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ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA)

MYSORE - 570 013

Affiliated to the Institute of Food Technologists, USA

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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.

The ultimate objective is to serve humanity through better food.

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7-11 September, 1993 at Mysore

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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Volume 29 Number 6 Nov./Dec. 1992

CONTENTS

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Biochemical Changes During Fermentation of Cocoa Beans Inoculated with *Saccharomyces cerevisiae* (Wild Strain)

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The overall pH profile of the cotyledon in the inoculated beans during fermentation was slightly higher than the control beans. The percentage of glucose remaining in the pulp of both the inoculated and control beans at the end of fermentation were 20% and 3% respectively. Ethanol and acetic acid production are generally higher in the inoculated beans as compared to the control beans. The intensity of chocolate flavour obtained from the inoculated and control beans was almost the same. However, in the inoculated beans, bitterness was reduced by 28%.

The acids and flavour produced during fermentation determine the overall quality of cocoa beans. Cocoa produced by Malaysia and Brazil are described in the world market as being characteristically acidic. Attempts have been made to overcome the acidity problems by means of aeration or regular turning over, of the bean mass using the shallow box fermentation or extracting the pulp sugar. Although these techniques might reduce the pH of the beans, the ultimate result would be a weak cocoa flavour. It appears that controlling of microbial activities is of significant importance in minimizing acidity problems. Under natural fermentation conditions, the dominant organisms play a major role in acids formation. In a majority of studies of this nature, the results always indicated that acetic and lactic are the major acids produced by a mixed flora[1]. Very little work had so far been done on the effect of employing a selective organism for cocoa fermentation. This paper illustrates the flavour potential including the changes in pH, glucose and other products formed during the cocoa fermentation using yeast inoculum as compared to a normal mixed flora fermentation.

Materials and Methods

Cocoa pods of mixed 'Sabah' hybrid, obtained from the estate of University of Agriculture, Malaysia, were processed by two methods: (i) the beans were inoculated with *Saccharomyces cerevisiae* (wild strain) and (ii) beans were left under a natural fermentation process as control. The yeast culture was obtained from cocoa beans that have been fermented for 5 days and identified by standard method[2]. It was propagated and maintained on Sabouraud Dextrose Agar (Merck, Darmstadt,Germany) and suspended in sterilized saline solution (0.85% NaCl) for adjusting the cell concentration to $1x10^6$ cells ml^{-1} and 20 ml of this suspension was then sprayed on to fresh cocoa beans weighing approximately 10 kg. Both the inoculated and control beans were placed separately in plastic bags (60x35 cm) with round holes (0.5 cm in dia), 2 cm apart on all sides instead of the traditional shallow fermentation box[3]. They were left to ferment 6 days in a dark room.

The temperature of beans mass was recorded at 24 h intervals. Ten grams of bean samples of both cotyledon and pulp were mechanically crushed for 3 min in 100 ml solution of 0.2% (w/v) benzoic acid and the pH measured. The respective solutions were filtered and the suspensions obtained were centrifuged at 12000 g for 30 min at 4°C (Model JA-2, Beckman centrifuge, USA). The supernatant was then filtered through a millipore filter (0.45 μm pore size membrane filter).

Acetic acid and ethanol were analysed by GLC in accordance with the procedure of Henderson and Steedman[4] (Pye Unicam 204 series, USA, steel column; porapak Q, 80-100 mesh, oven temperature: 180°C; injector and detector temperature: 200°C; gas carrier: flow rate of 40 ml nitrogen min^{-1}). Non-volatile organic acids were determined by HPLC (Shimadzu LC-6A, Chromatopac, Japan) fitted with a variable detector and injector (column: Hibar RP-18, 25x4.6 mm; mobile phase: water + H_2SO_4 0.008 N, pH 2.8, flow rate: 0.8 ml min^{-1}; the detector programmed at 230 nm, oven temperature at 40°C, gas carrier: 0.5 lb compressed air sq in^{-1}). Ethanol as well as non-volatile and volatile acids were determined from peak areas by referring to a standard curve prepared with known amounts of pure ethanol or acids.

Glucose was determined by total carbohydrate assay method[5]. Sensory evaluation test was conducted in accordance with the procedures established by MARDI Cocoa and Coconut Research Division, Malaysia[6]. All chemicals were of analytical reagent grade, obtained from Oxoid, Difco, Merck and Sigma.

Results and Discussion

pH changes: The overall pH profile in pulp and cotyledons in both the techniques was found to be similar, but the pH of the control (normal) beans was lower compared to the inoculated beans (Fig.1). Liau[7] reported that the internal pH of fermented beans was directly correlated to the acetic acid concentration in the beans.

Sugar content: The percentage of glucose decreased rapidly throughout the fermentation process (Fig.2). In the early stages of fermentation, there was a high concentration of sucrose present, but it decreased rapidly as the fermentation process proceeded. Sugars, in the pulp of cocoa beans, provided carbon sources for microbial activity. Because of low pH, higher sugar content and anaerobic environment in the pulp during the initial stages of fermentation, yeast activity is favoured, which converts sugars in the pulp into alcohol.

The glucose in the cotyledons increased when fermentation began (due to inversion) and reached a maximum stage on the second day of fermentation. After the first turning over which occurred on the second day of fermentation, it dropped gradually until the fourth day. The

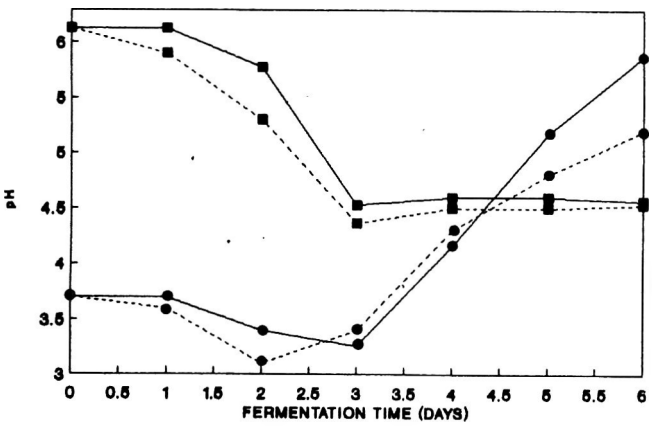

Fig.1. pH profile in the pulp and cotyledon of the beans inoculated with *S. cerevisiae* (wild strain) (—) and control (---). (■) cotyledon, (●) pulp.

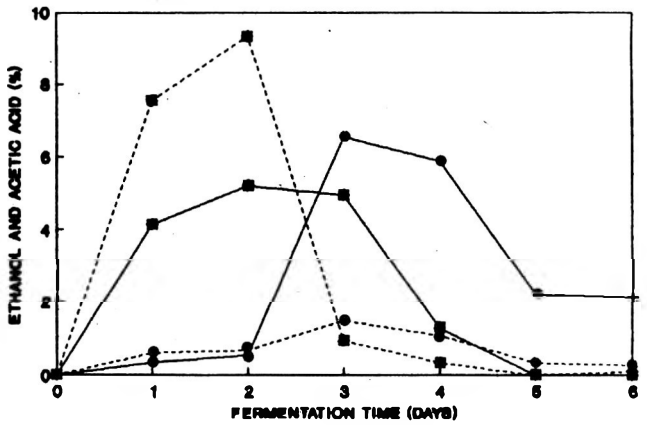

Fig.3a. The amount of ethanol and acetic acid present in the pulp (---) and cotyledon (—) of the bean inoculated with *S.cerevisiae* (wild strain). Ethanol and acetic acid production was expressed as percentage based on g of ethanol or acid produced/g wt of beans. (●) acetic acid. (■) ethanol.

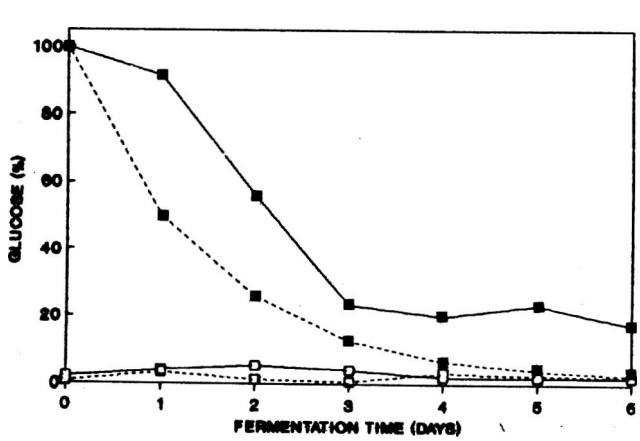

Fig.2. The level of glucose present in the pulp and cotyledon of the beans inoculated with *S. cerevisiae*. wild strain (—) and control (---). The amount of glucose was expressed as percentage of the amount in g/10 g of beans. (□) cotyledon, (●) pulp.

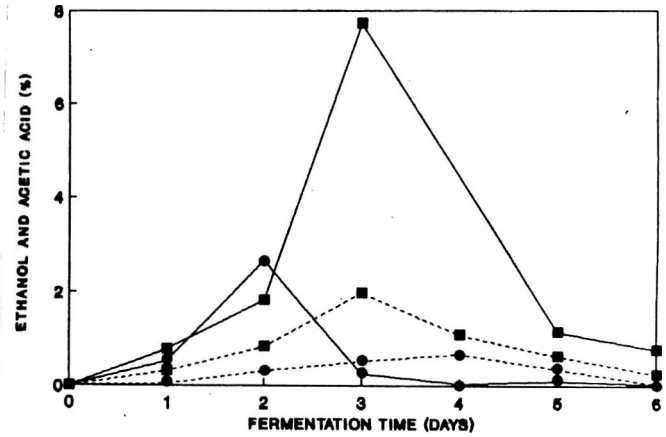

Fig.3b. The amount of ethanol and acetic acid present in the pulp (—) and cotyledon (---) of the control bean. Ethanol and acetic acid production was expressed as percentage based on g of ethanol or acid produced/g wt of beans. (●) acetic acid, (■) ethanol

TABLE 1. LEVELS OF ORGANIC ACIDS IN COCOA BEANS DURING FERMENTATION

Fermentation period, days	Salts of organic acids, mg/g dry cotyledon											
	Oxalate		Tartarate		Malate		Lactate		Citrate		Succinate	
	A	B	A	B	A	B	A	B	A	B	A	B
1	0.030	0.082	UD	UD	UD	0.0078	0.00014	0.417	0.043	0.622	0.022	0.521
2	0.013	0.078	UD	UD	0.072	0.00159	0.00085	0.256	0.0095	0.792	0.036	0.364
3	0.023	0.084	0.0099	0.677	0.041	0.105	0.040	0.993	0.475	0.325	0.023	0.320
4	0.034	0.065	0.116	1.709	0.083	0.815	0.077	2.280	0.303	0.635	0.057	0.861
5	0.024	0.063	0.0260	0.498	0.411	0.498	0.511	1.091	0.216	0.487	0.167	0.318
6	0.029	0.078	0.029	0.518	0.320	0.312	0.085	2.016	0.582	0.569	0.070	0.843

UD: Undetectable, A: Fermented with wild strain of *S. cerevisiae*, B: Control

increase of pH in the pulp (Fig.1) and also decrease of acetic acid (Fig.3a) during the third day of fermentation were in agreement with the above findings. It was also observed that the percentage of glucose in inoculated beans with yeast (wild strain) was slightly higher than control beans. This may be due to the oxidation of alcohol produced by yeast to acetic acid by the *Acetobacter.*

Production of ethanol and acetic acid: Ethanol level in the pulp of the beans inoculated with yeast reached a maximum level on the second day of fermentation and declined thereafter (Fig.3b). The ethanol concentration was higher (95%) than in the cotyledon (57%), but after the third day, it was approximately the same in both samples. The acetic acid was at the maximum level during the third day of fermentation. Maximum temperature of the bean mass observed was 46°C which occurred during the third day of fermentation. Generally, acetic acid is an essential component of the fermentation process as the acid contributes to bean death, prevents colonisation by microorganisms and creates environment conducive to the formation of flavour and aroma precursor within the bean cotyledon[8].

Other organic acids such as oxalic, tartaric, lactic, malic, citric and succinic were produced during fermentation (Table 1). The data showed no consistent pattern. Thus, the concentration of individual acids after their initial production may fluctuate considerably.

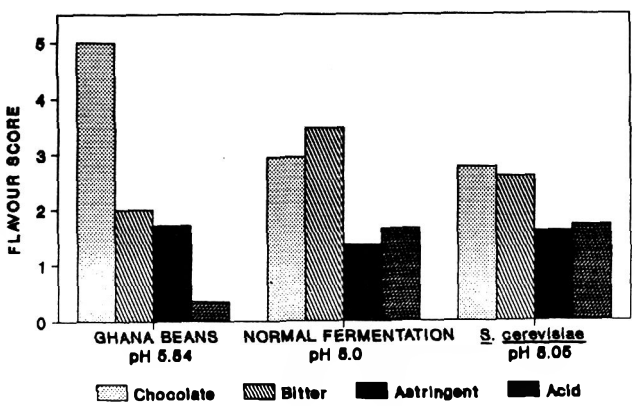

Fig.4. Mean of cocoa flavour score.

Flavour intensity analysis: Fermentation is one of the most important processes for the development of cocoa aroma and chocolate flavour. Fig 4 shows some of the parameters which affected the flavour within the Malaysian cocoa beans as compared to Ghana cocoa beans. The intensity of the chocolate flavour in Malaysian beans is low because of the acidic taste, astringency and bitter flavour. Production of chocolate flavour depends on many factors such as pH, hydrolytic enzyme activity and the fermentation process[9]. Quesnel[10] has shown that the C_3 to C_5 volatile fatty acids produced towards the end of fermentation and also during the drying phase, may also reduce the flavour.

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J. Fd. Sci. Technol, 1992, Vol. 29, No. 6, 344-347

Occurrence of *Bacillus cereus* and Other *Bacillus* Species in Indian Snack and Lunch Foods and Their Ability to Grow in a Rice Preparation

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***Bacillus brevis, B. cereus, B. circulans, B. coagulans, B. laterosporus, B. licheniformis, B. pumilus, B. stearothermophilus* and *B. subtilis* were isolated from Indian snack and lunch foods. Higher count of 4.2 $\log_{10}$ (cfu/g) and a lower count of 2.6 $\log_{10}$ (cfu/g) were observed in *bisibele bhath* and *uppuma*, respectively. The isolated cultures were positive for production of either one or more of the following: hemolysins, phospholipase, protease, lipase and amylase. A few selected cultures of *Bacillus* species occurring as post-processing contaminants in plain cooked rice reached cell populations which were sufficient to cause health hazards. These findings indicate the significance of *Bacillus* species as post-processing contaminants in processed foods.**

Several kinds of popular indigenous snack and lunch items of foods are being catered to a large group of consumers in our country. The various steps involved in the preparation and distribution of these foods provide ample scope for contamination with pathogenic and spoilage microorganisms. As a majority of these foods are cereal and/or pulse-based and involve restricted heat treatments, the commonly encountered contaminants are mainly *Bacillus* species. *Bacillus cereus* has been recognised as an agent of foodborne disease in early 1950s[1]. The close association of *B.cereus* with rice preparations (cooked, fried and dried products) have been well documented[2-5]. Besides, other *Bacillus* species namely, *B. licheniformis, B.subtilis* and *B. pumilus* have been implicated in foodborne illnessess[6] and it may not be prudent to ignore their role as causative agents. However, the low incidence of *B.cereus* poisoning may be attributed to the little attention it has received, as only small numbers of *B.cereus* are shed in the faeces following intoxication[6].

Only one report exists on the presence of *Bacillus* species in indigenous foods which are consumed on a large scale[7]. The present study reports the occurrence of *B. cereus* and other *Bacillus* species in a few selected Indian snack and lunch foods and their ability to grow in a rice preparation.

Materials and Methods

Enumeration of Bacillus *in foods:* Samples of *idli, vadai, uppuma*, plain cooked rice, curd rice and *bisibele bhath* from catering centres of institutions, restaurants and railway canteen located in Mysore City, were collected in sterile polythene bags (Stomacher Lab. blender bags), brought to the laboratory in an ice-box within 60 min of collection and enumerated for *Bacillus*. Fifty grams of sample was blended in a Stomacher using 450 ml sterile 0.1% peptone water, appropriate dilutions of the sample were surface inoculated in triplicate on pre-poured plates of polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA)[8] and incubated at 37°C for 20 to 36 h. Typical colonies of *Bacillus* appearing on the plates were counted and expressed in $\log_{10}$ colony forming units per gram (cfu/g).

Isolation and characterisation of Bacillus *cultures:* Distinct individual colonies appearing of PEMBA were isolated at random with preference to those colonies which had fimbriate margin and peacock blue colour with a surrounding zone of precipitation. Isolates were maintained on nutrient agar slants and sub-cultured at 15 day intervals.

Isolates were identified by morphological, cultural and biochemical characteristics[9]. The morphological tests included appearance of cells, Gram's reaction, motility and presence and position of spores. Isolates were tested for their growth in anaerobic agar, 7% NaCl, at 45, 55 and 65°C and for the Voges-Proskauer reaction. The biochemical characteristics included production of catalase and lecithinase, nitrate reduction, citrate utilisation, hydrolysis of starch, gelatin and casein and acid from refined sugars - glucose, mannitol, xylose and arabinose.

Production of extracellular enzymes: One isolate each of identified *Bacillus* species was grown in 20 ml brain heart infusion (BHI) broth for 20 h at 37°C, centrifuged aseptically at 8000 rpm for 30 min and the supernatant was stored in pre-sterilised screw capped tubes at 6°C, 50 µl was used for determining the activities of different extracellular enzymes by well-assay plate method (5 mm diameter wells) and the results expressed as zone diameter in mm. Productions of hemolysin, phospholipase, protease, lipase and amylase were tested in blood agar, PEMBA without polymyxin, milk agar, tributyrin agar and starch agar, respectively. For phospholipase activity, the diameter of opacity formed around the individual wells[10], while the diameters of the zone of clearance around the wells in blood agar, milk agar and tributyrin agar plates were measured. In case of starch agar plates, zone diameter of hydrolysis formed on exposure to iodine vapours was measured. In all these tests, the activity was categorised on the basis of zone diameter, less than 10 mm as weak, 10 to 15 mm as moderate, 15 to 20 mm as strong and more than 20 mm as very strong.

