypsin inhibitor activities are eliminated completely on heating the soaked seeds in boiling water for 5 min.

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Effect of Addition of Whey Protein Concentrate on the Sensory and Instron Texture Profile of *Khoa* made from Cow Milk

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Whey protein concentrate (5% solids) incorporated *khoa* showed improved sensory characteristics over the control cow milk *khoa* and compared well with the commercial one. Lower total solids content in whey protein concentrate incorporated *khoa* was necessary to counter adverse effect of whey protein concentrate on the Instron texture parameters.

Khoa is one of the most important heat dessicated products in India. Buffalo milk *khoa* serves as a base and a filler for the preparation of various milk-based sweets. Cow milk *khoa* is generally not favoured due to its excessive smooth and pasty body, slight sandy texture and salty taste (Rajorhia and Srinivasan 1979). Whey protein concentrates (WPC) are highly nutritious and known for their good functional properties (DeWit and DeBoer 1975). Our experience with WPC incorporation in buffalo milk *khoa* showed that grains in *khoa* grew with added WPC. The present investigation was undertaken to develop desirable grainy texture in cow milk *khoa*, and study the effect of WPC addition during its manufacture.

Fresh cheddar cheese whey in 100 l quantity was collected from the experimental Dairy of the Institute, clarified through cream separator at 28°C, pasteurized at 72°C for 15 sec. and held at this temperature for 30-40 min prior to ultrafiltration at 50°C using the laboratory module of hollow fibre membrane (Romicon, England, polysulphone membrane, type PM 50), with inlet and outlet pressures of 1.8 and 0.5 kg/cm², respectively. The WPC so obtained had 27.40% TS, 71.11% protein/TS, 9.12% fat/TS, 3.37% ash/TS and pH 6.22.

Pooled cow milk was procured from the Experimental Dairy of the Institute. The WPC was added at 0, 5 and 8% levels to cow milk, and the fat/TS ratio adjusted to 5.5/14 by adding 60-70% fat cream. *Khoa* was prepared in a stainless steel steam kettle employing an iron stirrer *(khunti)*. Samples were drawn at three different moisture levels during the last stages of *khoa* preparation. Last sample was that of fully prepared *khoa*. All the samples were collected in plastic trays and

tempered at 20°C for 12 h. All the experimental samples were analysed for total solids, fat and protein. For comparative studies, a commercial *khoa* sample was procured fresh from the Experimental Dairy of the Institute and tempered at 20°C for 12 h similar to other *khoa* samples. Cylindrical samples of *khoa* (19 mm dia, 20 mm height) were subjected to texture profile analysis, using an Instron Universal Testing Machine (Model 4301) fitted with a 100 N load cell and operated in a two bite compression (20%) mode with a cross head speed of 50 mm/min and chart speed of 100mm/min. Various Instron texture profile parameters were worked out from the force-distance curve as described by Bourne (1978).

Last sample from each batch of *khoa* representing fully prepared product, alongwith the commercial sample, was evaluated for sensory qualities by a panel of 8 experienced judges using a 9 point Hedonic scale.

It is apparent from Table 1 that the control cow milk *khoa* was graded much inferior to commercial *khoa* in all sensory characteristics. With the incorporation of WPC, all the sensory parameters showed considerable improvements, making cow milk *khoa*, though at lower total solids content, comparable with the commercial *khoa*. The WPC, having the property of gel formation on heating (Schmidt et al. 1984), might be producing a desired grainy texture in the WPC incorporated *khoa*. Further, whey proteins contain greater amounts of sulphur containing amino acids. During heat processing, these proteins become denatured and sulfydryl groups are released to impart a heated or cooked flavour on heating (Parry 1974). These flavours might be enhancing the flavour of *khoa*. Additional browning was also observed in the WPC incorporated *khoa* which partly masked the

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TABLE 1. SENSORY MEAN SCORES ON 9 POINT HEDONIC SCALE OF DIFFERENT *KHOA* SAMPLES.

Attributes	Control cow milk *Khoa*	Commercial *khoa*	WPC incorporated *Khoa*	
			5%	8%
Flavour	6.10	7.45	7.68	7.45
Body and texture	6.12	7.24	7.32	7.22
Appearance	6.42	7.20	7.10	7.25
Colour	6.52	7.34	7.10	7.32
Overall acceptability	6.38	7.38	7.31	7.24

Limits : 5.6-6.5 = Like slightly, 6.6-7.5 = Like moderately, 7.6-8.5 = Like very much. S.D. values ranged between ± 0.21 and ± 0.35

undesirable yellowish colour of cow milk *khoa*. However, WPC at 8% level did not give any better quality *khoa* than at 5% level, indicating that it was unnecessary to add more than 5% WPC in cow milk *khoa* for its improvement.

levels in *khoa*. At lower total solids, WPC incorporated *khoa* had comparable gumminess with that of control cow milk *khoa*. Springiness in the product showed the opposite trend. However, the chewiness value (a product of gumminess and springiness) of the product increased with the increase of total solid and WPC levels. A high chewiness value in *khoa* is undesirable, but it could be lowered with reduced total solids content. This would also be helpful in case of other texture parameters like hardness, cohesiveness and gumminess. Adhesiveness of control samples showed a sharp decline with the increase of total solids content, while WPC incorporated *khoa* exhibited substantial increase in this value. This might be due to the inherent interaction of added whey proteins in the system.

Based on this study, it can be concluded that

TABLE 2. CHEMICAL COMPOSITION AND INSTRON TEXTURE PROFILE OF DIFFERENT *KHOA* SAMPLES

Attributes	Control cow milk *khoa*			*Khoa* with 5% WPC			*Khoa* with 8% WPC		
	1	2	3	1	2	3	1	2	3
Total solids, %	76.9	77.6	80.0	67.5	74.6	76.8	63.1	64.7	66.8
Fat, %	30.0	30.3	31.2	25.8	28.6	29.2	23.2	24.2	25.0
Protein, %	17.5	17.6	18.2	15.9	17.6	18.3	15.7	16.1	16.6
Hardness, N	0.0464	0.0696	0.1148	0.0283	0.0698	0.0893	0.0343	0.0598	0.1080
Cohesiveness	0.2284	0.2038	0.1977	0.2565	0.1914	0.1725	0.2450	0.2370	0.1961
Gumminess, N	0.0106	0.0149	0.0220	0.0073	0.0132	0.0154	0.0084	0.0142	0.0212
Springiness, mm	4.00	3.25	3.00	4.00	3.50	3.25	4.50	4.25	4.00
Chewiness, N-mm	0.0424	0.0461	0.0678	0.0304	0.0467	0.0506	0.0378	0.0603	0.0847
Adhesiveness, N-mm	0.0052	0.0025	0.0001	0.0046	0.0073	0.0100	0.0039	0.0059	0.0072

1, 2 and 3 denote sample numbers given based on moisture levels of *khoa*.