Growth of Bacillus *species in plain cooked rice:* The test cultures included one isolate each of the identified species, *B.brevis, B.cereus, B.licheniformis, B.laterosporus and B.stearothermophilus.* A cell suspension of $1x10^2$ cfu in 1.0 ml normal saline was prepared[11] and inoculated into 100 g lots of plain cooked rice and placed in pre-sterilised glass beakers. Two types of cooked rice were used: (i) laboratory prepared rice, taking care to avoid post-processing contamination and (ii) cooked rice collected from an institutional catering centre. Inoculated and uninoculated (control) rice samples were stored at ambient temperature (15-26°C) and analysed for counts of *Bacillus*[8], mesophilic aerobes and enterobacteriaceae[12] and pH values at 12 and 24 h. Fresh samples of both the types of rice, prior to inoculation were also analysed for the respective bacterial counts. Bacterial colonies appearing on the respective incubated plates were counted and expressed in $\log_{10}$ (cfu/g).

Results and Discussion

Counts of Bacillus *species and their identification:* Counts of *Bacillus* species in the samples of snack and lunch foods did not show much variation (Table 1). A higher count was recorded in *bisibele bhath* in contrast to the lowest in *uppuma*, while *Bacillus* was absent in plain cooked rice. Out of 120 isolates of *Bacillus* obtained from the samples, 39 isolates were characterised to their species namely, *B.brevis* (6), *B.cereus* (2), *B.circulans* (4), *B.coagulans* (1), *B.laterosporus* (6), *B.licheniformis* (8), *B. pumilus* (2), *B.stearothermophilus* (3) *and B.subtilis* (7). The distribution of these species in relation to their sources is also shown in Table 1. The remaining 81 isolates had doubtful reactions.

In the absence of any reports on the incidence of *Bacillus* species in indigenous snack and lunch foods, the counts observed cannot be viewed either as very high or very low. Earlier studies [2,4,5,13] on *B. cereus* in selected dried food products, including different pulses and cereals, boiled and fried rice have shown the counts in the range of 2.0 to 5.0 $\log_{10}$ (cfu/g) as against those of more than 2.0 logs in 28 samples of various foods collected from Chinese "take-out" restaurants which were poorest in overall bacteriological quality[3].

In a study[7] conducted on outbreaks of food poisoning over a period of 5 years (1981-86) in Maharashtra, an average *B. cereus* count of 7.5 $\log_{10}$ (cfu/g) was recorded in samples of left-over foods such as cooked rice and *pulav* (spiced fried rice) which were implicated in food poisoning outbreaks. *Bacillus* counts recorded in the present study were on a lower side, as these foods were obtained from different catering centres and not involved in any food poisoning outbreak. Only two isolates of *B.cereus* were obtained, while there was a predominance of

TABLE 1. INCIDENCE AND DISTRIBUTION OF *BACILLUS* SPECIES IN INDIGENOUS SNACK AND LUNCH FOODS

Food sample	No. of samples analysed	pH of sample	*Bacillus* counts			Identified species
			Min.	Max	Aver.	
			[$\log_{10}$ (cfu/g)]			
Idli	8	5.6	3.3	3.7	3.5	*B. brevis (1); B. licheniformis (1); B. subtilis (1); B. stearothermophilus (1).*
Vadai	7	6.1	2.0	3.2	2.6	*B. licheniformis (1)*
Uppuma	10	6.0	2.5	4.4	3.4	*B. circulans (1); B. coagulans (1); B. licheniformis (2); B. subtilis (2).*
Plain cooked rice	6	7.1	Nil	Nil	Nil	Nil
Bisibele bhath	11	6.6	2.6	5.8	4.2	*B. brevis (4); B. cereus (1); B. laterosporus (3); B. Pumilus (1); B. stearothermophilus (1); B. subtilis (1).*
Curd rice	6	5.1	3.1	3.7	3.2	*B. brevis (1); B. cereus (1); B. circulans (3) B. laterosporus (3); B. licheniformis (4); B. pumilus (1); B. stearothermophilus (1); B. subtilis (3).*

Figures in parentheses indicate number of isolates of each *Bacillus* species

B. licheniformis, B.subtilis, B.laterosporus, B.brevis and *B. circulans* which are known to bring about spoilage of food. Other *Bacillus* species such as, *B.licheniformis, B. subtilis* and *B.pumilus* have also been implicated in foodborne illnesses[6].

Production of extracellular enzymes: Cultures of *B.cereus, B.laterosporus, B.licheniformis* and *B.stearo-thermophilus* were positive for production of hemolysins and phospholipase, with the activity ranging from moderate to very strong, indicating their toxigenic potentiality (Table 2). Apart from *B. cereus,* the production of hemolysin and phospholipase by *B. licheniformis* indicate their role in foodborne illnesses as reported earlier[6]. Cultures of *B. pumilus* and *B. subtilis* were only positive for production of hemolysins and negative for phospholipase. These two characters are considered as indicators of toxin production along with other positive animal model tests[10].

TABLE 2. PRODUCTION OF EXTRACELLULAR ENZYMES BY *BACILLUS* SPECIES

Cultures	Production of				
	Hemolysin	Phospholipase	Protease	Lipase	Amylase
	(Zone diameter, mm*)				
B. brevis	Nil	Nil	20	Nil	20
B. circulans	Nil	Nil	17	20	Nil
B. coagulans	Nil	Nil	Nil	20	Nil
B. cereus	22	20	16	Nil	20
B. laterosporus	20	14	16	Nil	Nil
B. licheniformis	20	18	16	Nil	Nil
B. pumilus	16	Nil	Nil	10	Nil
B. stearothermophilus	18	14	10	14	12
B. subtilis	20	Nil	10	17	14

*Includes initial diameter of agar well (5 mm)

Degree of activity: < 10 mm = Weak; 10 - 15 mm = Moderate; 15 - 20 mm = Strong; > 20 mm = Very strong

TABLE 3. GROWTH OF *BACILLUS* SPECIES IN PLAIN COOKED RICE

Bacillus culture inoculated	Bacterial counts, $\log_{10}$ (cfu/g)					
	Bacillus		Mesophilic aerobes		Enterobacteriaceae	
	Storage period (h)					
	12	24	12	24	12	24
	Plain cooked rice of Type (i)					
Control	<2.0	2.3	<2.0	3.2	<2.0	<2.0
B. brevis	4.3	7.9	<2.0	2.8	<2.8	<2.0
B. cereus	3.9	7.0	<2.0	2.8	<2.0	<2.0
B. laterosporus	5.1	7.1	<2.0	3.2	<2.0	<2.0
B. licheniformis	4.9	7.8	<2.0	<2.0	<2.0	<2.0
B. stearothermophilus	3.7	6.0	<2.0	3.6	<2.0	<2.0
	Plain cooked rice of Type (ii)					
Control	2.0	2.3	4.5	6.6	4.9	6.8
B. brevis	4.3	6.5	4.9	7.9	4.8	7.7
B. cereus	4.6	6.8	4.9	7.9	4.9	7.2
B. laterosporus	4.1	6.7	4.8	7.8	5.6	8.2
B. licheniformis	4.8	6.8	5.4	7.5	4.6	8.0
B. stearothermophilus	4.0	7.3	5.0	7.9	4.9	7.7

Fresh samples had < 2.0 $\log_{10}$ cfu/g count of all microbial types except that of 3.0 $\log_{10}$ cfu/g count of mesophilic aerobes in type (ii) samples. The initial pH was 6.4 and 6.2 for types (i) and (ii) samples, respectively and these varied between 5.9 and 6.4 during the storage.

Growth of Bacillus *species in plain cooked rice:* The results presented in Table 3 reveal the ability of a few selected cultures of *Bacillus* species to grow in plain cooked rice. In type (i) rice which was almost free from initial microflora, except for *B.stearothermophilus,* other test cultures of *Bacillus* species grew well, reaching counts of 7.0 to 7.9 $\log_{10}$ (cfu/g) in 24 h. The growth of *B. stearothermophilus* might have been slower due to the lower storage temperature (15-26°C).

In case of type (ii) rice, *B.stearothermophilus* recorded a count of 7.3 $\log_{10}$ (cfu/g), while with the other *Bacillus* test cultures, the count was 6.5 to 6.8 $\log_{10}$ (cfu/g). The lower counts of *Bacillus* in type (ii) rice may be attributed to the competitive microflora present initially, as counts of mesophilic aerobes and enterobacteriaceae in these samples were in the range of 7.2 to 8.2 $\log_{10}$ (cfu/g). The better growth of *B. stearothermophilus* may be due to slight increase in the storage temperature as a result of metabolic activities of other microflora. Further, the good growth of *Bacillus* cultures in type (i) rice may be due to the moisture content which was 82.3% as against 60.8% in type (ii) rice. The variation in pH levels observed was not significant.

The infective dose of *B.cereus* sufficient to induce illness is variable. It was shown that counts of *B.cereus* in foods ranging from 4.5 to 9.0 $\log_{10}$ (cfu/g) resulted in enteritis. Further, occurrence of *B. cereus* ($>10^4$ cfu/g) in food has been considered as one of the epidemiological criteria for implicating *B.cereus* in food poisoning outbreaks[14].

In both types of rice preparations, the count of *B.cereus* was sufficient to cause health hazards. The *Bacillus* counts recorded and the ability of different *Bacillus* species to produce extracellular enzymes indicate their toxigenic and spoilage potentialities.

The results indicate a need for proper hygienic and sanitation principles to avoid any post-processing contamination of the processed foods. Further, a long storage

period between preparation and catering may lead to public health hazards when prepared foods are not adequately protected from post-preparative contamination. Several investigators[5,15,16] have studied hazards involved in the preparation of cereal-based foods and made a few recommendations: (i) prepare small quantities of rice as required, (ii) keep prepared rice hot (>55 to 63°C), (iii) cool cooked rice quickly and (iv) reheat cooked rice thoroughly before serving. However, reheating of cooked foods before serving will not eliminate preformed heat stable toxins.

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Cooking and Parching Characteristics of Chickpea (*Cicer arietinum* L.)

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Five improved and a local variety of chickpea were evaluated for their physical, chemical, hydrating, cooking and parching characteristics. 'PBG-1' variety had the lowest seed weight, seed volume and hydrating properties; but required the highest force for breaking and crushing. The varieties 'PBG-1' and 'GL-769', after 60 minutes of cooking, indicated low cooked kernel yield, solid losses, lower overall acceptability and were rated poor for cooking. These varieties had poor puffing properties, while the local variety was found to be the best for puffing purposes.

Production of chickpea (*Cicer arietinum*) ranks fifth among legumes in the world[1]. In 1989, it constituted 37% of the total pulses production of 137 lakh tonnes in India[2]. Chickpea is consumed in different forms e.g. cooked, parched and canned. However, the quality of processed chickpea is lowered by the presence of variable quantities of "Hardshell" seeds which resist cooking and even do not germinate. The resistance to cooking may be due to

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hardshell impervious to water or the seed may become harder to cook due to the non-softening of cotyledons even if the seed imbibes water[3-5]. Plant breeders are engaged in developing varieties free of hardshell seeds. However, some seeds develop harder-to-cook-character during storage[6-8]. Though wide variations in the suitability of different varieties of legumes for cooked and parched products have been reported, the studies with respect to chickpea varieties are grossly lacking. The present investigation was undertaken to evaluate physico-chemical, cooking and parching characteristics of some important varieties of chickpea cultivated in Punjab.

Materials and Methods

Representative samples of five improved chickpea cultivars viz. 'C-214', 'C-235', 'GNG-146', 'GL-769' and 'PBG-1' from 1989-90 harvest were obtained from the Department of Plant Breeding of Punjab Agricultural University, Ludhiana. One local variety ('LV'), not grown in Punjab but used for commercial parching, was obtained from the local market.

Physical characteristics: Seed volume, density, hydration capacity, hydration index, swelling capacity and swelling index were evaluated using methods of Williams *et al.*[9] Percent hardshell grains were calculated after 10 h soaking at room temperature. Physical hardness of grains (moisture content 10.5 ± 3%) was determined using Instron Universal Testing Machine (Model 1111). Mean force required to break and crush the grains was determined using crosshead speed of 5 cm/min, chart speed of 20 cm/min, force range of 490.5(N) full scale and a die diameter of 2.5 cm with 4 mm deformation. It resulted into two peaks for each of the sample, the first peak corresponding to the force required to break and the second to the force required to crush the grains.

Composition: Ash and protein contents were determined as per AACC[10]. Cellulose was determined according to the procedure of Crompton and Maynard[11]. Method described by Goering and Van-Soest[12] was used for the estimations of neutral detergent fibre (NDF) and acid detergent fibre (ADF).

Cooking: Cooking quality was evaluated using the method of Williams *et al.*[9] with minor modification. Fifty grains were boiled in 200 ml water in 500 ml flask under reflux. After 60 min of cooking, sample was drained and percent cooking grains free of white core were noted. The samples were organoleptically evaluated by panelists for overall acceptability using 9-point hedonic scale.

Parching: One hundred g sample, uniformly mixed with calculated amount of water to bring the moisture content of grain to 12.5 ± 0.3% was rested for 10 min. Thereafter, it was parched in sand for 40 sec. at 190°C. Then the sample was taken out and rested for a period of 5 min and again parched (40 sec.) in hot sand (190°C). The moisture content of parched samples was 3.8 ± 0.5%. Seed volume, puffing capacity and puffing index were determined as stated earlier.[9] Percent unpuffed (hard) grains was calculated by counting. The samples were organoleptically evaluated as above.

Statistical analysis: All the results were expressed on a 14% moisture basis unless otherwise stated. The data were statistically analysed using randomized block design as described by Steel and Torrie[13]. Least significant differences (LSD) were calculated.

Results and Discussion

Physical and chemical properties: Chemical composition varied significantly among varieties. The protein content ranged from 18.75 – 24.00%, lowest being in 'LV' and highest in 'C-214' (Table 1). 'PBG-1' and 'GL-769' contained lower amounts of acid and neutral detergent fibres,

TABLE 1. CHEMICAL COMPOSITION AND PHYSICAL CHARACTERISTICS OF VARIOUS VARIETIES OF CHICKPEA

Attributes	Chickpea variety						
	'C-214'	'C-235'	'GNG-146'	'GL-769'	'PBG-1'	'LV'	LSD (0.05)
				Chemical composition[a]			
Protein (%)	24.0	21.8	22.9	22.3	20.8	18.7	0.3
Acid detergent fibre (%)	9.4	9.6	7.7	5.5	5.6	8.3	0.2
Neutral detergent fibre (%)	14.2	15.4	12.0	9.8	10.1	13.3	0.3
Lignin(%)	1.9	1.8	2.4	1.3	1.2	1.7	0.1
Cellulose(%)	7.5	7.8	5.3	4.2	4.8	6.6	0.2
Hemicellulose (%)	4.8	5.8	4.3	4.3	3.6	5.0	0.2
				Physical characteristics			
Seed weight[a] (g/100 seeds)	13.2	14.0	12.9	14.3	12.6	31.4	0.45
Seed volume[a] (ml/100 seeds)	11.0	12.1	12.6	13.6	11.0	25.7	0.70
Seed density[a] (g/ml)	1.2	1.2	1.0	1.1	1.1	1.2	0.04
Breaking force[b] (N)	126.0	134.9	76.5	162.8	153.0	166.8	20.50
Crushing force[b] (N)	248.2	274.7	227.6	343.3	387.5	137.3	11.50
Total force[b] (N)	374.2	409.6	304.1	506.1	540.5	304.1	23.50

a,b values are means of three and five replicates, respectively
LSD: Differences between two means exceeding this value are significant.

TABLE 2. HYDRATING, COOKING AND PARCHING CHARACTERISTICS OF VARIOUS VARIETIES OF CHICKPEA

Attributes	Chickpea variety						
	'C-214'	'C-235'	'GNG-146'	'GL-769'	'PBG-1'	'LV'	LSD (0.05)
Hydrating characteristics[a]							
Swelling cap/seed	0.135	0.104	0.114	0.054	0.040	0.340	0.02
Swelling index	122.7	85.95	90.5	39.7	36.5	132.5	2.7
Hydration cap/seed	0.13	0.10	0.12	0.06	0.05	0.33	0.02
Hydration index	98.5	71.0	93.0	42.0	39.5	105.1	2.5
Hardshelled grain(%)	3.7	24.5	8.0	60.0	64.0	0.0	1.4
Cooking characteristics							
Cooked grains[a] (%)	96.0	78.0	87.0	74.0	7.2	19.0	4.8
Gruel solid losses[a] (%)	8.8	5.8	6.7	5.5	5.0	3.9	1.1
Overall acceptability[b] (9-0)	8.6	8.0	8.0	7.2	7.0	4.1	0.5
Hydration index[a]	102.6	98.7	101.3	95.0	94.5	77.4	2.5
Parching characteristics							
Seed volume[a] (ml/100, seeds)	16.8	17.2	19.1	16.7	16.2	40.2	2.0
Puffing cap/100 seeds[a]	5.8	5.2	6.5	5.0	5.0	14.5	0.2
Puffing index[a]	52.7	42.1	51.6	46.8	47.3	56.6	1.6
Hardshelled grains[a] (%)	55.0	50.0	16.2	38.5	59.5	12.5	1.4
Overall acceptability[b] (9-0)	5.0	5.3	6.5	5.1	4.8	8.8	0.6

a, b values are means of three and six replicates, respectively
LSD: Differences between two means exceeding this value are significant

lignin, cellulose and hemicellulose. The varieties showed significant differences in physical characteristics of grains (Table 1). The variety ('LV') had highest seed weight, seed volume and seed density, 'PBG-1' showed lowest seed weight and volume. The seed density was lowest in variety 'GL-769'.

Very high force was required to break and crush 'PBG-1' and 'GL-769' grains, the total force being highest for 'PBG-1'. The total force required was lowest for the local variety and 'GNG-146'. Grains of local variety and 'GNG-146' required least force to crash and break, respectively. The data revealed that the grains of varieties 'PBG-1' and 'GL-769' were very hard and those of local variety and 'GNG-146' were soft. It seems that the constituents like fibre, lignin, cellulose and hemicellulose are the factors responsible for hardness of grains. Varieties with lower contents of these constituents had more harder grains and *vice versa.*

Hydrating properties: Swelling capacity per seed, swelling index, hydration capacity per seed and hydration index were lower for 'PBG-1' followed by 'GL-769', while the local variety had highest value for all these characteristics possibly due to the absence of hardshell grains, large seed size and soft grains texture (Table 2). Williams *et al.*[9] and Singh *et al.*[14] have also reported strong positive correlation between seed and weight size with hydration capacity. Hardness of grains and the amount of hardshell grains seem to be the other factors which accounted for variations in hydration and swelling properties. Bourne[3] also reported that the hardshell grains do not swell during soaking.

Cooking and parching quality: Cooking time has several definitions such as the time at which 50% of seeds becoming soft (cooked) or 100% softening[9,15]. Actual cooking time should be the time between the beginning of the test and when the seeds are ready for eating. This means that seeds are soft enough to masticate without having to chew. Trials conducted showed that the varieties differed considerably for the cooking time. Cooking for 60 min was considered to be the most appropriate for comparative evaluation of cooking behaviour of different varieties.

Percent cooked grains in local variety after 60 min cooking was lowest, thereby indicating its longer cooking time requirement. This could be attributed to its larger seed. Williams *et al.*[9] reported positive correlation between seed size and cooking time. Seed size governs the distance to which water must penetrate in order to reach the innermost portion of seeds. Due to its under cooking, the variety showed least gruel solids and lowest acceptability score for cooked kernels (Table 2). Among other varieties, 'PBG-1', and 'GL-769' had low hydration index and cooked grains, possibly due to their higher contents of hardshell grains. Various workers have reported that beans resist cooking mainly because of the presence of hardshell grains[4,5,8]. These varieties also showed comparatively lower gruel solids and poor acceptability scores. 'C-214', on the other hand, had highest hydration index and number of cooked kernels as well as showed the greateat gruel solids and acceptability score. Thus, 'C-214' was rated the best followed by 'GNG-146' and 'C-235' in terms of cooking time.

Local variety showed the highest puffed seed volume, puffing capacity, puffing index, least number of harder

grains on puffing and registered the highest acceptability score for puffed grains (Table 2). The variety was, therefore, rated best for puffing purposes, while PBG-1 with the least volume of puffed seeds, puffing capacity per seed, puffing index and overall acceptability was rated poorest. Varieties 'GL-769' 'C-214' and 'C-235' also showed very poor puffing quality, but 'GNG-146' was comparatively better. The results showed that the proportion of hardshelled grains is a most important factor in determining the hydrating, cooking, parching characteristics and consumer acceptability.

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Effect of Dehulling Methods and Physical Characteristics of Grains on *Dhal* Yield of Pigeonpea (*Cajanus cajan* L.) Genotypes

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Two traditional methods of dehulling - manual and home-processing (stone *chakki*); and two laboratory methods - barley pearler and tangential abrasive dehulling device (TADD) - were employed to study the dehulling quality of eight pigeonpea genotypes. *Dhal* yield by TADD was the highest (80.0%) for 'ICPL 87052' and the lowest (54.1%) for 'ICPL 87049' indicating significant (P<0.01) differences among genotypes. These results were further substantiated by *dhal* yield values obtained by the barley pearler. The stone *chakki* gave highly variable and erroneous results on *dhal* yield. The TADD and barley pearler methods were comparable and reliable. The theoretical *dhal* yield (manual method) was not correlated with dhal yields obtained by stone *chakki,* barley pearler and TADD. Grain hardness and grain volume were negatively correlated with the *dhal* yields obtained by the TADD and barley pearler methods, whereas swelling capacity and grain floatation values were not correlated with *dhal* yields obtained by these methods.

Considering the production and consumption, pigeonpea or redgram, is the second largest pulse crop in India and accounts for nearly 85% of the World's supply[1]. In India, it is mostly consumed after dehulling in the form of *dhal* (decorticated split cotyledons) and cooking in water to a desirable softness. Most of the nearly 2 million tonnes of pigeonpea produced annually in India is converted into *dhal*[2]. Not only does dehulling improve palatability and

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digestibility of pigeonpea; but it also reduces remarkably its cooking time[3]. Several methods are employed for dehulling pulses in India[4-6].

Variability in dehulling characteristics of pigeonpea may be influenced by variety and agro-climatic factors, but the role of these factors has not been established. In additon, several factors such as pre-treatments like soaking in salt solutions, water and oil application and sun-drying influence the dehulling of pigeonpea[7]. Some laboratory methods have been used to study the dehulling quality of pigeonpea genotypes[8-9]. A machine that removes barley bran was used to study the variability in dehulling characteristics of 19 pigeonpea genotypes[8]. In another study, variability in dehulling quality of 23 pigeonpea genotypes was described using the TADD method[9]. The objectives of this study were to compare different methods of dehulling to evaluate dehulling quality of pigeonpea genotypes, and to examine the relationship between the physical characteristics of the grain and dehulling quality of pigeonpea genotypes.

Materials and Methods

Grain samples of eight genotypes '(C-11', 'BDN 2,' and 'T 15-15' as control, and 'ICPL 87049,' 'ICPL 87052,' 'ICPL 87053,' 'ICPL 87066 and 'ICPL 87075' as newly developed genotypes) of pigeonpea were supplied by the pigeonpea breeding unit of Legumes Program at ICRISAT.

Pre-treatment of whole grain for dehulling: Soaking in water at room temperature followed by drying in the oven was the pre-treatment employed for dehulling. Grains of all genotypes were separately soaked in excess distilled water for 4 h at room temperature (25 ± 1°C). After soaking, excess water was discarded and the samples were dried in an oven at 55°C for 16 h and used for dehulling.

Dehulling methods: Two traditional methods, i.e., manual method and home-processing method (stone *chakki*), and two laboratory methods, barley pearler (Scott Seedburo, USA) and tangential abrasive dehulling device (TADD) as described by Ehiwe and Reichert[9] were used.

Manual method: The *dhal* yield was determined by manually separating the husk from the cotyledons. The seed coat and *dhal* fractions (cotyledons) were dried separately in the oven at 55°C overnight (16 hr) and weighed to calculate *dhal* and husk percentages.

Stone chakki (quern): A stone *chakki* consisting of lower (immovable) and upper (rotating) stone pieces each of 34.5 cm diameter and 5.5. cm thickness were used. A 100-g grain sample of pre-treated pigeonpea was slowly and uniformly added through a central hole in the upper stone which was gently and continuously rotated manually until the material was processed. The upper stone was removed and the processed grain material was collected and separated into *dhal*, brokens, powder and husk fractions. Both unsplit and split decorticated cotyledons were included as *dhal*.

Barley pearler: A 100-g grain material was dehulled for 4 min and the processed material was separated into *dhal*, brokens, powder and husk fractions. As mentioned earlier, both unsplit decorticated and split decorticated cotyledons were included together as *dhal*.

Tangential abrasive dehulling device (TADD): After standardizing the TADD for dehulling of pigeonpea, a 100-g grain sample was dehulled for 1 min by putting an approximately equal mass of grain material in 12 cups/holes of the TADD plate. After dehulling, the processed material was separated into *dhal*, brokens, powder, and husk fractions. As above, both unsplit and split decorticated cotyledons were included as *dhal*.

Physical characteristics: Moisture content was determined by drying the grain at 110°C for 18 h. Grain colour was visually recorded. The 100 grains were weighed in five replicates and the mean 100-grain mass of the sample recorded. For determination of grain volume, 20 ml of water was taken in a measuring cylinder and 50 grains were transferred into it. The increase in volume by the addition of grains was recorded as the volume of the grains. A floatation test was carried out by using sodium nitrate solution of 1.303 g/cc density. Fifty grains were dropped into the solution and shaken well. The number of floating grains was determined and calculated as the floatation percentage. Swelling capacity was determined by soaking 5 g of grains in distilled water at room temperature (25 ± 1°C) for 16 h. Excess water was discarded, traces of water wiped out and the samples weighed. Swelling capacity was expressed as g increase in mass per g of the grain material. An Instron food testing machine (Model 1140, High Wycombe, Buckinghamshire, UK) was used to measure the grain hardness. Fifty grains of each genotype were randomly selected and compressed to a breaking point at a crosshead speed of 80 mm per min with a 2:1 ratio. An average Instron force (Kg) was recorded as the grain hardness of the sample.

Statistical analysis: All the determinations were done in 3 to 15 replicates. Standard errors (SE) were determined by a one-way analysis of variance[10] and are indicated in the Tables as the pooled error of replicates.

Results and Discussion

The theoretical yields of dehulled grain determined by the manual method ranged from 85.2 to 88.4% with mean being 86.7% showing a small variation among the genotypes (Table 1). These *dhal* yield values primarily depend on the content of seed coat (husk) of pigeonpea genotypes as shown in Table 2. Excluding manual method, average *dhal* yield was highest (71.3%) in TADD followed by barley pearler (67.6%) and lowest in stone *chakki* (50.5%), (Table 1). The average *dhal* yield of pigeonpea genotypes analysed by TADD is comparable with that of the commercial *dhal* mills (70.1% *dhal*) in India[5], but is considerably lower than that of the improved commercial dehulling

TABLE 1. *DHAL* YIELD, BROKENS, POWDER AND HUSK FRACTIONS OF PIGEONPEA GENOTYPES OBTAINED BY DIFFERENT METHODS OF DEHULLING

Genotype	*Dhal* yield (%)				Broken (%)			Powder (%)			Husk (%)		
	MNM	SNC	BRP	TADD	SNC	BRP	TADD	SNC	BRP	TADD	SNC	BRP	TADD
'C-11'	85.8	45.6	71.8	75.7	27.7	4.0	2.5	6.0	5.3	5.9	9.9	13.1	14.6
'BDN2'	85.2	49.9	66.9	76.7	25.9	9.9	4.1	4.0	5.8	5.3	11.9	12.4	13.2
'T 15-15'	88.4	51.4	73.2	78.5	25.0	4.3	1.9	4.7	6.7	6.0	9.7	11.4	12.6
'ICPL 87049'	86.4	46.7	55.6	54.1	25.9	17.7	27.8	4.8	7.7	6.0	11.3	12.8	11.4
'ICPL 87052'	86.6	54.0	73.7	80.0	22.3	5.1	2.2	4.4	3.7	5.6	11.4	13.0	11.6
'ICPL 87053'	85.9	42.6	72.5	75.5	23.6	5.5	2.5	10.4	5.8	6.2	10.9	13.1	14.9
'ICPL 87066'	88.2	54.5	57.6	56.6	25.5	19.8	24.8	3.2	8.5	7.3	10.0	9.5	10.9
'ICPL 87075'	87.0	59.0	69.2	73.5	20.8	9.0	6.6	6.0	4.4	6.8	9.5	10.5	12.6
Mean	86.7	50.5	67.6	71.3	24.6	9.4	9.0	5.5	6.0	6.1	10.6	12.0	12.7
SEM	±0.36	±1.84	±0.51	±0.28	±2.12	±0.42	±0.23	±1.04	±0.32	±0.14	±0.46	±0.25	±0.20

MNM = manual method. SNC = stone *chakki*, BRP = barley pearler, TADD = tangential abrasive dehulling device.
Means of three independent determinations.

TABLE 2. PHYSICAL CHARACTERISTICS OF GRAINS OF PIGEONPEA GENOTYPES

Genotype	Grain colour	Moisture (%)	100-grain mass (g)	Grain volume (ml)	Floatation value (%)	Swelling capacity (g/g)	Grain hardness-force (Kg)	Husk (%)
'C-11'	Brown	9.4	10.0	8.0	8.8	1.08	17.4	14.2
'BDN2'	Cream	11.0	7.7	6.2	6.4	1.08	17.3	14.8
'T 15-15'	Cream	10.1	8.8	6.4	12.0	1.09	18.6	11.6
'ICPL 87049'	Cream	10.1	11.6	9.2	5.2	0.99	19.7	13.4
'ICPL 87052'	Brown	10.1	10.3	8.0	3.2	0.75	19.1	13.4
'ICPL 87053'	Brown	10.4	8.1	7.4	13.6	1.04	18.0	14.1
'ICPL 87066'	Cream	10.3	13.4	10.4	8.0	1.11	19.8	11.9
'ICPL 87075'	Cream	9.6	11.0	8.0	9.2	1.23	19.4	13.0
Mean		10.2	10.1	8.0	8.3	1.06	18.6	13.3
SEM		±1.2	±0.1	±0.1	±0.4	±0.02	±0.5	±0.38

Means of five independent determinatons.

technology developed for dehulling of pigeonpea[11]. The value for *dhal* yield was the highest (80.0%) for 'ICPL 87052' and the lowest (54.1%) for 'ICPL 87049' when dehulled in the TADD. Similar variations in *dhal* yield of these genotypes were observed when dehulled by using the barley pearler (Table 1). This indicated significant ($p < 0.01$) differences in dehulling quality of pigeonpea genotypes. The dehulling losses in terms of brokens were the highest (24.6%) in the stone *chakki* and this might have been due to the attrition action of the stones employed for dehulling in this method. A large variability in dehulling quality of pigeonpea genotypes was observed when they were dehulled by the TADD[9] and the machine that removes barley bran[8]. Eventhough the *dhal* yield primarily depends on the type of machine employed for dehulling, other characteristics such as size, shape and hardness of the grain seem to play an important role in determining dehulling losses and these have been discussed in the following sections. Some newly developed genotypes of pigeonpea '(ICPL 87049' and 'ICPL 87066') produced *dhal* yield lower than the control genotypes, 'BDN 2,' and 'C 11,' which yielded 76.7% and 75.7% *dhal* respectively when dehulled by TADD (Table 1). No large variability in *dhal* yield of these genotypes was obtained when dehulled in the stone *chakki* that also produced the lowest *dhal* yield.

A statistical comparison between dehulling methods indicated that the standard error (SE) and coefficient of variation (CV) of the procedures were the highest for stone *chakki* and the lowest for TADD. Not only did the stone *chakki* produce the highest percentage of brokens as dehulling losses (Table 1), it also produced highly variable and erroneous results on the *dhal* yield. Further, *dhal* yield obtained by a stone *chakki* was neither correlated with the TADD nor with the barley pearler. But there were significant ($P<0.01$) and highly positive correlations ($r=0.97^{**}$) and ($r=0.95^{**}$) between TADD and barley pearler for *dhal* yield and broken fractions, respectively. These results indicated that TADD and barley pearler methods are highly comparable.

Seed coat colour of genotypes varied widely from white to light brown to dark brown. There was no large variation in moisture content of these genotypes (Table 2). The 100-grain mass, grain volume and floatation value of these genotypes showed significant differences ($P \leq 0.01$). Grain hardness ranged between 17.3 and 19.7 kg (Instron force) indicating a small variation. Also, the grain coat content of these genotypes did not reveal a large variation.

The moisture content did not influence the *dhal* yields as there were no significant correlations between these characteristics (Table 3). Although the correlations are not

TABLE 3. CORRELATION COFFECIENT BETWEEN PHYSICAL CHARACTERISTICS AND *DHAL* YIELD OF PIGEONPEA GENOTYPES.

	1	2	3	4	5	6	7	8	9
1. Moisture	1.00								
2. 100-seed mass	-0.38	1.00							
3. Grain volume	-0.28	0.94**	1.00						
4. Floatation value	-0.10	-0.36	-0.28	1.00					
5. Swelling capacity	-0.17	0.05	-0.07	0.46	1.00				
6 Grain hardness	-0.20	0.81*	0.72*	-0.29	-0.11	1.00			
7. *Dhal* yield[a]	-0.03	-0.76*	-0.82*	0.24	-0.12	-0.65	1.00		
8. *Dhal* yield[b]	-0.20	-0.67	-0.71*	0.36	-0.16	-0.57	0.97**	1.00	
9. *Dhal* yield[c]	-0.21	0.52	0.34	0.20	0.17	0.65	-0.25	-0.15	1.00

a. *Dhal* yield by TADD
b. *Dhal* yield by barley pearler
c. *Dhal* yield by manual operation

** Significant at 0.01 level, * Significant at 0.05 level.

statistically significant, grain hardness was negatively correlated with the TADD and barley pearler *dhal* yields, whereas it was positively correlated with the *dhal* yield obtained by the manual method (Table 3). It has been shown that greater than 75% of the variability in dehulling efficiency or yield could be accounted for by grain hardness and resistance to splitting of the grain into individual cotyledons[12]. The present results suggest that losses in terms of brokens and powder fraction would be more, if grains of genotypes are hard, requiring more abrasive force during the operation. Grain volume was negatively and significantly ($P \leq 0.05$) correlated to *dhal* yields, obtained by the TADD (r=-0.82) and the barley pearler (r=0.71). There was a positive and significant ($P \leq 0.01$) correlation (r=0.94) between grain volume and 100-grain mass of these genotypes (Table 3). It appeared that *dhal* yield in TADD and barley pearler depended on the size of grains, implying that bolder grains would reduce the *dhal* yield. Swelling capacity and the floatation values of these genotypes were not noticeably correlated with the *dhal* yields obtained by different methods (Table 3). Further, the theoretical *dhal* yield obtained by the manual method was not correlated with the *dhal* yields obtained by the TADD and barley pearler. This indicated that the seed coat content of a genotype obtained by the manual method cannot be used to predict the *dhal* yield of mechanical methods, which are commonly employed for dehulling pigeonpea in India. However, it is emphasized that the observations of this study may be used with caution, as these are based on the analysis of a limited number of genotypes.

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Isolation of a Lectin from *Amaranthus paniculatus* Seeds

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A N-acetyl-α-D-galactosamine specific lectin from the seeds of *Amaranthus paniculatus* was purified to homogeneity by affinity chromatography on immobilized desialyated porcine gastric mucin and found to be a homo dimer and a glycoprotein (10.5% carbohydrate w/w) with molecular weight of the subunit being 27,000 (± 1410). Its amino acid composition revealed high contents of valine, leucine, and acidic amino acid residues. This lectin also had high contents of methionine, tryptophan and lysine. A. *paniculatus* lectin agglutinated normal and papain-treated rabbit and human A, B and O erythrocytes.