Instron texture profile indicated that the higher were the total solids and WPC levels, the greater was the hardness in *khoa* (Table 2), which might mostly be due to the reduction of free moisture content and the increase of protein proportion in the product. Whey proteins are known for their water-binding property (Mangino 1984). For achieving the same hardness value, the WPC incorporated *khoa* needed to be concentrated to a lower total solids level compared to control cow milk *khoa*. .. not only conserved heat energy, but also gave higher yields of the product.

Contrary to hardness, cohesiveness in *khoa* decreased substantially with the increase of its total solid and WPC contents. This might be due to the decreased free moisture content in the resultant product. Rajorhia et al. (1991) also observed that an increase in total solids was accompanied by a decrease in cohesiveness of *khoa*. Gumminess, which is the product of hardness and cohesiveness, decreased with the increase in WPC and total solid

a good quality *khoa* with reduced total solids content can be prepared by incorporating 5% WPC in cow milk *khoa* during its preparation. It will help in producing *khoa* at places where buffalo milk is not available.

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Studies on Viscosity-Molecular Weight Relationship of Chitosan Solutions

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The viscosity of chitosan solutions in 1% acetic acid of various concentrations (0.25-1.0% by weight of chitosan) was measured by Light scattering method. The data were fitted to Mark-Houwink equation as
$\eta_i = 4.74 \times 10^{-2} \, (\overline{M_w})^{0.723}$ which helps evaluate the average molecular weight (weight average) of chitosan samples with a knowledge of their intrinsic viscosity.

Keywords: Chitosan solutions, Viscosity-molecular weight relationship, Mark-Houwink coefficients.

Chitosan [(1,4) –2–amino–2–deoxy–β-D-glucan], a deacetylated product of chitin, is used for various industrial applications because of its polycationic nature (Muzzaralli 1977). Most of the industrial applications are dependent upon its solubility and viscosity which are, in turn, a measure of its molecular weight. The higher the molecular weight, higher is its viscosity and hence, better is its grade (Filar and Wirick 1978). Therefore, there is a need to measure the molecular weight of various soluble fractions present in the deacetylated product. Molecular weight distributions of chitosan were measured by various techniques viz., HPLC (Wu et al. 1976), laser light scattering at 632.8 nm with a Malvern system 4700-c instrument using a He-Ne Spectraphysics 35 mw Laser and an Olivetti M-24 Computer (Muzzaralli et al 1987) and gel permeation liquid chromatography (Mima et al. 1983). In the present investigation, the average molecular weight was measured by light scattering technique because of its ease and simplicity in apparatus (Spectrophotometer and a refractometer), as compared to laser light scattering method.

Chitosan samples were made from prawn waste by CFTRI process which is almost akin to the one reported earlier (Moorjani et al. 1975). Their solution viscosities and molecular weights were measured and they were related by Mark-Houwink equation (Allcock and Lampe 1981). Chitosan solutions of known concentrations (0.25-1.0% by weight) were prepared by dissolving chitosan in a 2% solution of acetic acid (v/v) and making it up to the volume with distilled water (Filar and Wirick 1978). The solutions were stirred for 30 min and undissolved chitosan particles were removed by squeezing the solution through a cloth. The viscosity of the solutions was measured by Cannon-Fenske viscometer by noting the time taken by solutions to pass through the capillary tube maintained at 30°C $\pm$ 1°C. The refractive indices (RI) of various chitosan solutions were measured by a refractometer using a mono-chromatic source of sodium vapour lamp (λ = 5893A°)

The intensity of light scattered through chitosan solutions was measured as the percentage of light transmitted through chitosan solutions as compared to 1% acetic acid solution by spectrophotometer (Bausch and Lamb, Spectronic 20). The experimental measurements were made at three concentrations viz., 0.25, 0.5, 1.0 gm per 100 ml of 1% acetic acid and the results are presented in Table 1. The turbidity (γ) of the solution was measured as the decrease in intensity of light because of scattering (Allcock and Lampe 1981). The average molecular weight of the polymer ($\overline{M_w}$) can be calculated by equation 1 :

$$\frac{1}{\overline{M_w}} = \lim_{C \to 0} \frac{Lt. \quad HC}{\gamma} \qquad \qquad (1)$$

$$\text{Where } H = \frac{32 \, \pi^3 n_0^2}{3 \, \lambda^4 \, N_0} \quad \frac{(n-n_0)^2}{C} ;$$

$\overline{M_w}$ = average molecular weight (weight average); n_0 = RI of the solvent (1% acetic acid) = 1.322; n = RI of solution; λ = wave length of light = 0.5983 x 10^{-4}cm; N_0 = Avagodro No.= 6.023 $\times$ 10^{23}; C = Concentration of polymer in solution, gm/ml of solution; γ = 1– (I/I_0); I/I_0 = fraction of light transmitted through 1 cm length of solution, $\overline{M_w}$ was measured by plotting HC/γ vs. C and interpolating to zero concentration and noting the value of intercept. The reciprocal of the intercept is the weight average of molecular weight. (Allcock and Lampe 1981) The results are presented in Table 1.

TABLE 1.   DATA FOR CALCULATING AVERAGE MOLECULAR WEIGHT AND INTRINSIC VISCOSITY

Property	Sample No. 1			Sample No. 2			Sample No. 3		
	Concentration of solutions								
	0.25	0.5	1.0	0.25	0.5	1.0	0.25	0.5	1.0
RI, n	1.336	1.338	1.346	1.336	1.339	1.346	1.336	1.339	1.346
Transmission, %	93.0	90.4	91.5	90.0	82.5	74.0	78.8	65.0	47.2
Turbidity γ	0.070	0.091	0.185	0.100	0.175	0.260	0.212	0.350	0.528
H $\times$ 10^4	1.5834	1.1745	1.4723	1.5834	1.5833	1.4723	1.5834	1.5833	1.5834
HC/$\gamma \times 10^6$	5.565	6.482	7.958	3.959	4.523	5.663	1.872	2.260	2.999
Density g/ml	0.990	0.981	0.962	0.990	0.981	0.962	0.993	0.985	0.970
t_1, sec	11.6	25.2	75.0	13.8	34.0	107.3	27.0	76.3	273.0
η_r	2.76	6.0	18.04	3.3	8.34	25.8	6.70	18.8	64.3
η_{sp}	1.76	5.0	17.04	2.3	7.34	24.8	5.7	17.8	63.3
$\eta_{sp/c}$ ml/g	704	1000	1704	920	1468	2480	2280	3560	6325
η_i		340			428			765	
M_w	2.046 $\times$ 10^5			3.076 $\times$ 10^5			6.574 $\times$ 10^5		