Grain amaranth is cultivated as a minor food crop in Central and South America, Mexico and in some areas of Asia and Africa. It is considered as potentially high food and feed resource due to its high quality and quantity of proteins[1-3]. *Amaranthus paniculatus*, an Asiatic species, is cultivated throughout India. The tender shoots and leaves are used as a leafy vegetable, while the seeds are used to prepare sweets consumed especially on fasting days[4]. Presence of anti-nutritional factors such as phenolic substances, trypsin inhibitors and cytoagglutinins have been reported in grain Amaranth[1,3,4]. From the Amaranthaceae family, only two lectins from the seeds of *Amaranthus leucocarpus* and *Amaranthus caudatus* have been reported [1,5]. In the present paper, the purification, physical and chemical characterization of the lectin from the seeds of *Amaranthus paniculatus* are reported.

Materials and Methods

Chemicals: Amaranthus paniculatus seeds were obtained locally. Porcine gastric mucin, melibiose, N-acetyl-D-galactosamine (D-GalNAc), α- and β-methyl-D- galactopyranosides, raffinose, p-nitrophenyl α- and β-D- galactopyrano sides, ovomucoid, were obtained from Sigma, U.S.A. Sephadex G-100 was purchased from Pharmacia (Sweden), and all other chemicals used were of analytical grade. Human A, B and O type blood samples were obtained from K.E.M. Hospital, Pune. Rabbit blood was supplied by the Institute of Veterinary and Biological Products, Pune. Protein concentrations were determined by absorbance measurements at 280 nm using bovine serum albumin (BSA) as standard.

Preparation of the affinity matrix: Desialyated porcine gastric mucin was immobilized by entrapment in glutaraldehyde crosslinked gelatin gel granules essentially as reported earlier[6].

Lectin purification: Finely ground seeds of *A. paniculatus* (10 g) were suspended in 50 ml of saline solution I (0.145 M sodium chloride containing 0.02% sodium azide), and stirred for 4 h at 4°C. The extract was centrifuged and residue re-extracted with saline as before. The supernatants were pooled, pH adjusted to 4.0 (using 1.0 M acetic acid) at 4°C and allowed to stand for 1hr, followed by centrifugation (12080 × g). The supernatant thus obtained was dialysed exhaustively against saline solution I, until sugar-free and clarified by centrifugation (12080 × g). This solution (Fraction A) was used for isolation of lectin by affinity chromatography using immobilized desialomucin. Fraction A was applied on a column (18 cm length × 3 cm outer diameter) packed with the immobilized desialomucin gel. The unadsorbed proteins were removed by washing with saline solution I. The adsorbed lectin was eluted with D-GalNAc (10 mM). Fractions of 5 ml were collected. The lectin containing fractions were pooled, dialysed extensively against distilled water, lyophilized and preserved at –20°C.

Homogeneity of the purified lectin was determined by polyacrylamide gel electrophoresis (PAGE) at pH 4.5[7] and 8.38 and sodium dodecyl sulphate PAGE (SDS-PAGE)[9] under reducing and non-reducing conditions. Haemagglutination assays were done at room temperature (26-28°C) in PBS (0.02 M phosphate buffer pH 7.2, containing 0.1 M sodium chloride), using two-fold serial dilution procedure in microtitre plates. A 4% (v/v) suspension of normal or papain treated rabbit erythrocytes was used. For blood-group specificity studies, a 4% (v/v) suspension of normal as well as papain-treated human A, B and O erythrocytes was used. Inhibition of haemagglutination induced by A. paniculatus lectin was studied with different sugars and glycoprotein solutions in PBS as follows: 100 µl of pure

lectin solution containing 4 haemagglutination units was added to 100 µl of varying sugar concentrations and incubated at R.T. for 1 hr. Rabbit erythrocytes suspension (100 µl) was then added to the above mixture and the contents were further incubated for 30 min before observing agglutination under the microscope.

Physical and chemical studies: The temperature stability of the *A. paniculatus* lectin was studied by incubating the pure lectin samples (0.1 mg/ml) at different temperatures for 20 min. The pH stability of the lectin was studied by pre-incubating the pure lectin (0.1 mg) in buffers (0.25 ml) of different pH at room temperature for 1 hr. After 1 h, the haemagglutination activity of the lectin was determined at pH 7.2 in PBS by the two-fold serial dilution method. The buffers used were 0.2 M glycine adjusted to a desired pH with 0.2 M HCl or 0.2 M NaOH.

Structural studies: Molecular weight of the lectin was determined by SDS-PAGE and gel filtration. Gel filtration was performed on a column of Sephadex G-100 (45 cm length × 2 cm diameter) at 4°C and eluting the protein with saline solution I. For gel filtration as well as SDS-PAGE, the marker proteins used were BSA, ovalbumin, pepsin, trypsin, α-chymotrypsin and cytochrome c. Amino acid analysis was performed thrice by HPLC[10] using an amount of protein corresponding to 1.85 nm i.e. 50 g. Tryptophan content was determined spectrophotometrically according to the procedure of Edelhoch[11]. Isoelectric focussing was done on 5% polyacrylamide gels containing 2 % ampholine (LKB's of pH range 3.0 to 10.0), in glass tubes. Catholyte was sodium hydroxide (0.02 M), while phosphoric acid (0.01 M)[12] was used as anolyte.

Results and Discussion

Saline extract of *Amaranthus paniculatus* seeds showed the presence of a lectin which agglutinated human A, B and O erythrocytes as well as rabit erythrocytes. *A paniculatus* lectin was purified by affinity chromatography on immobilized desialomucin as described earlier. Purification of the lectin could be obtained by two steps, with an yield of 15 mg/10 g seeds (Table 1). On PAGE, the affinity purified lectin gave single bands at pH 4.5 and pH 8.3 (Fig. 1) indicating the homogeneity of the purified lectin. On SDS-PAGE, under reducing and non-reducing conditions, the lectin gave a single band, indicating presence of a single subunit and absence of disulphide bridges. On PAGE and SDS-PAGE, the lectin was stained with both Coomassie Brilliant Blue R-250 and the periodic acid - Schiff (PAS) reagent[13], indicating its glycoprotein nature.

TABLE 1. PURIFICATION OF THE *AMARANTHUS PANICULATUS* LECTIN*

Fraction	Protein (mg)	Total Activity (HAU x 1000)	Specific activity (HAU/mg)	Recovery (%)	Fold purification
Saline extract	1109.0	60.0	54.1	100.0	1.0
Fraction A	504.0	51.0	101.5	85.3	1.9
After affinity chromatography	14.8	38.4	2594.0	64.0	48.0

* from 10g of seeds HAU: Haemagglutinating units

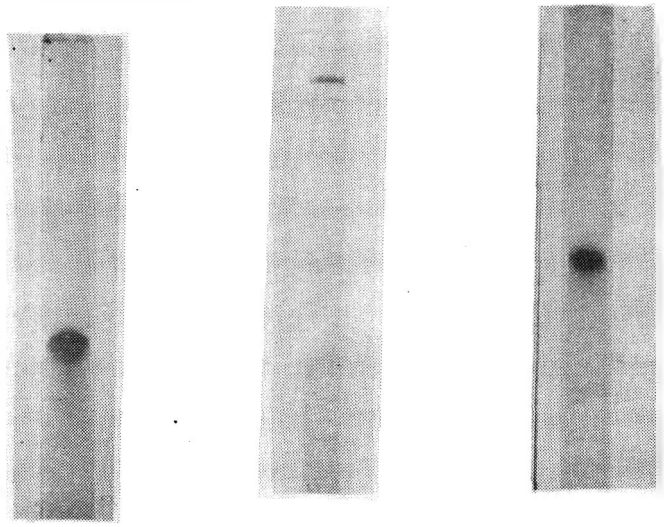

Fig. 1. PAGE and SDS-PAGE patterns of *A. paniculatus* lectin. (A) pH 4.5 (B) pH 8.3 (C) SDS-PAGE

Blood-group specificity: A. paniculatus lectin showed an equal haemagglutination activity towards normal and papain-treated human A, B and O erythrocytes indicating that it did not have blood-group specificity and papain treatment did not enhance the susceptibility of the erythrocytes to agglutination by the *A. paniculatus* lectin.

Sugar specificity: A. paniculatus lectin agglutinated normal and papain treated rabbit erythrocytes. Hence, for the study of sugar specificity of the lectin, by inhibition of haemagglutination induced by lectin, rabbit blood was used. D-GalNAc was the most potent inhibitor of the lectin (Table 2). A marked preference was shown for the α-anomer

TABLE 2. MINIMUM CONCENTRATION OF SUGAR/GLYCOPROTEIN NECESSARY TO INHIBIT FOUR HAEMAGGLUTINATING UNITS OF *A. PANICULATUS* LECTIN USING RABBIT ERYTHROCYTES

Sugar	Concn (mM)
D-GalNAc	1
D-Galactose	10
α -Methyl-D-Galp	8
β -Methyl-D-Galp	9
Melibiose	8
Raffinose	9
D-galactosamine. HCl	9
p-nitrophenyl- α–DGalp	7
p-nitrophenyl-β-D-Galp	8
Lactose	200
Glycoproteins (mg/ml)	
Ovomucoid	1.00
K-casein	1.00
Mucin	0.75

TABLE 3. AMINO ACID COMPOSITION OF *AMARANTHUS PANICULATUS* LECTIN

Amino acid	Residue %[a]	Residues/27,000[b]
Aspartic acid	5.9	12
Threonine	5.8	12
Serine	6.9	14
Glutamic acid	4.7	10
Proline	1.1	2
Glycine	5.8	12
Alanine	5.6	11
Valine	9.5	19
Methionine	1.8	4
Isoleucine	10.8	22
Leucine	19.1	39
Tyrosine	5.9	12 (13)[*]
Phenylalanine	5.6	12
Histidine	1.8	4
Lysine	2.7	6
Arginine	2.0	4
Tryptophan	4.8	(10)
Cysteine	N.D.	

N.D. not detectable
a Mean value of three independent analysis.
b Calculated to nearest integer.
* Values between brackets were determined spectrophotometrically

and the α–galactosides. Lactose, which is a β-galactoside is a poor inhibitor. Glycoproteins ovomucoid and mucin were also inhibitory.

Physical and chemical studies: The temperature stability studies showed that the *A. paniculatus* lectin activity was completely lost by heating at 70°C for 20 min. The lectin was stable in the pH range 4.0 to 9.0. On storage and under alkaline condition (pH > 8.0) the lectin had a marked tendency to aggregate.

Structural analysis: The *A. paniculatus* lectin was found to be a glycoprotein. The carbohydrate content was 10.5% (w/w) as determined by phenol-sulphuric acid method[14], using D-glucose as standard. On isoelectric focussing, the lectin focussed as three very closely spaced bands in the pH range of 6.2 to 6.4, probably due to microheterogenity. The molecular weight of the subunit determined by SDS-PAGE was 27,000 (±1410). The molecular mass of the native protein, determined by molecular sieving on Sephadex G-100, was 48,000 ± 1,130. Thus, the lectin is a dimer under physiological conditions, the two subunits being held together by non-covalent interactions.

Amino acid composition: The results of amino acid analysis of *A.paniculatus* lectin are summarized in Table 3. No half-cystine residues were detectable. The lectin contained valine, leucine, isoleucine and acidic residues at high levels. It also had unusually high amounts of methionine, tryptophan and lysine. *A. paniculatus* lectin was very similar to *A. caudatus* and *A. leucocarpus* lectins, with regard to blood-group specificity. *A. leucocarpus* lectin was found to be inhibited by only D-GalNAc, while other galactose containing saccharides were non-inhibitory. However, in addition to D-GalNAc, *A. paniculatus* lectin was inhibited by other D-galactose containing saccharides, though to a lesser extent, except lactose.

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Effect of Improvers on the Quality of Whole Wheat Flour Bread

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Effect of improvers on the quality of whole wheat flour bread showed an increase in the specific loaf volume to varied levels. Sodium stearoyl-2-lactylate showed highest response in improving the specific loaf volume followed by polyoxyethylene sorbitan monostearate, glycerol-monostearate, polyoxyethylene sorbitan monopalmitate, diacetyl tartaric acid esters of monoglycerides and soya lecithin. The crumb characteristics of whole wheat flour bread are also improved, while the crust shape, crumb colour and typical wheaty taste of whole wheat flour bread were unaffected.

Even though bread making potential is largely related to the quality and quantity of protein in the flour[1], the action of additives in increasing the loaf volume and crumb characteristics is well known[2]. The inclusion of emulsifiers or surfactants in bread is a well established practice in the baking industry. Sodium stearoyl-2-lactylate complexes with starch and proteins to form a huge aggregate and increases the dough stability[3]. Shogren *et al*[4] showed that addition of surfactant to the blend of wheat flour, wheat bran and vital gluten substantially improved the loaf volume.

Baking industry is one of the largest organised food industries in the country. The wheat production in India is expected to rise to 75 million tonnes by 2000 AD from the present production of 54.0 million tonnes. The production of bread in India is estimated to increase from 1.52 million tonnes in 1990 to 2.7 million tonnes by 2000 AD. There exists a considerable scope for introduction of newer bakery products. Whole wheat flour bread would be ideal since it offers wholesome, typical, wheaty taste. With this background, studies on the effect of improvers on the quality of whole wheat flour bread were undertaken and the results are presented in this paper.

Materials and Methods

Commercial sample of *Triticum aestivum* procured from local market was milled into whole wheat flour in hammer mill (Model: Apex). The chemical and rheological characteristics of the whole wheat flour have already been reported[5].

Preparation of whole wheat flour bread with improvers: Whole wheat flour bread based on sponge and dough method was prepared according to AACC[6] procedure with a reduced proof time of 40 min instead of 55 min. The loaves were prepared separately with guar gum (0.5%), potassium bromate (20 ppm), ascorbic acid (200 ppm), gluten (2.0%) enzyme active soya flour (0.5%), sorbitol (0.5%) and emulsifiers (0.5%) like soya lecithin, polyoxyethylene sorbitan monostearate (Tween-60), polyoxyethylene sorbitan monopalmitate (Tween-40), sodium stearoyl-2-lactylate (SSL), glycerol-monostearate (GMS) and diacetyl tartaric acid esters of monoglycerides (DATEM). Sensory evaluation of loaves after 24 h of preparation for crust shape and colour, crumb colour, grain and texture and eating quality was carried out by a panel of six judges.

Changes in quality of bread during storage: The loaves without and with 0.5% each of SSL and sorbitol were prepared and packed separately in polypropylene bags of 150 gauge after cooling for 4 h for determining the changes in quality of bread during storage. Swelling power of bread crumb was measured according to modified method of Cathcart and Luber[7]. The compressibility of bread crumb was measured in a General Foods Texturometer (Model GTX) equipped with brass plunger of 50 mm diam and flat aluminium platform using the following settings: attenuator-1, bites-6/ min, chart speed - 750 mm/min, and volts-0.5. The force required for 90% compression of a bread slice of 1.0 cm thickness was measured.

Results and Discussion

Effect of various improvers on the quality of whole wheat flour bread: The data on the improvement brought about by the addition of improvers on the quality of whole wheat flour bread are presented in Table 1.

The results indicate that improvers increased the specific

TABLE 1. EFFECT OF IMPROVERS ON THE QUALITY* OF WHOLE WHEAT FLOUR BREAD

Improver	Level	Specific loaf volume (ml/g)	Crumb texture	Crumb grain score**
Control	-	3.31[a]	Soft	7.0
Guar gum (%)	0.5	3.45[b]	Very soft	7.5
Potassium bromate (ppm)	20	3.37[a]	Soft	7.0
Ascorbic acid (ppm)	200	3.69[ef]	Soft	7.5
Gluten (%)	2.0	3.62[cd]	Soft	7.5
Enzyme active Soya flour (%)	0.5	3.47[b]	Soft	7.5
Sorbitol (%)	0.5	3.60[c]	Very soft	7.0
Soya lecithin (%)	0.5	3.47[b]	Very soft	7.5
Tween-60 (%)	0.5	3.74[ef]	Very soft	7.0
Tween-40 (%)	0.5	3.68[de]	Very soft	7.5
SSL (%)	0.5	3.75[f]	Very soft	7.5
GMS (%)	0.5	3.70[ef]	Very soft	7.0
DATEM (%)	0.5	3.60[c]	Very soft	7.5
SEM		±0.02		

Means of the same column followed by different letters differ significantly ($P < 0.05$)

* All breads had normal crust, dark brown crust colour, light brown crumb colour anc wholesome typical wheaty taste.

* Breads with gluten, enzyme active soya flour, soya lecithin, Tween-40, SSL and DATEM had fine uniform and all others had medium fine uniform crumb grain.

** Maximum score - 8.

loaf volume of whole wheat flour bread to varying levels. Ascorbic acid, gluten and sorbitol showed better improvement in specific loaf volume of whole wheat flour bread than enzyme active soya flour, potassium bromate and guar gum. The overall improvement in quality of whole wheat flour bread due to addition of guar gum was marginal when compared to the improvement reported by Venkateswara Rao *et al*[8] in white bread. Potassium bromate did not show any change in crust and crumb characteristics. Rogers and Hoseney[9] reported that whole wheat doughs did not respond to potassium bromate within the range of 0-50 ppm. Addition of ascorbic acid improved the overall quality of whole wheat flour bread. Addition of gluten improved the specific loaf volume, crumb grain and score. Gan *et al*[10] reported similar findings stating that the non-endosperm components play a prominent role in disrupting the continuous gluten protein matrix in whole wheat bread resulting in coarse and thick cells. But the gluten supplemented whole meal bread was thicker, smoother and more continuous indicating strengthening effect of gluten. Shogren *et al*[4] also reported that the adverse effects of 15 parts wheat bran per 85 parts of wheat flour were essentially eliminated by adding 2% vital gluten and an emulsifier. Improvement in the quality of whole wheat flour bread due to enzyme active soya flour could be attributed to the enzyme lipoxygenase. Faubion and Hoseney[11] reported that lipoxygenase present in enzyme active soya flour has strengthening effect on dough similar to that obtained by oxidation. Wu *et al*[12] obtained good results when 1.0% enzyme active soya flour was included in the recipe in short time bread making systems. Sorbitol, soya lecithin, Tween-60, Tween-40, SSL, GMS and DATEM improved the crumb softness. Improvement in loaf volume and crumb texture with addition of DATEM was reported by Galliard and Collins[13] and Lorenz[14]. SSL proved to be the best among the additives tried for improving the quality of whole wheat flour bread. It brought out the highest improvement in the specific loaf volume and showed marked improvement in other crumb characteristics.

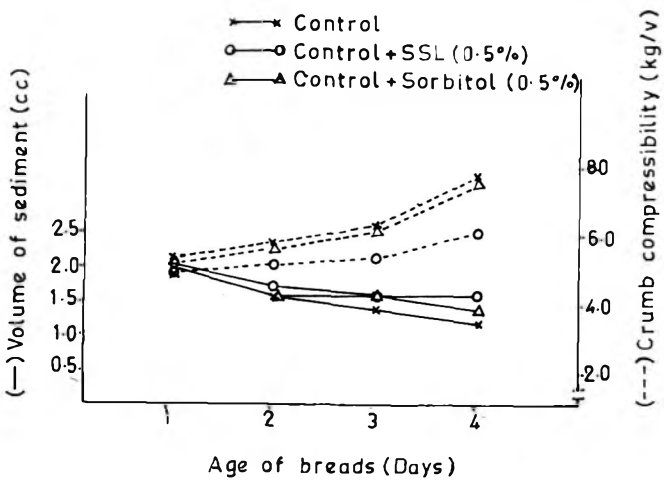

Fig.1. Effect of storage on the swelling power of crumb and crumb compressibility of whole wheat flour bread.

Changes in quality of bread during storage: The effect of storage on swelling power and softness of crumb from whole wheat flour bread is presented in Fig 1. Katz[15] reported that swelling power of bread decreases during staling and this change is attributed to starch retrogradation. The results showed that the volume of the sediment which was 2.0 on the first day for all the three breads, decreased by 0.8, 0.4 and 0.6 cc for the control, SSL and sorbitol containing breads respectively after four days of storage. The results confirmed that breads with SSL retained the capacity of highest swelling power compared to the control bread as well as sorbitol-containing bread. Lorenz[14] made similar observation that emulsifiers act as anti-staling agents.

The softness of breads improved with addition of SSL and sorbitol as indicated by the decrease in crumb compressibility values by 0.5 and 0.1 kg/v respectively measured by General Foods Texturometer. The crumb compressibility values increased by 2.4, 1.3 and 2.3 kg/v on four days of storage for control and breads containing SSL and

sorbitol respectively. The increase was gradual for all the breads during four days of storage. Szczesniak and Hall[16] illustrated the application of General Foods Texturometer to the quantification of the firmness of bread on staling and reported the increase in firmness values with the age of bread. The results indicated that the rate of firming of crumb was slower with the addition of SSL, while the effect was only marginal with the addition of sorbitol. According to Tenney[17], emulsifiers improve the softness and retain it for a longer period of storage and the maximum improving effect was found with SSL.

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ANNOUNCEMENT

A Section has been introduced under the head "Rapid Communications" in the Journal of Food Science and Technology in order to give importance to impact-making, innovative, newer research findings (please refer "Instructions to Authors", appearing in this issue). Such papers will be published within 5 months from the date of receipt of the manuscript, out of the order of submission. The maximum length of such papers has been fixed to 10 manuscript pages (all inclusive, typed in double spacing throughout). The authors have to append a note, indicating novelty, implications of the results and urgency in publication. The editor reserves the right to decide as to what constitutes a "Rapid Communication".

This section is expected to serve the interest of researchers and technologists for quicker publication of their important findings and will also attract quality manuscripts.

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Effect of Ingredients on the Quality of Whole Wheat Flour Bread

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The improvement in the quality of whole wheat flour bread with 8% fat, 0.5% sodium stearoyl -2-lactylate (SSL) and 20 ppm potassium bromate was better by sponge and dough method compared to that by straight dough and mechanical dough development methods. The specific loaf volume decreased with the increase in sugar content beyond 5.0% in straight dough and sponge and dough methods but remained unaffected in mechanical dough development method. Maximum increase in specific loaf volume was caused by 200 ppm ascorbic acid in mechanical dough development method and by 0.5% SSL in straight dough and sponge and dough methods. With optimum levels of all ingredients, maximum improvement in the quality of whole wheat flour bread was obtained by sponge and dough method.

Demand for whole wheat flour bread has increased considerably in last few years in advanced countries because of its better nutritional and sensory qualities[1]. Upto 80% of the wheat produced in India is consumed in the form of *chapati, puri* and *parota*. There exists a considerable scope for the introduction of newer varieties of bakery products in India to meet the increasing demands for the bakery products. Whole wheat flour bread would be one of the ideal choices since it offers a familiar wholesome, typical, wheaty taste. The results of studies on the effect of ingredients on the quality of whole wheat flour bread are reported in this paper.

Materials and Methods

Chemical and rheological characteristics: Commercial *Triticum aestivum* wheat from local market was milled into whole wheat flour in hammer mill (Model: Apex). Moisture, total ash, Hagberg's falling number, diastatic activity, damaged starch were determined according to AACC procedures[2]. Crude protein (N × 5.7) was estimated by micro-Kjeldahl method. Dough properties of whole wheat flour were studied using the farinograph, extensograph, amylograph and mixograph according to standard AACC procedures[2].

Effect of varying levels of ingredients on quality of whole wheat flour bread: The ingredients used for the preparation of whole wheat flour breads in all the cases were 100 g flour, 2% yeast, 0.1% yeast food, 2.5% sugar, 1.0% salt, 0.5% malt, 15 ppm potassium bromate and 66.3% water. The methodology of preparation of straight dough was according to remix procedure of Irvine and McMullan[3] with a reduced fermentation and proof times of 120 and 25 min for the dough respectively. For the sponge and dough method, standard AACC procedure was used with a reduced proof time of 25 min. The breads based on mechanical dough development method were prepared using Tweedy mixer (Type N 23 F-G) applying partial vacuum of 15 inches of mercury. The dough was developed by the expenditure of 5 watt h energy/pound, and floor time given was 20 min. The dough was moulded, proofed for 25 min and baked for 25 min at 450°F.

Breads were made by all the three methods by changing the ingredients with 3.0% yeast, 4.0 and 8.0% fat, 5.0, 7.5 and 10.0% sugar, 0.5% SSL, 10 and 20 ppm potassium bromate and 100 and 200 ppm of ascorbic acid separately.

Breads with optimum levels of ingredients: Breads based on straight dough, sponge and dough and mechanical dough development methods were prepared using optimum levels of ingredients. The ingredients used were flour: 100 g, yeast: 3.0%, malt: 0.5%, salt: 1.0%, sugar: 5.0%, fat: 8.0%, diammonium hydrogen phosphate: 0.1%, SSL: 0.5%, ascorbic acid: 0.02% and water: 66.3%.

Evaluation of breads: The evaluation of breads were carried out after 24 h by a panel of six judges.

Results and Discussion

Chemical and rheological characteristics of whole wheat flour: The protein content (11.9%), damaged starch (10.7%), farinograph water absorption (66.3%), valorimeter value (44), extensograph ratio figure (5.0), extensograph area

(83.0 cm^2), mixograph area (73.1 cm^2) indicated that the *Triticum aestivum* wheat used in the study was of medium strong variety.

Effect of various ingredients on the quality of whole wheat flour bread:

Yeast: Increasing the yeast from 2 to 3% improved specific loaf volume of bread in all the three methods (Table 1). The improvement in specific loaf volume was higher in the third method compared to the first two methods. This showed high requirement of yeast in mechanical dough development method. The other crust and crumb characteristics of breads were not affected. An improvement in crumb texture was found due to increase in volume of bread by mechanical dough development method. Lai *et al*[4] reported that yeast higher than the minimum level is required in sponge and dough method for the best loaf volume in whole wheat flour bread.

Fat: The results showed an increase in specific loaf volume of bread in the three bread making methods with the addition of 4 and 8% fat (Table 1). The increase in specific loaf volume was highest in straight dough method and least in mechanical dough development method. However, flying top was observed in case of straight dough and mechanical dough development methods due to undermaturation of dough. Breads made with addition of fat showed improvement in crumb texture and crumb grain score. Baldwine *et al*[5] reported that fat improves the gas retention capacity of the dough by plugging the voids in cell walls. The use of fat and sugar improves the quality of whole wheat dough for production of speciality whole meal breads[6]. Pomeranz[7] also reported an increase in loaf volume of upto 25% and improvement in crumb grain with the addition of 0.7 to 3.0% fat. Junge and Hoseney[8] showed that dough containing shortening remained expanded for longer time and hence produced higher volume.

Sugar: Increasing the sugar from 2.5 to 5% level increased the specific loaf volume due to increase in gas production during fermentation and proofing in case of breads made by straight dough and sponge and dough methods (Table 1). Further increase in sugar level had an adverse affect on the specific loaf volume with the values reversing almost to those of control. This may be attributed to the increased gas production and poor gas retention properties of the doughs. This observation was also supported by the reduction in dough raising capacity of flour[9] with increase in sugar content. Rogers and Hoseney[1] reported that with addition of 6% sugar, the gas production

TABLE 1. EFFECT OF INGREDIENTS ON THE QUALITY OF WHOLE WHEAT FLOUR BREAD* BY DIFFERENT METHODS

Ingredient	Level	Specific loaf volume (ml/g)			Crumb texture			Crumb grain score**		
		Method			Method			Method		
		I	II	III	I	II	III	I	II	III
Control	-	2.31	2.44	2.13	Soft	Soft	Slightly hard	7.0	7.0	6.5
Yeast (%)	3.0	2.38	2.49	2.56	Soft	Soft	Soft	7.0	7.0	6.5
Fat (%)	4.0	2.77	2.82	2.32	Very soft	Very soft	Soft	7.5	7.5	7.0
	8.0	2.84	2.88	2.49	Very soft	Very soft	Very soft	7.5	7.5	7.0
Sugar (%)	5.0	2.50	2.65	2.14	Soft	Soft	Slightly hard	7.0	7.0	7.5
	7.5	2.46	2.56	2.15	Slightly hard	Slightly hard	Slightly hard	7.0	7.0	7.5
	10.0	2.35	2.46	2.17	Slightly hard	Slightly hard	Slightly hard	7.0	7.0	8.0
SSL (%)	0.5	2.87	3.16	2.38	Very soft	Very soft	Soft	7.5	8.0	7.5
Potassium bromate (ppm)	10.0	2.31	2.43	2.13	Soft	Soft	Slightly hard	7.0	7.0	6.5
	20.0	2.39	2.50	2.37	Soft	Soft	Slightly hard	7.0	7.0	7.0
Ascorbic acid (ppm)	100.0	2.40	2.46	2.34	Soft	Soft	Soft	7.0	7.5	7.0
	200.0	2.47	2.51	2.58	Soft	Soft	Very soft	7.0	7.5	7.0

Method I. Straight dough method
II. Sponge and dough method
III. Mechanical dough development method

* Breads with fat in methods I and III had flying top and all others had normal crust shape.
* Breads with fat, sugar and SSL had dark brown and all others had brown crust colour.
* Breads with fat and SSL in method I, fat sugar and SSL in method II and sugar and SSL in method III had fine uniform and all others had medium fine uniform crumb grain.
* Breads with sugar had sweetish taste and all others had wholesome typical wheaty taste.
** Maximum score - 8

during fermentation was adequate; but gas retention failed to cope with the production thus resulting in low loaf volume of whole wheat breads. However, the specific loaf volume of breads by mechanical dough development method with different levels of sugar showed negligible effect on loaf volume. This could be attributed to the insufficient time for any increase in gas production. The increase in sugar level, generally changed the crust colour from brown to dark brown and adversely affected the crumb texture making it slightly hard. While the sweetness of breads increased gradually, crumb grain and crumb grain score of breads remained unaffected with increase in sugar level.

SSL: Surfactants are widely used in bread making for dough strengthening Sodium-stearoyl-2-lactylate complexes with starch and proteins to form a huge aggregate and increases the dough stability[10]. Shogren *et al*[11] showed that addition of surfactant to the blend of wheat flour, wheat bran and vital gluten substantially improved the loaf volume. Adding SSL at 0.5% level increased the specific loaf volume of breads prepared through straight dough, sponge and dough and mechanical dough development methods (Table 1). Highest crumb grain score, fine and uniform crumb, very soft crumb texture showing maximum improvement in the quality of breads were observed with the addition of SSL in all the three bread making methods.

Potassium bromate: Addition of potassium bromate at 10 and 20 ppm levels showed negligible effect on specific loaf volume, crust and crumb characteristics and sensory quality of breads from all the three methods. Rogers and Hoseney[1] also reported that whole wheat doughs did not respond to potassium bromate within the range of 0-50 ppm.

Ascorbic acid: Galliard[12] reported that a traditional whole meal loaf has a low specific volume and a dense crumb structure; but permitted use of ascorbic acid and emulsifiers produces good loaf volume bread and soft crumb texture. Bloksma[13] attributed the effect of oxidants on dough properties to the interchange reactions of sulphydryl and disulphide groups present in protein network. Ascorbic acid is widely used for improving the quality of bread. Though ascorbic acid is a reducing agent, it exerts the effect of an oxidising agent on the dough properties[14]. The mechanism involves the oxidation - reduction of ascorbic acid by enzymes, ascorbic acid oxidase and dehydro ascorbic acid reductase, respectively[15]. The improvement in specific loaf volume of bread was 0.02 to 0.21 ml/g and 0.07 to 0.45 ml/g with 100 and 200 ppm ascorbic acid, respectively in the three bread making methods. The maximum improvement was observed in mechanical dough development method as the requirements of oxidants in this method are known to be high. While all other bread characteristics remained same, the crumb score improved by 0.5 in breads from sponge and dough and mechanical dough development method.

Malt: Omission of 0.5% malt from the dough resulted in decrease in specific loaf volume for the breads made by all the three methods (Table 1). The brown crust colour changed to pale brown due to lack of enough enzyme activity and production of reducing sugars which are needed for browning reactions and caramelization. Crumb grain was fine, closed and uniform, while crumb grain score was 7.5 for the breads without malt when compared to those of medium fine and uniform grain and score of 7.0 for the breads with malt. The results indicated that the flour required malt supplementation due to deficiency in α-amylase activity. This was also supported by the high falling number value of 489, which indicated low α-amylase activity[9].

Effect of optimum levels of ingredients on the quality of whole wheat flour bread made with three different methods:

The sponge and dough method is the most suitable method for the preparation of whole wheat flour bread. Bread made by this method showed the highest specific loaf volume followed by mechanical dough development method and straight dough method (Table 2, Fig.1). The optimum levels of all ingredients together improved the specific loaf volume by 1.38 ml/g and also enhanced the crumb grain characteristics by the sponge and dough method. However, the mechanical dough development method had shown a very marked improvement in the specific loaf volume. It showed that an oxidant and an emulsifier are necessary for producing a good loaf volume in bread made by mechanical dough development method. A similar observation was made by Galliard and Collins[16]. The improvement in the specific loaf volume after incorporating optimum levels of all ingredients, was lowest in the straight dough method. The crumb grain improved from uniform medium-fine to uniform fine cells and the

TABLE 2. EFFECT OF OPTIMUM LEVELS OF INGREDIENTS ON THE QUALITY OF WHOLE WHEAT FLOUR BREAD* MADE WITH THREE DIFFERENT METHODS

Method	Specific loaf volume (ml/g)	Crust shape and colour	Crumb texture
Straight dough	3.02	Normal	Soft
Sponge and dough	3.82	Normal and dark brown	Very soft
Mechanical dough development	3.23	Normal and dark brown	Very soft

* All breads had light brown crumb colour, fine and uniform crumb grain, 7.5 crumb grain score out of a maximum of 8 and wholesome typical wheaty eating quality.

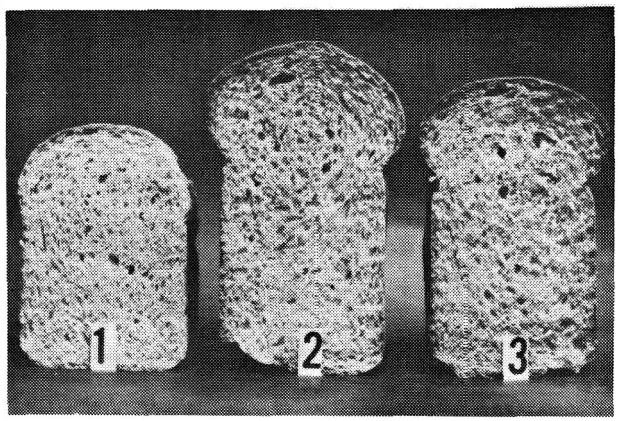

Fig.1. Photograph of breads showing the effect of optimum ingredients on the quality of whole wheat flour bread made by different methods. 1 - Straight dough method, 2-Sponge and dough method and 3 - Mechanical dough development method.

crumb texture was soft, with a crumb grain score of 7.5. The results clearly showed that very good quality of whole wheat flour bread can be prepared with optimization of ingredients and the application of the sponge and dough method.

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Effect of Phosphate and Spent Hen Yolk on the Quality of Chicken *Patties* and *Kababs* from Spent Hens

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Addition of phosphate and ova yolk to chicken *patties* and *kababs* formulated with deboned meat and by-products (skin, gizzard and heart) from spent hens significantly improved emulsion stability, reduced cooking losses and imparted better acceptability to both the products. Shrinkage in diameter of *patties* was small and significantly lower in phosphate added samples. Sensory scores indicated significantly higher overall palatability scores in both cases.

Profitable disposal of the tough meated spent hens has become a problem with the rapid development of broiler industry and increased availability of spent hens. Many workers[1,2] have attempted to develop ground or emulsion

type products for efficient utilisatioin of spent hen components. Addition of skin, neck meat, gizzard and hearts at various levels was tried to improve the texture of chicken frankfurters[3,4]. Ova yolk was found to have similar compositional and emulsifying characteristics as that of conventional egg yolk material[5]. Lu and Baker[6] demonstrated the antioxidant capacity of egg yolk phosvitin. Addition of salt and phosphate was found to improve the quality of several red meat and poultry products[7-9]. In the present paper, the results of studies on the effect of addition of phosphate and ova yolk on the quality of chicken *patties* and *kababs* formulated with meat and by-products (skin, gizzard and heart) from spent hens are reported.