The intrinsic viscosity of solution (η_i) is given by

$$\eta_i = \lim_{C \to 0} \frac{(\eta_{sp})}{C} \qquad \qquad (2)$$

Where η_{sp} = specific viscosity = $\eta_r - 1$; η_r = relative viscosity = $t_1 d_1 / t_2$; t_1 = time taken by solution in secs. for flow in Cannon–Fenske viscometer; t_2 = time taken by solvent (1% acetic acid) for flow in Cannon–Fenske viscometer = 4.2 sec; d_1 = density of polymer solution in gm/ml.

The average molecular weight of the polymer and its intrinsic viscosity in a solution are related by the Mark-Houwink equation (Allcock and Lampe 1981).

$$\eta_i = K \, (\overline{M_w})^a \qquad \qquad (3)$$

The coefficient K and exponent were calculated from the intercept and slope of the straightline fitted to $\ln(\eta_i)$ and $\ln \overline{(M_w)}$. The data, fitted with a regression coefficient of 0.95, are presented in Table 1.

$$K = 4.74 \times 10^{-2} \quad a = 0.723$$

It is to be noted that Mark-Houwink coefficients are specific to the type of solvent used and the values reported in the present investigation (K = 4.74×10^{-2}, and a = 0.723) are applicable for chitosan solutions in 1% acetic acid solvent alone, which limits the applicability of these coefficients. The reported parameters (of Mark-Houwink equation) would help determine the average molecular weight of chitosan samples simply by measuring its intrinsic viscosity.

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Relationship Between Fissured Kernels and Cooking Characteristics of Rice

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Regression models to predict relationship between % fissured kernels and cooking characteristics of rice have been formulated. Gruel solid loss and alkali spread value were correlated positively to fissured kernels with correlation coefficients of 0.66 to 0.93 and 0.94 to 9.6, respectively, for various varieties. Water uptake and cohesiveness scores were correlated negatively to % fissured kernels with correlation coefficients in ranges of 0.71 to 0.86 and 0.65 to 0.81, respectively.

Keywords : Rice, Fissured kernels, Cooking, Regression models.

The subject of fissuring in rice has been extensively investigated in relation to moisture variation (Kunze 1977; Kunze and Hall 1965; Nguyen and Kunze 1984; Srinivas et al. 1977; Srinivas et al. 1978 and Singh et al. 1990), varietal factors (Stermer 1968; Kunze and Prasad 1978), milling (Kunze and Hall 1965; Velupillai and Pandey 1990), drying (Nguyen and Kunze 1984), storage and cooking characteristics (Desikachar and Subrahmanyan 1961). The present study was conducted to obtain more information on the relationship of fissured kernels with cooking characteristics of rice by preparing regression models.

Represented samples of 'Jaya', 'PR-106', 'PR-103', Basmati Pusa No. 1' and 'PR-108' obtained from breeders were used in this study. To induce fissures in the grains, $1^1/_2$ kg rough rice of each variety was soaked in water at 30°C, in triplicate, for 6 h. After draining water, the samples were dried to original grain weight in a forced air cabinet drier at 30°C. Weighed samples (150 g each) of unsoaked and soaked paddy were dehusked and polished uniformly (6% degree of polish) as described earlier (Singh et al 1990). Head rice and brokens were separated using a laboratory rice-sizing device (Burrows Equipment Co., Evanston, IL, USA). Fissured kernels were, then, manually separated from the milled rice (Bhattacharya 1969) and were then blended with the non-fissured head rice kernels (obtained from unsoaked sample) at 10, 20, 30, 40 and 50% levels. Cooking, gruel solid loss, % water uptake and cohesiveness scores were determined by standard methods (Batcher et al. 1957; Bhattacharya and Sowbhagya 1971). Alkali-spreading values were determined by the method

of Little et al. (1958) using 1.5% potassium hydroxide. All the values reported are average of three replicates except for cohesiveness and were subjected to linear regression analysis on HP-1000 Computer system.

The fissured kernels required lesser cooking time than their sound counterparts. The cooking times for sound kernels of 'Jaya', 'PR-106', 'Pusa No. 1', 'PR-103' and 'PR-108' were 20.5, 22.0, 28.9

TABLE 1. LINEAR MODELS SHOWING THE EFFECT OF BLENDING FISSURED KERNELS IN MILLED RICE ON VARIOUS COOKING CHARACTERISTICS.

Variety	Linear models	R^2
Gruel solid loss		
'Jaya'	Y = 2.74 + 0.047 X	0.93
'PR-106'	Y = 1.20 + 0.025 X	0.91
'Pusa No. 1	Y = 2.10 + 0.025 X	0.88
'PR-103'	Y = 1.55 + 0.015 X	0.66
'PR-108'	Y = 1.47 + 0.015 X	0.84
Water uptake		
'Jaya'	Y = 5.7 + (-0.020) X	0.81
'PR-106'	Y = 3.4 + (-0.015) X	0.86
'Pusa No. 1	Y = 2.8 + (-0.015) X	0.74
'PR-103'	Y = 3.5 + (-0.015) X	0.71
'PR-108'	Y = 2.8 + (-0.014) X	0.74
Alkali-spreading value		
'Jaya'	Y = 3.0 + 0.058 X	0.94
'PR-106'	Y = 2.30 + 0.059 X	0.96
'Pusa No. 1	Y = 2.00 + 0.034 X	0.94
'PR-103'	Y = 2.30 + 0.049 X	0.96
'PR-108'	Y = 2.15 + 0.046 X	0.96
Cohesiveness score		
'Jaya'	Y = 8.15 + (-0.0688) X	0.61
'PR-106'	Y = 8.82 + (-0.0702) X	0.81
'Pusa No. 1	Y = 8.33 + (-0.567) X	0.77
'PR-103'	Y = 7.98 + (-0.0472) X	0.67
'PR-108'	Y = 7.65 + (-0.0465) X	0.65

X = Percent fissured kernels, Y = Characteristics

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25.5 and 26.0 min., respectively, whereas for fissured kernels, these were 19.5, 20.6, 26.2, 24.0 and 24.0 min., respectively. The presence of fissures might have facilitated easy penetration of water in the kernels, thus reducing the cooking time. The other possible factor could be the reduction in grain hardness due to fissuring (Singh et al. 1990).