Materials and Methods

Layer type spent hens of above 500 days were slaughtered and dressed. Hot boning was done within 3 h for separation of components. The meat was frozen in 1 kg lot in polythene bags at -10°C for 3-5 days, tempered at 5°C for 15 h and coarse minced (8 mm hole plate). Skin, gizzard and heart were also similarly frozen and minced (5 mm hole plate) twice to reduce the particle size. Frozen chicken fat was coarse-minced. Ova yolk of different size follicles was collected by hand and kept at 5°C for 3-5 days before use.

Patties formulation: Four formulations are presented in Table 1. Emulsion type mix was prepared using a bowl chopper. *Patties* of 75 g mix each were prepared using a mould of 75 mm diameter and 15 mm height by oven cooking at 160°C for 20 min to attain an internal temperature of 75°C. *Kababs* were prepared by traditional procedure using 40 g mix for each *kabab*, moulding on to skievers and charbroiled till done as evaluated by outer appearance. Yields of cooked products were recorded and kept at 5°C before sampling for analysis.

To record emulsion stability, 20 g of the emulsion mix was placed in a polythene bag and heated at 80°C for 30 min in a water bath. The cookout was drained, the cooked mass was cooled and weighed to determine the weight loss. The cooking loss of *patties* and *kababs* was proportioned into % loss of solids, % loss of moisture from the solids and moisture contents of raw and cooked *patties*. Shrinkage of cooked *patties* was expressed as % loss in diameter of the cooked *patties* compared to raw *patties*. Shear force values were recorded using Warner-Bratzler shear press where rectangular strips of the samples (2x1x1 cm) were cut and sheared at 3 points and the average force was calculated per gram of sample. Sensory evaluation was done by an experienced panel of 8-10 members and *patties* or *kababs* were served after reheating in vegetable oil. A structured 8 point hedonic scale was used to record sensory scores (8: extremely desirable and 1: extremely undesirable). TBA values and sensory quality were evaluated on refrigerated stored chicken *patties* at 5 day intervals upto 20 days storage. Histological sections were cut from the cooked *patties* and stained with haemotoxylin and eosin stain to evaluate the emulsion quality. Three trials were conducted using different batches of meat.

TABLE 1. FORMULATIONS OF CHICKEN *PATTIES* AND *KABABS*

Ingredients, g	Control	Phosphate[1]	Ova yolk	Phosphate + yolk
Deboned meat	997.5	997.5	997.5	997.5
SGH mix[2]	370.5	370.5	361.5	361.5
Chicken fat	132.0	132.0	120.0	120.0
Yolk	-	-	45.0	45.0
Phosphate	-	7.5	-	7.5
Salt	30.0	22.5	30.0	22.5

All the formulations contained 37.5, 22.5, 75 and 105 g each of maida, spices mix, condiments and ice flakes respectively.

[1]Tetra sodium pyrophosphate (anhydrous).

[2]Skin, gizzard and heart in the natural proportions of 115:26:7

Results and Discussion

Phosphate containing samples showed a considerable increase (about 0.3 units) in pH compared to control or yolk samples (Table 2). Effect of alkaline phosphates to increase the pH of meat products has been reported[7,10,11]. Addition of yolk alone has little effect on pH compared to control. Phosphate and phosphate + yolk significantly improved emulsion stability (indicated by lower values) compared to control or addition of yolk alone. Even though Baker *et al*[1,3]. observed that egg yolk (4% level) was

TABLE 2. EMULSION QUALITY, COMPOSITION AND SENSORY SCORES OF CHICKEN *PATTIES*

Parameters	n	Control	Phosphate	Yolk	Phosphate + yolk
pH	3	5.6	6.0	5.7	6.0
Emulsion stability %	11	22.6^{a}	4.5^{c}	13.0^{b}	5.7^{c}
Cooking loss %	30	24.3^{a}	5.1^{c}	12.2^{b}	5.0^{c}
Shrinkage %	25	16.4^{a}	9.1^{c}	14.7^{a}	11.7^{b}
Shear force kg/g	6	0.08^{a}	0.06^{ab}	0.06^{b}	0.06^{b}
Raw emulsion*					
Moisture %	9	62.1	61.2	61.6	61.5
Protein %	3	15.0	14.9	15.8	15.8
Fat %	6	20.9	21.6	19.5	19.8
Cooked *patties**					
Moisture %	8	56.8	59.8	57.9	59.3
Protein %	3	19.5	17.5	18.1	17.3
Fat %	6	21.9	21.4	21.6	21.6
Sensory scores	35				
Appearance		6.2^{c}	7.2^{a}	6.7^{b}	7.1^{a}
Flavour		6.1^{c}	6.9^{a}	6.5^{b}	6.7^{ab}
Juiciness		6.0^{b}	6.9^{a}	6.2^{b}	6.8^{a}
Texture		5.9^{b}	6.7^{a}	6.1^{b}	6.7^{a}
Mouth coating		7.0	7.0	7.0	7.0
Overall palatability		5.9^{c}	6.7^{a}	6.3^{b}	7.0^{a}

Means with the same superscript do not differ significantly ($P<0.05$).

* Mean differences were not tested.

TABLE 3. COOKING YIELD AND SENSORY SCORES OF *KABABS*

Parameters	n	Control	Phos-phate	Yolk	Phosphate + yolk
Cooking yield %	13	71.0^{b}	80.2^{a}	77.5^{ab}	83.1^{a}
Sensory scores	23				
Appearance		6.0	6.3	6.3	6.3
Flavour		5.9	6.2	6.3	6.2
Juiciness		5.3^{b}	5.9^{a}	5.7^{ab}	6.0^{a}
Texture		5.6	6.0	5.8	6.1
Mouth coating		7.0	7.0	7.1	7.0
Overall palatability		5.6	6.2	6.0	6.4

Means with the same superscript do not differ significantly ($P<0.05$)

detrimental to the stability of the emulsion, addition of ova yolk (3% level) was found to improve the emulsion stability compared to control. Kondaiah and Panda[10] also reported the desirable effect of yolk addition in chicken sausages particularly in the formulation containing meat and by-products. The trend of cooking loss of chicken *patties* was found nearly similar to that of emulsion stability. When the cooking loss % was partitioned into solids loss % and moisture loss %, the respective losses in the four treatments were: control 5.15, 19.14; phosphate 0.56, 4.50; yolk 1.43, 10.74 and yolk + phosphate 0.13, 4.90. This indicated that the lower cooking losses in phosphate added samples were due to lower losses in both solids and moisture which was mainly due to combined effect of phosphates on water and fat binding[7].

Per cent shrinkage in the diameter of the *patties* was least ($P<0.05$) in phosphate added samples followed by phosphate + yolk, yolk added samples and control. The latter two were not significant ($P>0.05$). Shear force values were significantly higher in control compared to other treatments. The increased firmness in control was due to the higher cooking losses resulting in less juicy product[11]. The formulations were developed to have similar composition. However, in cooked *patties* higher moisture and lower protein contents were observed in phosphate or phosphate + yolk containing samples due to lower cooking losses. TBA values indicated very little differences between treatments. Since the values, in general, were very low (0.09 to 0.20 mg malonaldehyde/kg during 0 to 20 days storage period), the desirable effect of one treatment over the other could not be demonstrated. The uniformly low TBA values and high sensory scores of chicken *patties* observed in all the treatments during the 20 days of storage could be attributed to the anti-oxidant and anti-microbial effect of different spices incorporated[12].

Histological sections of the cooked *patties* indicated that phosphate or phosphate + yolk added samples showed better emulsification of fat, the fat globules being well surrounded by protein matrix and there was no evidence of fat coalescence compared to control and yolk added samples.

Sensory evaluation of *patties* (Table 2) indicated that phosphate or phosphate + yolk containing samples scored better compared to other formulations. Yolk alone added samples scored better than control. In general, the effect of treatment on *kababs* (Table 3) was similar to that on *patties*.

The study has demonstrated that addition of phosphate or phosphate + yolk has increased the quality and acceptability of chicken *patties* and *kababs* from spent hens. Even though addition of yolk with or without phosphate did not result in significant extra desirable effect compared to addition of phosphate, it has facilitated utilisation of ova yolk, an edible by-product from spent hens.

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Glycolipid Composition of Some Seed Varieties of Cotton

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Glycolipids, from six varieties of cottonseeds showed the prominence of diglucosyl and monoglucosyl diglycerides which accounted for 62-71% in addition to the presence of steryl glucoside (SG), acylated steryl glucoside (ASG) and an unidentified component. The fatty acid composition of total and component glycolipids showed the presence of myristic, palmitic, oleic, stearic, linoleic acids as major acids. Glucose was identified as a sugar moiety. The ratio of sugar-sterol-fatty acid was nearly 1:1:1 in ASG and that of sugar-sterol was nearly 1:1 in SG.

Glycolipids, the compounds with solubility properties of a lipid, have a complex structure. The function of the glycolipids is still obscure but it is assumed that they provide sugar molecules to the cells for their functioning[1]. Glycolipids exert a strong influence in the biosynthesis of oils and fats in the seeds[2]. The activities of many membrane-bound enzymes are controlled by the extent and quality of the lipid environment within the membrane. Some work[3-5] has been reported on the fatty acid composition of oils from varieties of cottonseed belonging to different gossypium species. This paper reports results on the glycolipid composition, fatty acid composition of total and component glycolipids and sterol composition of SG and ASG components with component molar ratios of varieties of cottonseed belonging to the *G. arboreum, G. hirsutum* and *G. barbadense* species.

Materials and Methods

Varieties of cottonseeds namely 'Y-1' (*Gossypium arboreum*), 'Laxmi' (*Gossypium hirsutum*), 'B-1007' (*Gossypium hirsutum*), 'CJ-73' (*Gossypium arboreum*), 'Varlaxmi' (*Gossypium barbadense*) and 'MCU-5' *(Gossypium hirsutum)* were obtained from Maharashtra State Oilseed Corporation Ltd., Akola. Standard glycolipids and methyl esters were obtained from Analabs, U.S.A. The decorticated and powdered seeds were extracted with chloroform: methanol (2:1, v/v) by the procedure of Folch *et al*[6]. The neutral lipids, glycolipids and phospholipids were separated by silicic acid (>200 mesh) column chromatography using chloroform, acetone and methanol (2.2:2:2,v/v/v). The glycolipids were eluted on acetone.

The glycolipids were separated into individual components such as monoglucosyl diglyceride (MGDG), diglucosyl diglyceride (DGDG), steryl glucoside (SG) and acylated steryl glucoside (ASG) by preparative TLC, using the solvent system[7], chloroform-methanol- 28% ammonia (70:20:2, v/v/v). The bands were visualised by iodine vapours, appropriate areas scrapped off, eluted with acetone and weighed. These fractions of glycolipids were spotted on TLC plate and developed with solvent system[7]. The spots were visualised with the periodate-benzidine reagent. The R_f values were compared with authentic standards. Sugars were identified[8] by hydrolyzing the samples with sulphuric acid for 12 h at 100°C. SG and ASG were analysed[9] for their components.

The fatty acids were converted into their respective methyl esters by the procedure of Christi *et al*[10] and analysed by gas liquid chromatography (GLC) having a flame ionisation detector at 240°C, the column being packed with 15% EGSS-X on chromosorb-W (40-60 mesh). The conditions of GLC were: chart speed 60 cm/h. injection port temperature 300°C, column temperature 200°C and nitrogen flow rate 60 ml/min. The quantitation was carried out by a programmed computer after identification.

Results and Discussion

The glycolipid composition of lipids from different varieties of cottonseeds (Table 1) shows the presence of MGDG, DGDG, SG and ASG as component glycolipids along with some unidentified components. Variations in the glycolipid composition are due to the different varieties. The fatty acid composition of total and component

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TABLE 1. GLYCOLIPID COMPOSITION OF VARIETIES OF COTTONSEEDS

Seed var	Glycolipids (wt.%)[a]				
	MGDG	DGDG	SG	ASG	Unidentified
'Y-1'	23.8	42.8	15.8	16.3	1.3
'Laxmi'	25.4	44.4	16.0	12.6	1.6
'B-1007'	20.8	43.5	19.8	14.5	1.4
'CJ-73'	24.1	42.2	18.5	13.9	1.3
'Varlaxmi'	24.7	43.5	17.8	12.8	1.2
'MCU-5'	24.4	42.8	18.5	13.0	1.3

a :-Means of triplicate analysis,
MGDG :-Monoglucosyl diglyceride,
DGDG :-Diglucosyl diglyceride,
SG :-Steryl glucoside,
ASG :-Acylated sterylglucoside

TABLE 2. FATTY ACID COMPOSITION OF TOTAL GLYCOLIPIDS OF VARIETIES OF COTTONSEEDS

Seed var	Fatty acids (wt %)[a]				
	16:0	18:0	18:1	18:2	Others
'Y-1'	29.5	2.0	26.2	40.2	2.1
'Laxmi'	29.2	1.2	20.4	44.2	4.6
'B-1007'	33.1	1.4	19.1	43.2	3.2
'CJ-73'	29.4	1.5	22.1	43.4	3.6
'Varlaxmi'	30.1	1.2	20.4	44.2	4.1
'MCU-5'	32.2	2.5	21.6	43.2	0.5

a: Means of triplicate analysis
Others: Fatty acids 12:0, 14:0, 16:1, 20:0, 22:0, 24:0

TABLE 3. FATTY ACID COMPOSITION OF COMPONENT GLYCOLIPIDS OF VARIETIES OF COTTONSEEDS

Seed var	Glycolipid	Fatty acids (wt.%)[a]				
		16:0	18:0	18:1	18:2	Others
'Y-1'	MGDG	30.1	2.5	23.1	43.1	0.2
	DGDG	30.8	2.2	25.3	40.1	1.1
	ASG	31.2	3.7	20.2	44.9	-
'Laxmi'	MGDG	30.4	2.2	30.0	36.4	1.0
	DGDG	31.3	1.8	33.4	32.5	1.0
	ASG	31.6	2.0	26.2	40.2	-
'B-1007'	MGDG	30.5	1.5	26.1	40.1	1.8
	DGDG	32.7	1.3	25.2	38.2	2.6
	ASG	33.0	1.7	23.1	42.2	-
'CJ-73'	MGDG	29.2	1.3	26.2	41.2	2.1
	DGDG	29.2	2.2	25.2	40.0	3.4
	ASG	30.1	2.1	20.2	47.2	0.6
'Varlaxmi'	MGDG	28.5	1.5	22.1	42.2	5.7
	DGDG	30.3	2.6	22.2	40.3	4.6
	ASG	30.2	2.1	20.2	47.1	0.5
'MCU-5'	MGDG	31.1	1.5	22.2	45.2	-
	DGDG	30.3	2.5	23.1	40.2	3.9
	ASG	27.6	2.0	22.8	47.1	0.5

a: Means of triplicate analysis
Others: Fatty acids 12:0, 14:0, 16:1, 20:0l 22:0

TABLE 4. STEROL COMPOSITION OF STERYL GLUCOSIDE (SG) AND ACYLATED STERYL GLUCOSIDE (ASG) FRACTIONS OF LIPIDS FROM VARIETIES OF COTTONSEEDS

Seed var	RRT of SG				RRT of ASG			
	I	II	III	IV	I	II	III	IV
'Y-1'	71.0	5.3	18.5	5.2	3.1	71.1	20.4	5.4
'Laxmi'	70.5	5.5	18.7	5.3	3.3	73.5	17.1	6.1
'B-1007'	72.4	5.7	16.8	5.1	2.5	74.3	17.2	6.0
'CJ-73'	71.6	5.9	17.1	5.4	3.1	72.5	18.6	5.8
'Varlaxmi'	73.3	5.1	15.7	5.9	2.8	74.7	16.8	5.7
'MCU-5'	70.8	5.2	18.9	5.1	3.6	70.9	20.0	5.5

I : β-Sitosterol (RRT=1.00)
II : Stigmesterol (RRT=0.88)
III : Campesterol (RRT=0.71)
IV : Brassica-sterol (RRT=0.70)
RRT : Relative retention time

glycolipids (Table 2) shows the fatty acids as palmitic, stearic, oleic and linoleic acids along with minor quantities of 12:0, 14:0, 20:0, 22:0 and 24:0 fatty acids. The differences regarding the changes in the fatty acid composition of total and component glycolipids may also be attributed to varietal differences. 'Y-1' variety contained the highest percentage of unsaturated fatty acids followed by 'MCU-5' variety. ASG components (Table 3) contained the highest unsaturation followed by MGDG and DGDG for all varieties. Sterol compositions of SG and ASG components were found to be nearly similar, β-sitosterol being the major component. Glucose was identified as a sugar moiety. Molecular ratio of sugar-sterol-fatty acid was nearly 1:1:1 for ASG and that of sugar-sterol was nearly 1:1 for SG (Table 4).

The glycolipid composition of these seeds from different cotton varieties agreed well with the general glycolipid pattern of other seeds such as *Behada*[9], *Karanja*[11], *Briza spicata*[12], rice bran[13], *kenaf*[14], palm[15,16] and *Elaeis guineensis*[17].

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Packaging of Some Fruit and Vegetable Products in Glass Containers

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Suitability of indigenously available glass containers has been studied for packing mango juice, banana puree, tomato puree and processed peas. Reduction in β-carotene was found to be less in amber coloured bottles compared to colourless bottles. Corrosive products like banana and tomato puree could be safely packed in glass bottles. Acidification of brine was found essential for packing processed peas in glass bottles.

Heat sterilizable glass containers are used as an alternative to tinplate containers for packing processed food products. In some of the developed countries, about 40% of the processed food products are packed in glass containers[1]. In view of the high cost of metal containers and corrosion problem, there is a good scope for introducing such glass containers for packing processed food products in India.

The quality of the product in glass containers was comparable with products packed in tinplate containers except for slight colour deterioration due to the passage of the light through the walls of the containers which can be reduced by incorporating cerium oxide in composition or by producing coloured glass.