The gruel solid loss showed a positive relation with the extent of fissuring (Table 1). A unit increase in fissured kernels resulted in an increase of 0.047, 0.025, 0.025, 0.025, 0.016 and 0.015 of gruel solid loss, in 'Jaya', 'PR-106', 'Pusa No. 1', 'PR-103' and 'PR-108', respectively. A higher gruel solid loss in fissured kernels has also been reported earlier (Desikachar and Subrahmanyan 1961; Singh et al. 1990). Water uptake in all the varieties exhibited a negative correlation (0.84 - 0.90) to % fissured kernels. 'Jaya' exhibited highest and 'PR-108' least reduction in water uptake as expressed by regression equation (Table 1).

Alkali-spreading in all the varieties increased with the increase in the number of fissured kernels. Spreading was highest in 'Jaya' and lowest in 'Pusa No. 1'. The correlation between cohesiveness and fissuring was highly significant in all the varieties (Table 1). As the % fissured kernels increased, the sample became more prone to disintegration on cooking. The cohesiveness score showed highest reduction in 'Jaya' and lowest in 'PR-108' with a unit change in fissured kernels. A high score for cohesiveness of cooked rice indicated that the kernel did not clump (Batcher et al. 1956, 1957). A negative correlation coefficient between alkali-spreading and clearing value and cohesiveness has

also been reported earlier (Little et al. 1958).

This study establishes the strong effect of fissuring in rice kernels on cooking characteristics.

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BOOK REVIEWS

Proceedings of Nutrition Society of India by Kamala Krishna Swamy and Leela Raman (Eds). National Institute of Nutrition, Hyderabad, Vol.37, 1991; pp 405; Price: Rs. 250, US $ 50.

It was a pleasure for me to go through the book which is the outcome of Silver Jubilee Celebrations of Nutrition Society of India which dealt with a broad range of subjects in clinical, experimental and public health nutrition. The publication is a result of expert opinions emanating from world authorities on topics of major social issues for third world countries in the field of nutrition. The book is a kind of monograph which reflects the current information on the interdisciplinary nature of the field of Nutritional Sciences. Speakers of national and international eminence covered a wide range of topics such as new dimensions of nutrition problems; energy metabolism and requirements; modern methods of assessment for measuring nutritional status; nutritional modulations of cancer and the newer approaches for solving public health problems and on the impact of changing dietary patterns and life styles on health and nutrition.

The changing profile on national nutrition has clearly indicated that the time has come when nutrition scientists must turn their attention away from intellectual exercises that merely seek to assess the precise magnitude of the problem of hunger in the country and instead, concentrate on a consideration of what is practicable and feasible with current resources to mitigate the problem. Presentations are made in great detail on the measure of population growth on a whole range of public services. Female literacy holds the key to the success of health, nutrition, family planning and education programmes in all developing societies. The other notable contribution is that kwashiorkor and marasmus were not two different problems calling for entirely different strategies for their prevention and control. Instead, these two factors are just one and the same central problem of PEM requiring identical approaches for their solution. Thus, changes in the profile of under-nutrition are enlightened very clearly. The report provides a flood of information on various deficiency diseases. Prof. J.E. Dutra-de-Oliveira pointed out very rightly that there is a greater interest in malnutrition than in nutrition.

The book deals with the information on nutritional aspects of palm oil in terms of fatty acid composition and the effect on blood cholesterol level. The hypocholesterelomic and beneficial effects of palm oil are mainly due to lack of lauric acid (12:0) and myristic acid (14:0) as well as moderate levels of oleic and linoleic acids. Prof. J.C. Waterlow has done a commendable job of going through, in detail about all specific theories on challenges and controversies of PEM. Trends in global undernutrition and poverty are being discussed at length. The responsibility of the scientific nutritional community to set the desired intervention strategy to alleviate undernutrition of the poor is emphasised.

Vitamin-A deficiency continues to be a significant health problem in many developing countries. Role of vitamin-A in immuno-competence is discussed well. Dr. K.N. Agarwal has stressed that the deleterious effects of iron deficiency are seen earlier than the anaemia stage. The latest iron deficiency is likely to be made widespread and changes induced in placenta, brain etc., are mostly irreversible.

Endemic goitre is widespread, but varies in prevalence. The clear possibility exists that, nutritional iodine deficiency and socio-economic backwardness are usually interlocked in a vicious cycle. Attention has been focussed on the technology for double fortification of edible salt with both iron and iodine. This fortified salt contains 1 mg/g iron in the form of ferrous sulphate, 40 mg/g iodine as KI or KIO_2 and a new polyphosphate iron chelating agent which acts as both stabilizer and iron absorption promoter. This will be a challenge in reducing both iron deficiency anaemia and goitre.

Dr. P.S. Setty focussed on the intimate relationship between the energy restricted state and sympathetic nervous system (SNS) activity. The energy deficiency results in a reduction in SNS drive enabling conservation of energy by diminishing metabolism and heat production. The SNS, thus, modulates many physiological functions during situations when energy intake is deficient and thereby, helps to maintain energy homeostasis.

It is rightly pointed out by Drs. S.K. Nair and E.T. Poehlman that protein turn-over is a determinant of energy expenditure in humans. It may account for 20% of resting metabolic rate (RMR) and valuable proportion of thermic effect of a meal (TEM) and thermic effect of activity (TEA).

Protein turn-over is a major contributor of TEM after a protein meal but it contributes none after a carbohydrate meal. It is necessary to gather accurate information on the energy expenditure of typical occupational groups in the country before revising the energy allowances for Indians. During the last decade, significant refinement has occurred in evaluation of iron status in different disorders. Limitations of many earlier investigations have been realised.

The review focusses attention on assessment of essential fatty acid status in Indians (1) by comparing intake with requirement and (2) by measuring functional indices of EFA from plasma lipid fatty acid composition. Dietary, bio-chemical and histological methodologies have been developed for assessing vitamin-A nutritional status in human at sub-clinical levels of nutriture. The application of the preparative technique to the analysis of cholesterol synthesis using deuterium oxide is illustrated. It is also pointed out that cancer primarily is a nutritionally determined disease.

Changing trends in community nutrition indicate that there has been a significant reduction in the production of "severe" and "moderately malnourished" rural pre-school children, thereby indicating an improvement in their nutritional status; but the food consumption pattern remained essentially similar.

The chapter on rapid and participatory appraisal for health and nutrition outlines some of the history, principles and methods of rapid assessment or appraisal and actual and potential applications in health and nutrition. Three intermingling streams of activities can be identified, i.e., rapid rural appraisal (RRA), rapid assessment procedures (RAP) and participatory rural appraisal (PRA).

The research papers on breast feeding, diarrhoeal disease, growth and development during infancy, effect of mango consumption on vitamin-A nutrition, impact of income generation by women on family nutritional improvement and finally, the fourth Silver Jubilee Lecture on the agricultural future are very interesting.

The editors have done a commendable job in compiling different papers and bringing out this volume. The quality of each chapter is high and the book would be of practical use to the nutritionists. As it happens in most edited books,

the style of writing in this compilation varies from article to article; text book style from one end to research publication style to other end. The book would be an useful addition in the university libraries and research institutes which offer various courses in the field of nutrition.

The book has a vast bibliography containing about 600 references which will be useful to the scientist engaged in clinical, experimental and public health nutrition and for policy planners for developing programmes to overcome malnutrition.

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'Handbook of Applied Mycology-Mycotoxins in Ecological systems' Vol. 5, by D. Bhatnagar, E.B. Lillehoj and D.K. Arora (Eds), Marcel Dekker Inc New York 10016, 1992; pp 464; Price: US $ 150

The discovery of mycotoxins dates back to 1940s. Their involvement in the etiology of human and animal health drew more and more attention after the discovery of aflatoxins in England in 1960. The research on various facets of mycotoxin problem gained momentum, once the toxicity of the mould metabolites was established. As a result, thousands of research papers appear every year. Among these, reports on aflatoxins are most pivotal due to their higher toxicity and wide prevalence. The involvement of *Fusarium* toxins in biological warfare and their recognition as causative factors in certain human and animal diseases is a revelation of the research work by various scientists. The ramification of the work is so wide that it will become difficult for a student or a research worker to go through different journals, whenever an information on a specific topic is required. The editors, who themselves are the experts working on mycotoxins, have reduced the burden very much by writing on multifarious aspects of research in a nutshell. The book covers the most modern topic such as "A molecular approach towards understanding aflatoxin production", "Development and use of Immunoassays in the Detection of Ecologically Important Mycotoxins". It also deals with the biochemistry and biosynthetic mechanism involved in the synthesis of aflatoxins and other *Fusarium* toxins, their tracing in detail and the steps involved in biosynthesis. The enzyme responsible in biosynthesis

of aflatoxin at every stage has been discussed. The discussion on DNA-adduct formation which is the basis of carcinogenic ability of aflatoxin has been included. The chapter on "Species and diet-related differences in aflatoxin biotransformation' focusses on the biotransformation as determined by the species and dietary factors which, in turn, modulate the extent of toxicity of mycotoxins. There was always a confusion about the stage at which the problem of mycotoxin and *A. flavus* starts. This has been very much cleared by reviewing all the research work done in this context under the title "Aflatoxin in maize....." and "Insect interactions with mycotoxin-producing fungi and their hosts". The topic "Aflatoxins: Their biological effects and ecological significance" exposes the seriousness of the aflatoxin problem.

Surprisingly, the book has not reviewed the work on detoxification methods of aflatoxins and other mycotoxins, which is extremely important to the industrialists, food and feed manufacturers and the research workers for improvising the existing methods.

The book is well edited. It is recommended for libraries of the Universities and research institutions which have "Environmental problems" and 'Food safety' in their curricula.

T. SHANTHA
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"International Food Data Bases and Information Exchange" Concepts, Principles and Designs. A.P. Simopoulos and R.R. Butrum (Eds) Published by S. Karger, AG Basel. Allschwilerstrasse 10, P.O. Box Postfach, CH-4009, Basel, Switzerland XII + 160 pp, 22 figs, 21 tabs., hard cover 1992; Price : US $ 149.00, S. Fr 186.

The concept of generating food composition tables is not new and every food research laboratory will have evolved its own data on surveys of food consumption of communities or societies or will try to generate their own source-tables. Doctors and nutritionists, depend on such tables to assess the sufficiency or otherwise of nutrients of diets. Demographers use them for studies of national food policies.

But it is unfortunate, that such data are not freely available. We have only a few sources which provide the information. Food composition tables

prepared by FAO, for different regions of the world, have been the source for many surveys and studies. These have been compiled, on the information provided by many laboratories all over the world. A similar compendium prepared by ICMR. "Nutritive Value of Indian Foods" is more restrictive, but provides data on diverse components of food. However, the information available on recipes and local food preparations, or even on packaged foods is very meagre. The reasons are obvious. The task of compilation of data on such diverse products is indeed colossal, and no one laboratory or institution can ever hope to complete such a task. It is in this context, that computerization of collected data, becomes relevant.

The book under review, provides basis for a classification system under which all foods, both raw materials and prepared foods, could be computerized. If the data are placed on such a system, retrieval of data becomes easy and end users, i.e., the food and nutrition researchers, the food processors and manufacturers, food analysts, demographic surveyors and governments could be benefitted.

The book is divided into 8 chapters and each one is written by different set of authors. The first chapter by one of the editors, Dr. Butrum, presents the scope of the subjects covered and more specifically defines the end users of such data. For example, apart from those mentioned earlier, international agencies, which provide food aid, need to know the nutrients available in the target country, as also, the constituents of the food that they are supplying. The composition of foods in the local market may not be available in any published data. Moreover, even the names of foods and raw materials used in the making of the foods may be in the language of the country and not recognized elsewhere. An international system of codifying foods, thus, becomes manifest.

The second chapter by Srivastava and Butrum, deals with international food data base. The factors which have to be considered in preparing an international data base and in choosing the software which could meet these and other considerations (The chosen software is "Oracle") are described in detail.

This is further amplified in the next chapter by Southgate and Greenfield. Factors for consideration in choosing foods to be included in

the system, are discussed. The nutrients to be included in the system are divided into categories like, high priority, desirable or for future development. The constituents for categorization are the proximate composition, the vitamins, the minerals, fatty acid composition, sugar composition, trace elements, vitamins and other minor constituents.

In defining the function of computers in nutrition sciences, Feinberg et al., observed: "Computer Science also indicates fruitful and scientific ways to organize and improve information management. Nutrition is deeply related to social and cultural habits; this, in turn, influences food science. Computer science provides possible methodological approaches to a more rigorous way of reasoning". Feinberg et al. discussed the data collection and validation of the same, for developing computer models. Standardization of analytical methods amongst different laboratories has first to be achieved before any useful computerization of data is possible. Codification of characteristics of foods and criteria for classification are well exemplified by selecting some foods, both raw and processed. "Langual" which can be translated to mean the language of food, developed by US FDA, to retrieve data on food, is further described in some detail by Hendricks.