In India, at present, light weight heat sterilizable glass containers are not available and hence, attempts have been made in this investigation to utilize the existing glass bottles for packing some of the fruit and vegetable products. The results of these studies are presented in this paper.

Materials and Methods

Bananas (*Musa cavendishii, pachabale* variety), tomatoes (*Lycopexsicum esculentum*) and processed peas (*Pisum sativum*) were obtained from the local market. For preparation of mango (*Mangifera indica*) juice, canned and stored (6 months at 0°C) Alphonso mango pulp was used.

Containers used for packing the above products were:

i) A_1 tall plain cans (77 x 116 mm) - [(D 100/50) (D 11.2/5.6 g/m^2, higher coating inside)]
ii) Used amber-coloured beer bottles (680 ml)
iii) Colourless honey jars. [(350 ml); Height: 125 mm; Diameter: 65 mm].

Mango juice: Mango juice was prepared so as to contain 35% pulp, 20° Brix (total soluble solids) and 0.3% acidity (as citric acid).

Banana puree: Bananas were peeled, blanched in boiling water until the temperature in the centre of the fruit reached 88°C. The fruits were macerated in Stephen's machine. The Brix, acidity and pH were determined in the pulp

and the final Brix and pH were adjusted to 34° and 4, respectively[2].

Tomato puree: Tomato juice was extracted by hot break method. To prepare tomato puree, the juice (3° Brix) was concentrated to 12° Brix in a forced circulation evaporator under vacuum at a temperature of 42°C.

The prepared juice/puree was heated to 85°C, puree was heated to 85°C, filled into different types of containers, sealed and processed in boiling water for 25 minutes and then cooled in water.

Processed peas: Peas were soaked in water overnight (18 h). The soaked peas were washed in running cold water, blanched for 5 min in boiling water, cooled and filled into cans and bottles. In one set, the peas were covered with 2% hot brine (85°C), and in the other set, the peas were covered with acidified brine (0.2% acetic acid in brine), exhausted and sealed. The sealed containers were processed in a retort using a mixture of steam and water for 1 h at 115°C, (11 psig) and cooled with the application of overhead pressure for processing and cooling.

Storage conditions: The products packed in tin cans and glass bottles were stored at 37°C. The samples were withdrawn periodically for analysis of various physical, chemical parameters and sensory qualities.

Methods of analysis: A piercing type vacuum gauge was used to measure the vacuum in inches of Hg, while a hand refractometer was used to measure °Brix. Titratable acidity was determined by the standard method of AOAC, while the salt content in brine was determined by titrating a known quantity of the sample with standard silver nitrate solution, using potassium chromate solution as the indicator[3].

β-carotene was determined according to the procedure described by American Assay of Vitamin Chemists[4]. Ascorbic acid was determined by titrating against 2-6, dichlorophenol indophenol dye, while nitrate was determined colorimetrically after reducing it to nitrite[5]. Chlorophyll content was determined by following the procedure described by Ranganna[6].

For sensory evaluation of the products, a numerical scoring scale (5 point scale) was prepared and samples were scored by ten panelists with respect to colour, flavour, taste and overall acceptability.

Results and Discussion

Shelf-life studies:

Mango juice: The cans retained good vacuum during storage. There was no significant difference among different types of containers during storage with respect to total soluble solids, pH, acidity, colour, taste and flavour upto 6 weeks of storage. At the end of 8 weeks, there was slight reduction in ascorbic acid content in mango juice packed in bottles as compared to cans. Similarly, β-carotene content was less in bottles as compared to cans, while the reduction was higher in mango juice packed in honey jars (Table 1).

Although the product was giving caramelised taste and flavour, it was acceptable in all the three containers. Medium feathering was noticed in the can interior and the condition of the bottle was good. Similar observations were noticed by Wartenburg[7] in the case of orange and apple juices

TABLE 1. RESULTS OF ANALYSIS OF PRODUCTS PACKED IN GLASS BOTTLES

Physical and chemical characteristics	Mango juice (8 weeks at 37°C)			Banana puree (6 weeks at 37°C)			Tomato puree (6 weeks at 37°C)			Peas in non-acidified brine (10 weeks at 37°C)			Peas in acidified brine (10 weeks at 37°C)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Vacuum (inches of Hg)	12	-	-	5	-	-	10	3	5	13	-		7	-	-
Total soluble solids%	21	20	21	30	35	32	14	13	13	-	-	-	-	-	-
pH	3.35	3.35	3.35	4.27	4.23	4.21	3.95	3.86	3.88	5.90	5.85	-	5.48	5.2	-
Acidity (% citric acid)	0.37	0.36	0.30	-	-	-	1.61	1.52	1.49	-	-	-	-	-	-
Ascorbic acid (mg/100 g)	6.97	6.51	5.71	4.24	3.71	3.71	-	-	-	-	-	-	-	-	-
Total chlorophyll (mg/100 g)	-	-	-		-	-	-	-	-	1.562	1.382	-	0.864	0.892	-
β-carotene (µg/100 ml)	2987	2656	1695	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate (ppm)	-	-	-	57.05	93.96	93.96	-	-	-	-	-	-	-	-	-
Overall quality	Slight caramelised taste and flavour		Slight metallic taste and flavour	N	N	N	N	FL	FL	N	N	-	N	N	-

N: Normal FL: Flavour loss

A - Can (control), B - Amber- coloured beer bottles, C - Colourless honey jars.

packed in glass containers and laminated board without headspace.

Banana puree: Vacuum in the can was considerably less from the initial stage. However, there was no significant change in total soluble solids, acidity and pH during storage among different types of containers. Reduction in ascorbic acid was found to be more in both types of glass bottles as compared to cans. Banana pulp was rich in nitrate, the initial concentration being 95.25, 98.23, 98.40 ppm in can, beer bottle and honey jar, respectively. After 6 weeks storage, a significant reduction of nitrate was found in cans as compared to bottles (Table 1). This may be attributed to the consumption of nitrate during corrosion process in cans.

In glass bottles, there was no role of nitrate in such corrosion process and hence the product was quite safe. After 6 weeks storage, the colour of the product was light brownish yellow in cans, while more brownish tinge was noticed in the bottles near the headspace. Slight metallic taste and flavour were found in the canned product, whereas it was normal in both the types of bottles. In the can interior, there was heavy feathering and medium detinning. Overall quality of the product in glass bottles was better acceptable than in tin containers.

Tomato puree: Low vacuum was noticed in bottles as compared to cans. There was no significant change in total soluble solids, pH and acidity.

Initially, the colour of the product was bright brick red. After 4 and 6 weeks, the colour deteriorated slightly in the glass bottles. At the end of 6 weeks storage, the taste and flavour of the product in honey jar were not satisfactory. During storage, feathering in the can interior gradually increased. At the end of 6 weeks, heavy feathering with slight detinning was noticed (Table 1). The studies carried out by Ali[8] suggested that it was not pigment degradation that caused the decrease in redness, but an increase in non-enzymatic browning as indicated by the overall colour and appearance. The chemical methods of evaluation gave results that closely tallied with the sensory evaluation techniques.

Processed peas in non-acidified brine: There was a slight reduction in vacuum in can, whereas there was no significant difference in pH and salt content. There was a significant loss in chlorophyll in glass bottles as compared to canned products during storage (Table 1). At the end of 10 weeks storage, peas showed slight brownish green colour, turbidity in brine and slight off-flavour in glass bottles. In cans, the quality of the product was acceptable and the brine, as compared to bottle was relatively free from turbidity. Heavy purple feathering was noticed in can interior.

Processed peas in acidified brine: In view of the problem faced in the above experiment, peas were packed in brine with 0.2% acetic acid.

There was a slight reduction of vacuum in cans from 10" to 7" at the end of 10 weeks storage; but no significant difference in pH and salt content was observed. In terms of chlorophyll, the quality of the product in glass bottles was comparable with the products packed in cans and it was acceptable. The purple feathering in the can interior gradually increased during storage. Thus, the peas in acidified brine could be successfully packed in glass containers (Table 1). Similar method has been followed by the National Canner's Association of USA for packing peas in glass bottles[9].

In the present investigation, wrinkled varieties of peas were used and similar results were obtained in metal cans as well as glass bottles. The quality of the product was quite acceptable indicating that the acceptable quality could be obtained by packing peas in glass containers.

To summarize, mango juice packed in amber coloured beer bottle and honey jar was comparable with canned product and it was acceptable. However, there was more reduction of β-carotene content in mango juice packed in honey jar. Banana puree, rich in nitrate content, which is considered as potential accelerator of corrosion (detinner) in cans can be safely packed in glass bottles, although there is a slight colour deterioration. Tomato puree is also a corrosive product having short shelf-life in internally plain cans. Glass bottles (especially beer bottles) were found to be more suitable for tomato puree for a satisfactory shelf-life. Acidified brine was found to be essential to pack peas in glass bottles.

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J. Fd. Sci. Technol, Vol. 29, No. 6, 371–374

Storage Stability of Canned Jackfruit *(Artocarpus heterophyllus)* Juice at Tropical Temperature

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Internal plain tinplate can corrosion and depletion of vitamin C in canned jackfruit juice, with or without added nitrate, were found to follow zero-order kinetics during storage at 30°-50°C. The activation energy followed the order of iron dissolution > tin dissolution > vitamin C depletion. The presence of nitrate accelerated all three processes without significantly ($P<0.05$) affecting the E_a values. Based on a maximum permissible tin level of 250 mg/kg, tin dissolution appeared to be the predominant factor limiting the shelf-life of the product rather than depletion of vitamin C. It was estimated that properly processed jackfruit juice packed in plain tinplate cans could keep well for > 17 months at storage temperatures < 30°C and in the absence of corrosion accelerators such as nitrates.

Several types of canned exotic tropical fruits and their juices are produced and processed in Malaysia. However, information on their shelf-life especially under tropical conditions of storage is lacking.

Iron and tin dissolution and vitamin C loss are important parameters to determine the keeping quality of canned fruit juices. Excess levels of iron and tin in canned fruit juices limit their shelf-life by causing unacceptable changes in colour, taste or clarity[1,2]. The possible toxicity of high levels of tin to humans[3-5] also makes it prudent to establish a maximum permissible limit, normally 250 mg/kg, for this metal in such products[6,7]. Lowering of this limit to 200 mg/kg is now being considered in Malaysia. In terms of nutritional quality, it is the retention of the total vitamin C potency or anti-scorbutic activity, contributed by the combined presence of L-ascorbic acid, and dehydroascorbic acid, which is of primary concern.

This paper discusses the results of studies concerning internal can corrosion and vitamin C retention in jackfruit juice packed in plain tinplate cans during storage at temperatures (30°-50°C) typical of those prevailing in non-air-conditioned warehouses in the tropics.

Materials and Methods

Preparation and canning of jackfruit juice: The edible portion of ripe jackfruit (*Artocarpus heterophyllus*) was initially steam-blanched at atmospheric pressure for 2 min to inactivate enzymes and to soften the flesh. Jackfruit puree was prepared by adding water to the fruit in the ratio of 3:1 followed by grinding through a pulper. The resulting puree was then mechanically pressed to obtain the juice. The juice so obtained was passed through a fine screen, mixed with cane sugar (22° Brix), acidified with citric acid to give a total titratable acidity of 0.5% (based on citric acid), and enriched with vitamin C to a level of 35 mg/100 ml of juice. The pH of the juice was 3.8 and this did not change appreciably during storage.

The juice so prepared was divided into two lots. Nitrate in the form of $NaNO_3$ was added to one lot to a level of 15 mg/kg. The other lot without nitrate served as control. Each lot of the juice was heated to 85°C, followed by hot-filling into 211×301 plain electrolytic tinplate cans (with enamelled ends) with gross headspace of 5 mm. The cans were then seamed and processed for 20 min in boiling water. After cooling, the cans were stored at 30°, 40° and 50°C. Two cans from each lot of the juice and at each temperature were withdrawn at appropriate intervals of time and the contents analysed for dissolved iron and tin and total vitamin C.

Methods of analysis: Dissolved iron and tin were determined following the procedures outlined by Seow *et al*[2]. An IL 251 atomic absorption spectrometer at 248.3 or 235.4 nm was used for the analysis. Total vitamin C was determined using the micro-fluorimetric method of the AOAC[8]. All analyses were carried out in duplicate.

Results and Discussion

Iron and tin dissolution: Fig.1 and 2 show the concentrations of dissolved iron and tin, respectively, after various periods of storage at the three temperatures studied.

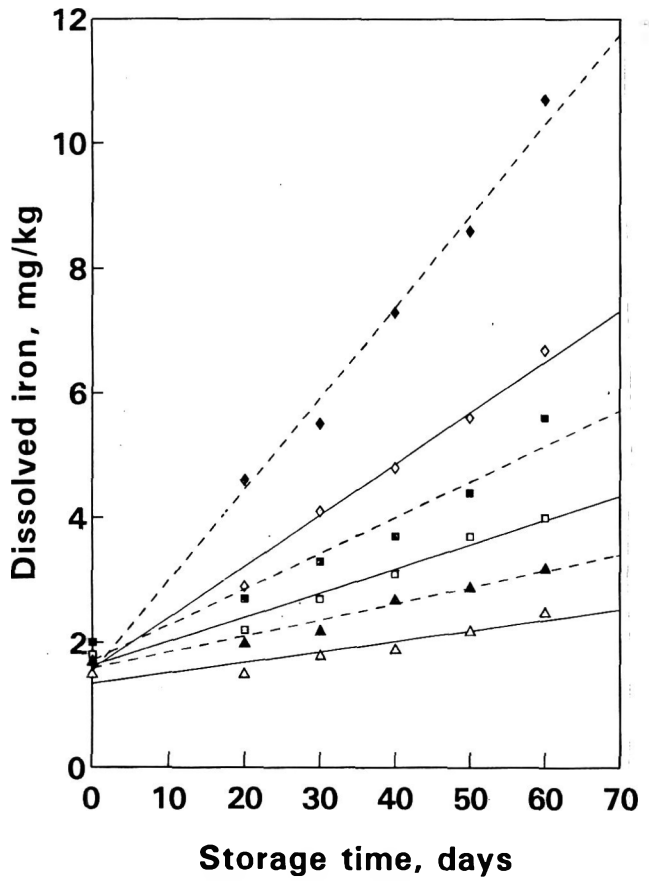

Fig.1. Changes in dissolved iron concentration in canned jackfruit with [*closed symbols*] or without [*open symbols*] added nitrate during storage at 30° [▲, Δ], 40° [■ □] and 50°C [♦, ◊]

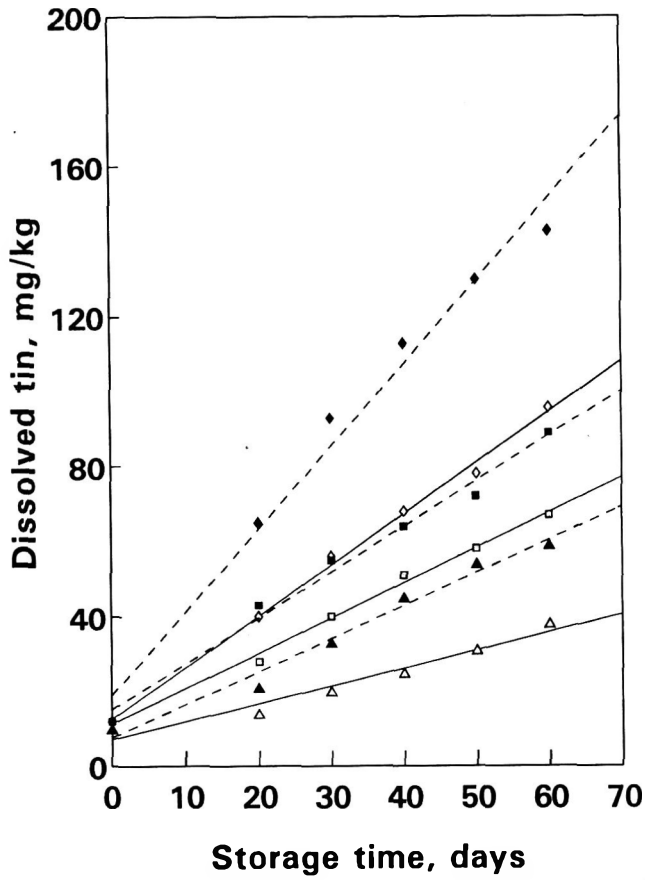

Fig.2. Changes in dissolved tin concentration in canned jackfruit juice during storage. [Symbols as in Fig.1)

Over the 60-day storage period, the kinetics of iron and tin dissolution may be taken to be of zero-order in nature which is in good agreement with the findings of Nagy and Nikdel[9] on canned single-strength grapefruit juice. The oxygen entrapped within the headspace of the cans and that remaining dissolved in the juice probably accounted for the initial dissolution of tin and also iron. In the freshly canned jackfruit juice, the dissolved tin content was 10-12 mg/kg.

The dissolution of both metals was temperature-dependent and conformed to Arrhenius kinetics (Fig. 3). Rate constants for iron and tin dissolution were calculated from the slopes of the plots in Fig. 1 and 2 by linear regression analysis and activation energy (E_a) values from the slopes of Arrhenius plots as shown in Fig. 3. As an approximation, the rate of internal corrosion would double with every 10°C rise in storage temperature. The activation energy for iron dissolutioin (54-70 kJ/mol) was considerably higher than that for tin dissolution (38-43 kJ/mol). Statistical analysis showed that the presence of added nitrate at a level of 15 mg/kg significantly increased ($P< 0.01$) the rate of internal corrosion by about 1.6 times, but had no significant effect ($P<0.05$) on the E_a values of either iron or tin dissolution. It is a well-known fact that active depolarizers such as nitrates promote rapid detinning in plain tin-plate cans of acid products[1,10-12].

Previous studies of internal tinplate can corrosion have shown that a linear relationship existed between dissolved tin and iron[9,12]. However, the results of the present study show a somewhat curvilinear relationship which neither the presence of nitrate nor storage temperature seems to affect. It is likely that, as detinning (whether normal or nitrate-induced) progresses, the degree of protection afforded by the tin coating to the base steel increasingly diminishes, thus leading to progressively larger iron/tin uptake ratio.