The historical development and present status of codifying and recording of food data in Nordic Countries (Anders Moller), in Latin America and Caribbean Islands (Bressani and Flores), and in East European Countries (Dobrzycki, J.H. and Maria LosKuczera), are dealt with, in the following chapters. Perhaps, paucity of information with the editors, has been the excuse for not including any of the Asian or South East Asian Countries in any part of the discussion.

For a country like India, where foods have a wide diversity, based on regional, cultural and ethnic considerations, the data available are very meagre. We have only the "Nutritive Value of Indian Foods" published by the NIN, as the source book. Even this deals more with raw materials than ready-to-serve foods. Computerization and the use of computers in every-day life, are now catching up. Considering the variety and plethora of foods available in the country, it is necessary to prepare such data base tables on Indian foods. But when, preparing such tables, or even when collecting such

data, it is necessary to follow international methods of collections, codification and means of retrieval so that the information will be of universal acceptance.

The book under review, provides an excellent guide for such a system. The authors of the different topics have discussed the subject matter in easily understandable manner, giving concrete examples. The book is a very desirable addition to libraries in food research institutes, home science colleges and even industrial food laboratories, where food analytical data get generated. It is invaluable for those who want to create food analytical data banks, from data generated in other laboratories.

It is needless to comment on the excellent get-up and editing of the book. The price of the book is a deterrent for individuals, but considering the value of information contained in the book, libraries, national and international organizations, and research institutes can ill-afford not to possess the book.

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Isobenzan : Published under joint sponsorship of the United Nations Environment Programme, the International Labour Organisation and the World Health Organisation (WHO) as a part of the IPCS (International Programme on Chemical Safety); World Health Organisation, Geneva, 1992; pp 62; Price: Sw.Fr. 10 and Sw. Fr. 7 in developing countries.

The book is mainly concerned with the environment, health hazards and toxicological effects of the insecticide, Isobenzan. It is a publication of International Programme on Chemical Safety (IPCS) under the series: Environmental Health Criteria 129, published jointly by above agencies. This is a small monograph of 40 pages of running matter with about six pages of various references (88 in number) at the end. I am particularly delighted to read the summary, conclusions and recommendations in a nutshell at the beginning of the text running over three pages with its translation into two international languages at the end.

Isobenzan is a chlorinated cyclodiene insecticide,

which was marketed under the trade mark Telodrin. It was manufactured by Shell in Netherlands during 1958-65 and was used for several years thereafter from the existing stocks. At present, the only major source of exposure is believed to be the original waste disposal sites of industrial wastes or dredgings from contaminated sediments. However, no isobenzan was detected in the rivers of Netherlands during 1979-80 and the only source feared to be existing might be root crops.

It seems that all efforts were made to present the data as accurately as possible in the book. Studies were made for the toxicological effects on rats, mice, dogs, domestic fowl and cattle. The studies were extended to two or three generations in case of rats, mice and dogs. The milk of the cattle grazing in the pastures treated with isobenzan, and eggs of the leghorn hens were considered to be potential sources of contamination and were analysed for isobenzan.

Data on human beings were limited to studies on workers and operators exposed to isobenzan in manufacturing and formulation plants. No cases of skin irritation and carcinogenicity were reported. No data are available on the levels of isobenzan in the blood or adipose tissues of general population. Poisoning incidents or untoward effects of long term exposure of general population have not been reported. The monograph concludes with the words that the available information on the hazards of isobenzan is incomplete but is adequate to say that no human or environmental exposure of this substance, used as an insecticide or for any other purpose, should be allowed.

Though the toxicological effects of isobenzan may not be persistent today, the information contained in the book is a manifestation of hazardous nature of organo-chlorinated insecticides, and the histological or clinical tests one has to carry out to evaluate the hazardous nature after making any such insecticide or pesticide. Hence, the book is useful for organochemical industry, research workers engaged on chlorinated insecticides and to those organic chemists and Government officials involved in formulating the regulatory aspects of pesticides and insecticides.

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Safe Use of Pesticides : WHO Technical Report Series 813; WHO, Geneva-1991; pp. 28; Price: SW fr.6 SW. fr 4.2 in developing countries.

This booklet is the fourteenth report of the WHO Expert Committee on Vector Biology and Control and contains recent developments (till 1990) in the toxicology of pesticides used in vector control. The information is presented under 11 titles; Introduction, Trends in pesticide use, International activities for promoting pesticide safety, The WHO Pesticide Evaluation Scheme (WHOPES), Current research on pesticides for use in public health, Aircraft disinfection, Exposure of the public to pesticides, Classification of pesticides, Education and training, Poisoning by pesticides, Conclusions and Recommendations. This is followed by Annexure 1, wherein details of treatment of poisoning due to organophosphorus, carbamate and organochlorine insecticides, anti-coagulant rodenticides and paraquat are given.

Some of the main conclusions and recommendations of the expert committee include a) chemical pesticides will continue to play a dominant role in disease vector control in the near future, b) the pyrethroid insecticides seem to be free from adverse effects if normal precautions are taken, c) permethrin added to drinking water as a larvicide at a concentration of 15 μg/l is considered safe. Substituted urea larvicides should not be added to drinking water until the possibility of the diabetogenic side effect has been excluded, d) the testing of pyrethroids other than already recommended for aircraft disinfestation should be encouraged, e) non-absorbent containers of active ingredients of pesticides and formulations in Hazard class III can be reused after a serial washing for purpose other than storage of food, drink and animal feed, f) the problem of acute pesticide poisoning is serious in some countries and urgent action is required and g) poison control centres should be strengthened so as to have information available on poison control and to deal with pesticide poisoning at all times.

In addition to these general recommendations, there are recommendations made to WHO. A simple toxicological study to test for the synergism between deet and permethrin and biomonitoring that may lead to new methods for measuring exposure to pesticides and its effects are recommended for future research. The booklet has 24 references of importance and a list of selected WHO publications of related interest.

In view of the diverse and authentic information covered in the document, there is no doubt that it is a ready reckoner on vector control and useful for research workers, pesticide handlers and policy makers.

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Starch Hydrolysis Products : World Wide Technology Production and Applications : by F.W. Schenck and R.E. Hebeda (Eds), VCH Publishers, Inc. New York, 1992, pp: 650, Price: £ 84

This book presents complete up-to-date details on various aspects of starch hydrolysis products and covers aspects such as production, analysis, quality control, metabolism, regulatory aspects, downstream processing, plant design, industry, economics and waste treatment. This book is the first in the Food Science and Technology series to be published by VCH publishers. Hydrolysis products of starch are mainly sweeteners and many articles and books have been published on specific hydrolysis products, manufacturing process and the equipment used for their production. This is the first book where complete coverage of all facets of this complex industry has been nicely brought out. This book written by international experts contains extremely useful information on hydrolysis products of starch. It gives enough details in terms of principles and actual methodologies involved and fulfils the objectives set and the target groups for which it is meant.

The contents of the book are organized in 21 chapters. The opening chapter gives an excellent introductory survey on the history of starch hydrolysate production, along with a worldwide list of starch hydrolysate products. Chapter 2 deals with sources, production and chemistry of starch. Various commercial enzymes used for the hydrolysis of starch for the production of specialized products such as glucose syrups, maltose syrups, high fructose corn syrup and cyclodextrins are described in chapter 3 followed by the details of the processes and equipments used in the hydrolysis of starch in chapter 4. Chapters 5 to 11 deal with individual products of starch hydrolysis. In each of these chapters, a general introduction to the product, description of the production methodology, flow diagram, the physico-chemical and physiological properties of the product, their commercial applications in various industries and practical aspects of storing, shipping and handling of the

product are described in good detail.

Chapter 12 deals with various analytical methods involved in the quantitative analysis of hydrolysed products of starch. The metabolism and metabolic effects of products of starch hydrolysis, with special reference to absorption and digestion in animals and humans are described in chapter 13. Toxicological and physiological effects of hydrolysis product on ingestion are also briefly mentioned in this chapter.

In many countries, starch-derived products for various applications have become, subject to requirements of law. Few people are aware of the existence of government regulatory organizations to enforce laws relating to the use of these products. The legal aspects covering the governmental regulations that make these products uniform around the world are described in chapter 14. The economic aspects of manufacture are covered in chapter 15, while chapter 16 gives the details of production facility, design and construction.

The next 4 chapters deal with downstream processing aspects involved in the production of starch hydrolysate products. All the equipments involved in clarification, concentration, refining and chromatographic separation are described in detail, with illustrations, design and flow charts. Waste water treatment and disposal are of critical importance in any industrial set up. The last chapter gives an overview of waste treatment, as it applies to the manufacture of starch hydrolysis products. The generation of starch hydrolysis-related waste water and the available waste water disposal operations, treatment applicable to the starch hydrolysis industry and sludge handling and solid waste disposal are described in this chapter. The book ends with an appendix wherein worldwide suppliers of equipment, ingredients and services are listed.

The book covers all major aspects and is well presented. The extensive references given at the end of each chapter are comprehensive. In conclusion, I would like to emphasize that this book is a valuable source of information of immense practical interest, It may also remain as a very useful reference book for many years. I strongly recommend this book to all scientists and technologists who would be interested in getting a quick and authoritative glimpse of the products of starch hydrolysis.

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INSTRUCTIONS TO AUTHORS

Manuscript, in triplicate should be typed/printed in double-space on one side of A_4 size/bond paper, leaving 2.5 cm margin on all four sides of the page. The data reported in the manuscript must be original with clear definition of objectives, material used, methods employed and without repetition. It should not have been published or offered for publication elsewhere. The manuscript must be as per format of the journal and authors should consult a recent issue of the journal for style and layout. The manuscript will be returned to authors, if it departs in any way from the required format and style. Papers essentially of an advertising nature will not be accepted. Footnotes for text are to be avoided. All submissions will be reviewed by two referees and an appropriate editorial board member.

Four different types of papers are published :

1. Research Papers with a maximum length of 16 manuscript pages, including figures, tables and references.

2. Research Notes which are limited to a maximum length of 8 manuscript pages, all inclusive.

3. Rapid Communication of the size of the maximum length of 10 manuscript pages (all inclusive) will be published rapidly, out of order of submission. Such communications must be based on new results of impact-making quality. The authors have to append a note, indicating novelty, implications of the results and urgency in publication. The editor reserves the right to decide, what constitutes a Rapid Communication.

4. Reviews on specific topics of higher utility and current trends (by invitation only)

Materials and Methods must give sufficient details for the work to be repeated. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Any hazard must be mentioned and the relevant safety precautions described or reference made to safety procedures. The use of proprietary names should be avoided.

Each paper should be provided with an abstract of maximum ten line length of the manuscript, reporting concisely on the principal findings of the paper and in the form acceptable to abstracting agency. This should be followed by upto 6 keywords and this should include all the main topics incorporated in the paper including any already given in the title.

The manuscript of the research paper should be divided into sections viz. Abstract, Introduction, Materials and Methods, Results and Discussion and References. The research notes will be without these sections, except for Abstract and References. The chemicals are to be referred by names and not by formula in the text. All vernacular names e.g., *pulav, puree, chapati* must be underlined and briefly described when appearing for the first time. Similarly, all varietal names such as 'IR 150', 'Pb-56' must be put in single inverted commas.

The title of the paper is to be typed in capital and small letters for all types of papers. Authors' names should be in capital and the affiliation in capital and small letters. This should be followed by Abstract and Introduction (without heading).

Tables, numbered consecutively with Arabic numbers are to be typed on separate sheet and placed after references section. No vertical lines be used and the table should not have more than 12 columns. Nil results should be indicated by using ND (not detected), while absence of data sign, '–'.

Graphs and line drawings must be in a style and standard of draughtsmanship. These should be drawn in Indian ink, with stencilled lettering, on tracing paper or white drawing paper or preferably art paper. The lettering should be twice the size of the printed letter. Photographs should be submitted as clear black-and-white prints on glossy paper and must have good contrast. High quality computer-generated line diagrams or glossy prints are also acceptable. Legends for all the figures are to be typed on separate page with details of symbols. The graphs, line drawings and photographs must be protected adequately against damage and bending of the envelop during transit. The manuscript will be returned to authors, if these requirements are not satisfied.

In case of sensory evaluation results, the objectives should be stated clearly. Adequate details and references should be provided of the sensory evaluation methods used, along with the details of the collection, analyses and interpretation of data. The experimental designs used are to be clearly stated, e.g. randomized block, Latin squares, factorials, fractional factorials, incomplete blocks, etc. The source of panel, whether in-house or from outside is to be indicated. The number of panelists should be stated along with the composition (age, sex, status etc.) of the panel. It is necessary to state that same or different panel has participated in different sensory evaluation trials. The data involving less than 15 panelists and also where the panelists are not trained to function as a human analytical instrument (with periodic re-orientation) as well as the members without required sensitivity are not acceptable. For effective tests, the data involving less than 200 panelists and without information on the target population selected are unacceptable.

Sensory evaluation studies should report the data on physical and environment factors (taste booth type, colour of walls/separator, extent of distraction from external sound/odour, room temperature, relative humidity, illumination specification etc. The equipment and methods of sample preparation, testing temperature conditions, sample size, number of samples evaluated per session, nature of palate clearing agents used, time of evaluation, sequence of testing and data entry carriers should be reported. For affective test, the location of testing (stores, homes, central location etc.), the instructions given regarding sample preparation/processing and the questionnaire used for collection of data should be specified.

Statistical procedure used for handling sensory evaluation data should be clearly indicated including any transformations or deviations that are carried out, e.g. assignment of numbers to intervals, categories and the like. The type of analysis carried out, categories, the level of significance and the decisions made are to be provided with appropriate tables and graphs. Appropriate and adequate data on the tests of significance, (like F, x^2, t, r, Rank sum, Mannwhitney, Rank Correction etc.) should be provided to justify conclusions and enable repeatability. The probability levels, degrees of freedom, the observed value of the test criterion, the direction of the effect and the decision based on these are to be indicated.

References should be cited at the appropriate point in the text by giving authors' names and year in bracket i.e. (Smith 1990; Smith and Gibbons 1990; Smith et al. 1992) as per Harvard System. A list of references, in alphabetical order, should appear at the end of Results and Discussion section. In case of single author reference, the alphabetical arrangement should be by date while it should be by co-author when the reference with one co-author is cited. The alphabetical arrangement should be by date in case of the reference with more than one co-authors. Abbreviations such as *et al., ibid, idem* must be avoided. The titles of all scientific periodicals should be abbreviated as in *Chemical Abstracts, Biological Abstracts, Annual BIOSIS List of Serials* and *Serial Sources for the BIOSIS Data Base.* If the journal is not included in these sources, then the name of the journal is to be given in full. Unpublished data or private communications should not appear in the list, but can be indicated in the text. The examples of layout of typical references are as below :

Published papers/notes/reviews

a) Tairu AO, Omotosu RA, Bamiro FO (1991) Studies on oxidative stability of crude and processed yellow nutsedge tuber and almond seed oil. J Food Sci Technol 28:8-11

Books/approved methods

a) Hacking AJ (1986) Economic aspects of Biotechnology. Cambridge University Press, Cambridge.

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

Chapters in edited books/book series/papers in symposium proceedings/souvenir

a) Kurtzman CP, Phaff HJ, Meyer SA, (1983) Nucleic acid relatedness among yeasts. In: Spencer JFT, Spencer DM, Smith ARW (eds) Yeast Genetics, Fundamental and Applied Aspects. Springer-Verlag, New York, pp 139-166

b) Gross E (1975) Subtilin and nisin: The chemistry and biology of peptides with $\alpha - \beta$-saturated amino acids. In: Walter R, Merenhoper J (eds) Peptides, Chemistry, Structure and Biology: Proceedings of the Fourth American Peptide Symposium, Ann Arbor, Michigan, USA, pp 31-42

c) Bhalerao SD, Mulmulay GV, Potty VH (1989) Effluent management in food industry. In : Souvenir, National Symposium on Impact of Pollution in and from Food Industries and its Management. Association of Food Scientists and Technologists (India), Mysore, pp 1-31

Reports by specified authors/institutions

a) Andress EL, Kuhn (GD (1983) Critical Review of Home Preservation Literature and Current Research, Co-operative Agreement No. 12-05-300-553. USDA and Pennsylvania State University, Pennsylvania.

b) USDA (1977) Home Canning of Fruits and Vegetables, Home and Garden Bulletin 18, United States Department of Agriculture, Washington, DC.

Patents

a) Schmidt GR, Means WJ (1986) Process of preparing algin/calcium gel-structured meat products. US Patent 4 603 054

Thesis

a) Ramesh MV (1989) Production of heat stable alpha-amylase. Ph.D. Thesis, University of Mysore, Mysore, India.

Papers presented at symposia

a) Stevens KA, Klapes NA, Sheldon BW, Klaenhammer TR (1991) Anti-microbial action of Nisin against *Salmonella typhimurium* lipo-polysaccharide mutants. Paper 7-501 presented at 91st American Society for microbiology, Annual Meeting, Dallas, Texas, USA, 5-9 May.

Corresponding author will receive a set of page-proofs for correction of type-setting errors. No other major changes in the paper should be made at this stage.

There are no page charges. 25 off-prints of the paper are provided free of charge to first author of each published paper. Additional off-prints can be ordered, on payment, once the paper is accepted.

SPECIAL ANNOUNCEMENT
3rd International Food Convention
IFCON 93
7-11 September, 1993 at Mysore

Your are already aware from our first circular that the IFCON 93 was scheduled to be held between 5-9 July, 1993. As this period coincides with many important National and International Conventions, the National Steering Committee after deep consideration, has postponed IFCON 93 to 7-11 September 1993. Please make a note of the revised dates for registration, which are indicated below:

Class of Registration	Registration Fee upto 31-3-1993	Registration late fee upto 31-8-1993
AFST Members	Rs. 300	Rs. 500
Non-Members	Rs. 600	Rs. 800
International Participants	US $ 250	US $ 350
Students	Rs. 200	Rs. 300

No Registration fee will be accepted after 1st September 1993.

The Registration Form is attached herewith for the convenience of those who have not received the folder. Please send the duly filled registration form along with the requisite fee in the form of DD/cheque drawn in favour of IFCON-93 to the Secretary, AFST(I), CFTRI Campus, Mysore-570 013 (India) at the earliest. The second circular, giving the details of technical sessions, format for submission of poster presentations, reservation of accommodation, travel, etc., will be issued during March'93.

REGISTRATION FORM

Name (in Capital Letters) Prof./Dr./Mr./Mrs./Ms. ....

Affiliation .... Title ....

Postal Address ....

City .... Postal Code ....

State/Province .... Country ....

Name of Spouse/Guest(s) ....

Telephone (Office) .... (Res.) ....

Telex .... Fax. ....

Registration   US $/Rs. ....

Spouse/Guest(s)   US $/Rs. ....

                                            Total

                                            ....

Enclosed DD/Cheque No. ....

Date .... Bank ....

for US $/Rs. ....

Payable to IFCON-93.

Date ....

I intend to present a paper in the Poster Session   YES   NO

                                                            Signature