Vitamin C retention: It is generally accepted that ascorbic acid degradation in canned fruit juices follows a primarily slow anaerobic pathway, usually after an initial sharp aerobic decrease over a matter of a few days by reaction

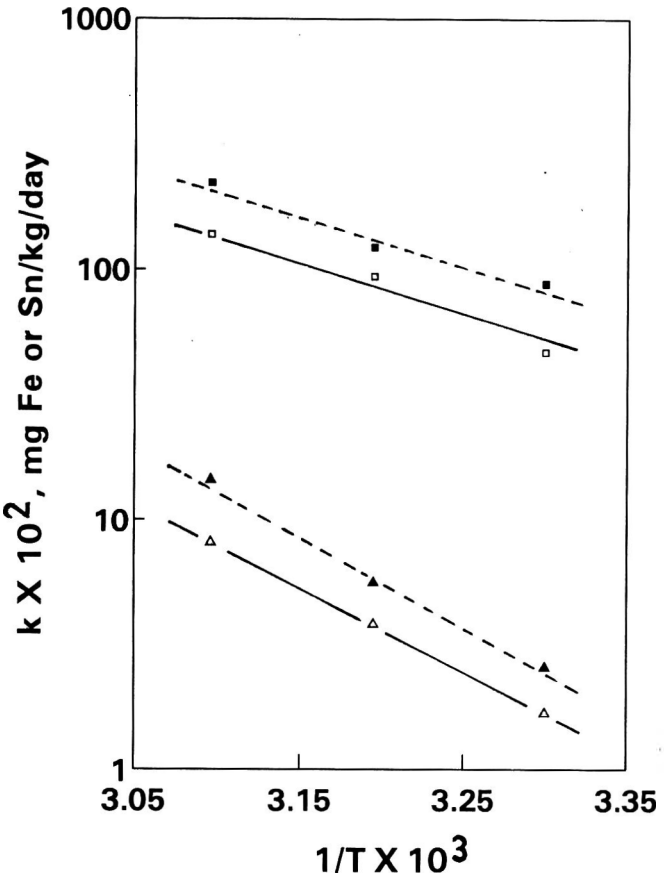

Fig.3. Arrhenius plots of (A) iron dissolution and (B) tin dissolution in canned jackfruit juice with [▲] or without [Δ], added nitrate.

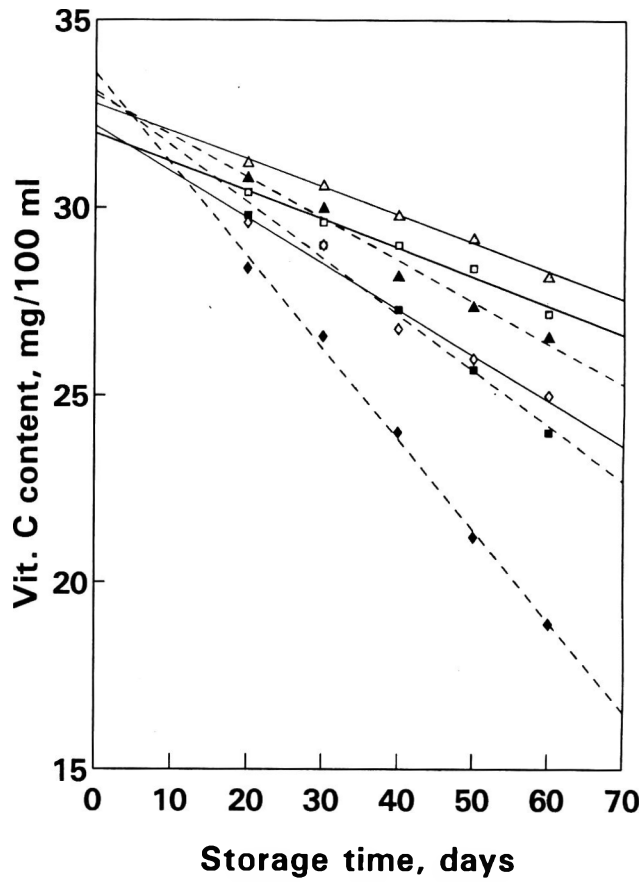

Fig.4. Changes in total vitamin C content in canned jackfruit juice during storage. [Symbols as in Fig. 1]

with residual headspace and dissolved oxygen[13]. As Fig. 4 shows, vitamin C concentration in jackfruit juice packed in plain tinplate cans, with or without added nitrate, appeared to decrease linearly with time at any particular storage temperature. Zero-order kinetics for ascorbic acid degradation in canned fruit juices have been similarly observed by several other investigators[13-16].

Nagy and Smoot[14] have shown that the content of dehydroascorbic acid in canned grapefruit juice was unaffected by storage time or temperature and that the retention of total vitamin C followed the same pattern of retention of reduced ascorbic acid alone. There is no reason to believe that this will not also hold true for other canned fruit juices.

The rate of loss of vitamin C, as expected, increased with an increase in storage temperature and it is obvious from Fig. 5 that the degradation reaction conforms to the Arrhenius equation. The presence of added nitrate was also observed to increase the rate of ascorbic acid degradation at any particular storage temperature without significantly ($P< 0.05$) altering the activation energy. Nitrate acts as an electron acceptor or depolarizer in the corrosion process and is reduced to nitrite[11] which is known to react readily with L-ascorbic acid[17,18]. This could account for the accelerative effect of nitrate on ascorbic acid degradation. The E_a value of 29-30 kJ/mol (6.9-7.2 kcal/mol) obtained in the present study is considerably lower than that (18.2 kcal/mol) reported by Nagy and Smoot[14] for canned grapefruit juice.

Predicted shelf-life: Tin dissolution and vitamin C depletion may be used as criteria in determining the shelf-life of properly processed canned fruit juices, based either on the time required for accumulation of tin to a certain level[19] or for complete depletion of ascorbic acid[13,16]. A product may thus be considered unacceptable on such grounds even before external signs of spoilage (such as swelling of the cans) are evident. The predicted shelf-lives of canned jackfruit juices with or without added nitrate at the three storage temperatures studied, based on a maximum permissible tin content of 250 mg/kg under Malaysian food legislation[7] or complete depletion of vitamin C, are given in Table 1. The figures were calculated using the linear regression equations obtained in the storage studies.

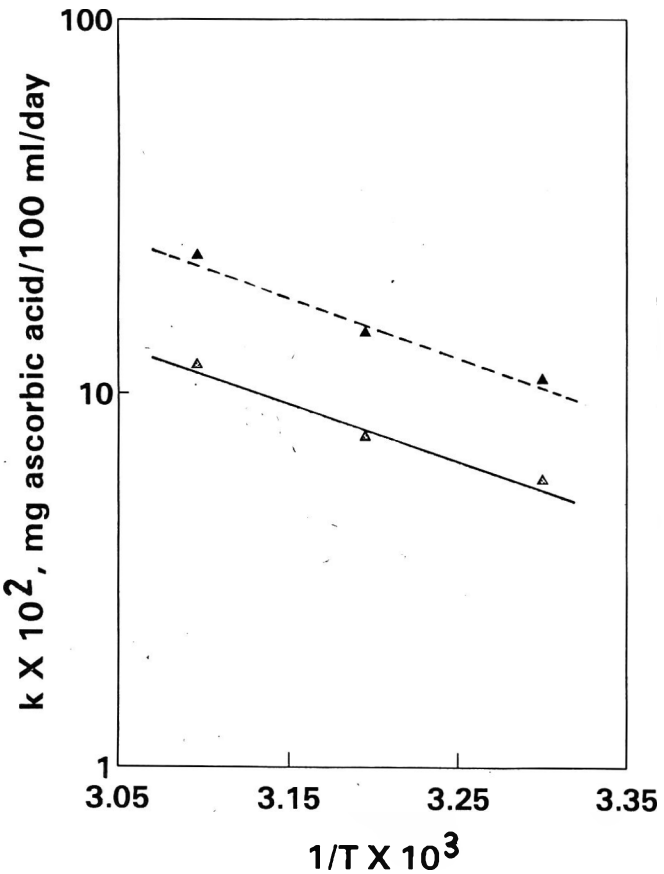

Fig.5. Arrhenius plots of vitamin C depletion in canned jackfruit juice [▲] or without [Δ], added nitrate.

TABLE 1. PREDICTED SHELF-LIFE OF CANNED JACKFRUIT JUICE (WITH OR WITHOUT ADDED NITRATE) BASED ON TIN DISSOLUTION OR VITAMIN C DEPLETION

Temp (°C)	Predicted shelf-life (days)			
	Tin dissolution		Vit. C depletion	
	With NO_3	Without NO_3	With NO_3	Without NO_3
30	517	276	537	300
40	254	193	400	220
50	173	105	268	138

It is apparent from Table 1 that the product would become unacceptable by virtue of its reaching the maximum permissible tin content of 250 mg/kg before complete exhaustion of vitamin C occurred. The difference in predicted shelf-life based on these two criteria is small at 30°C, but becomes more pronounced when the product is exposed to higher storage temperatures. This is to be expected since the E_a for tin dissolution is much higher than that for ascorbic acid degradation. Tin dissolution is, thus, the predominant shelf-life determining factor, particularly at storage temperatures >40°C.

Jackfruit juice packed in plain tinplate cans exhibits satisfactory stability only when stored at temperatures not exceeding 30°C. In the tropics, where storage temperatures are higher than 30°C, rapid tin dissolution would take place, thereby shortening the shelf-life considerably. The problem would get aggravated if corrosion accelerators are present.

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J. Fd. Sci, Technol Vol. 29 No. 6, 375–380

Nutritional and Cooking Quality Evaluation of Dry Cowpea (*Vigna sinensis* L.) Grown Under Different Agricultural Conditions. 2. Effect of Soaking and Cooking Processes on the Physical, Nutritional and Sensory Characteristics of Cooked Seeds

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Cooking time, volume and firmness of cooked cowpeas were not affected by foliar application of plants with gibberellic acid, cycocel and white wash under non-saline conditions. Salinization increased cooking time and decreased volume and firmness of cooked seeds. Soaking in water for 12 h before cooking, decreased cooking time and firmness, but increased volume of cooked seeds, whereas the effect of soaking in hot water was more pronounced. Rate of water imbibition during cooking of dry and soaked seeds was linear to cooking time. Soaked seeds in distilled water gave the highest weight at any time of cooking. More solids were leached out due to cooking in 2% NaCl solution compared to cooking in plain water subsequent to hot water soaking for 12 h. Soaking in hot water before cooking retained more nutritional components. Soaking in hot water improved markedly the sensory properties particularly appearance, texture, colour and reduced the beany flavour.

Egypt has a limited cultivable area accounting to about 6.3 million acres. Concentrated efforts are put up to meet the needs of the increasing population. The main problem facing the country is the shortage of irrigation water. The use of drainage water directly or indirectly by mixing with irrigation water has been proposed, which might cause soil salinization due to its high salt content. However, available information indicates that certain growth regulators could improve the salt tolerance of some plants through modifications of the growth pattern[1]. Furthermore, use of anti-transpirants may save water by minimizing its loss via transpiration[2]. In this regard, leguminous plants would be of special importance, because of their high nutritional value as fodders and food, besides their ability to improve soil fertility through nitrogen fixation[3]. About 93% of the Egyptian calorie intakes come from vegetable food items. Legumes form a basic ingredient, being second to cereals (used for preparing bread) in supplying protein in daily diet. Cooking quality of legume affects the final acceptability and the firmness of cooked cowpeas was found to be influenced by soaking time and cooking conditions[4]. Therefore, this investigation was undertaken to evaluate physical characteristics, nutritional components and sensory quality of cooked cowpea seeds grown under different agricultural practices.

Materials and Methods

Cultural treatments: A pot experiment was carried out at the Agricultural Experimental Station of the University in two summer seasons of 1988 and 1989. As plants become well-stabilized i.e. produced 3-4 true leaves (about 40 days after sowing), plants were thinned again to 3 plants/pot. Also, at this time, salinity, as NaCl, was applied to half the number of the pots. Pot salinization was carried out gradually i.e., the total amount of NaCl was added in three equal instalments at 2 day intervals to a final concentration of 60 mM (3480 ppm), when the soil was at the field capacity. Forty four days after sowing, the salinized pots received the final NaCl instalment to reach the ultimate salt concentration (60 mM). All pots (salinized and unsalinized) were sprayed with the following three different substances: a) gibberellic acid (GA_3) as Berelex at 100 ppm, b) chloroethyl trimethyl ammonium chloride (cycocel) CCC, 1500 ppm, and c) white wash: Reflecting anti-transpirant calcium carbonate (chalk): A suspension of chalk 6% in water.

Other plants were sprayed with water and used as unsalinized control. Foliar application of these substances was carried out 4 times in 10 day intervals. Both surfaces of leaf were treated.

Pre-treatment of seeds: A 100 g seeds were steeped in

300 ml distilled water for 12 h (The amount of water absorbed per unit weight of cowpea increased with the increase in soaking time and reached equilibrium condition after 12 h of soaking[4]) at room temperature. Another sample was soaked in distilled water after heating upto boiling temperature, then heating source was removed and soaking continued to 12 h. After rinsing the soaked samples, seeds were cooked under atmospheric pressure either in distilled water or in 2% NaCl until 50% of seeds were split.

Nutrient composition: Starch, thiamin and riboflavin were determined according to AOAC[5]. Reducing sugars were determined in 70% ethanol extract by the method of Dubois *et al*[6]., using pure glucose as standard. Available lysine was analyzed according to the method of Conkerton and Frampton[7]. Nutrient concentrations were calculated as means from triplicate determinations on dry matter weight basis.

Physical characteristics: Firmness was determined using fruit and vegetable tester (John Chatillon and Sons Inc., Kew Garens, New York, U.S.A.), using 516-500 MRPER gauge with 0.07 cm plunger diameter. The volume of the cooked seeds was determined by seed displacement method as modified by Griswold[8]. The loss of total soluble solids in water remaining after soaking and cooking was determined by using Abbe' refractometer. The loss in total solids (soluble and insoluble solids) was determined by drying water remaining after soaking and cooking in an oven at 105°C, according to AOAC[5]. The loss of total insoluble solids during soaking and cooking in water was determined by difference. The rate of imbibition of both soaked and cooked seeds was determined by weighing the sample after soaking and cooking. The increase in weight of soaked and cooked sample indicated the quantity of water imbibed by the seeds.

Sensory evaluation: A taste panel of 10 members from the Food Science and Technology Dept. of the University, evaluated the effect of different treatments of cooked seeds for appearance, texture, colour and flavour using the score sheet as used by Molander[9].

Results and Discussion

Effect of soaking and cooking on some physical characteristics of treated and untreated cowpea seeds: Results in Table 1 illustrate the effect of the interaction between salinity and foliar applications, besides soaking and cooking treatments on some physical characteristics of cooked seeds such as cooking time, volume and firmness of cooked seeds. No considerable variations were observed in cooking time as well as volume and firmness of cooked seeds between control and all foliar treatments under non-saline conditions. However, 60 mM NaCl salinization increased cooking time and decreased both volume and firmness of cooked seeds. The foliar substances tested showed a favourable effect under saline conditions i.e. decreasing cooking time and slight increase in volume and firmness of cooked seeds. Soaking in distilled water for 12 h at room temperature decreased cooking time and firmness of the cooked seeds, but it increased volume of cooked seeds. On the other hand, seeds cooked after soaking in hot water were slightly lower in their cooking time, volume and firmness compared to those cooked after soaking in distilled water. It is important to point out here that prior to cooking, it is advisable that the legume seeds be soaked in hot water, since this process remarkably decreases cooking time which, in turn, decreases the loss in the nutritive components[10]. In addition, cooking seeds in water containing 2% NaCl decreased cooking time, volume and firmness of cooked seeds. The action of sodium salts on bean softening occurs by ion exchange and possibly

TABLE 1. EFFECT OF SALINITY AND FOLIAR APPLICATION SUBSTANCES ON SOME PHYSICAL CHARACTERISTICS OF UNSOAKED AND SOAKED COOKED COWPEA SEEDS

Foliar application substances	Cooking time (min)						Vol. of cooked seeds (cm^3)						Firmness (g./0.17 cm^2)					
	A_1	A_2	B_1	B_2	C_1	C_2	A_1	A_2	B_1	B_2	C_1	C_2	A_1	A_2	B_1	B_2	C_1	C_2
							0 mM NaCl											
Unsalinized control	14	13	13	12	22	20	4.1	3.9	3.9	3.7	3.6	3.4	352.4	348.1	349.8	337.7	358.3	355.9
GA_3 100 ppm	13	12	11	10	20	18	4.3	4.0	4.0	3.8	3.6	3.5	348.6	347.4	346.2	336.9	356.4	353.5
CCC 1500 ppm	15	13	14	13	24	22	4.3	4.1	4.0	3.8	3.7	3.6	344.8	343.7	340.9	336.7	353.2	350.2
White wash 6%	13	12	12	11	21	20	4.4	4.2	4.1	3.9	3.8	3.6	342.1	340.0	339.2	335.6	349.1	347.7
							60 mM NaCl											
Salinized control	16	14	15	13	25	24	3.8	3.6	3.5	3.4	3.4	3.3	340.5	337.9	338.3	328.6	345.3	343.4
GA_3 100 ppm	14	13	13	12	23	21	3.9	3.6	3.5	3.4	3.5	3.4	338.9	335.3	336.1	328.9	347.3	344.9
CCC 1500 ppm	15	14	14	12	25	23	4.0	3.8	3.6	3.5	3.4	3.4	340.7	333.5	339.3	327.7	349.8	346.6
White wash 6%	14	12	12	10	21	19	4.1	3.9	3.8	3.7	3.6	3.5	338.6	333.9	336.5	330.2	350.2	347.6

A_1 = Cooking without 2% NaCl under atmos. pressure after soaking in d.w. for 12 hr., A_2 = Cooking with 2% NaCl under atmos. pressure after soaking in d.w. for 12 hr., B_1 = Cooking without 2% NaCl under atmos. pressure after soaking in hot d.w. for 12 hr., B_2 = Cooking with 2% NaCl under atmos. pressure after soaking in hot d.w. for 12 hr., C_1 = Unsoaked, cooking without 2% NaCl under atmos. pressure., C_2 = Unsoaked, cooking without 2% NaCl under atmos. pressure.

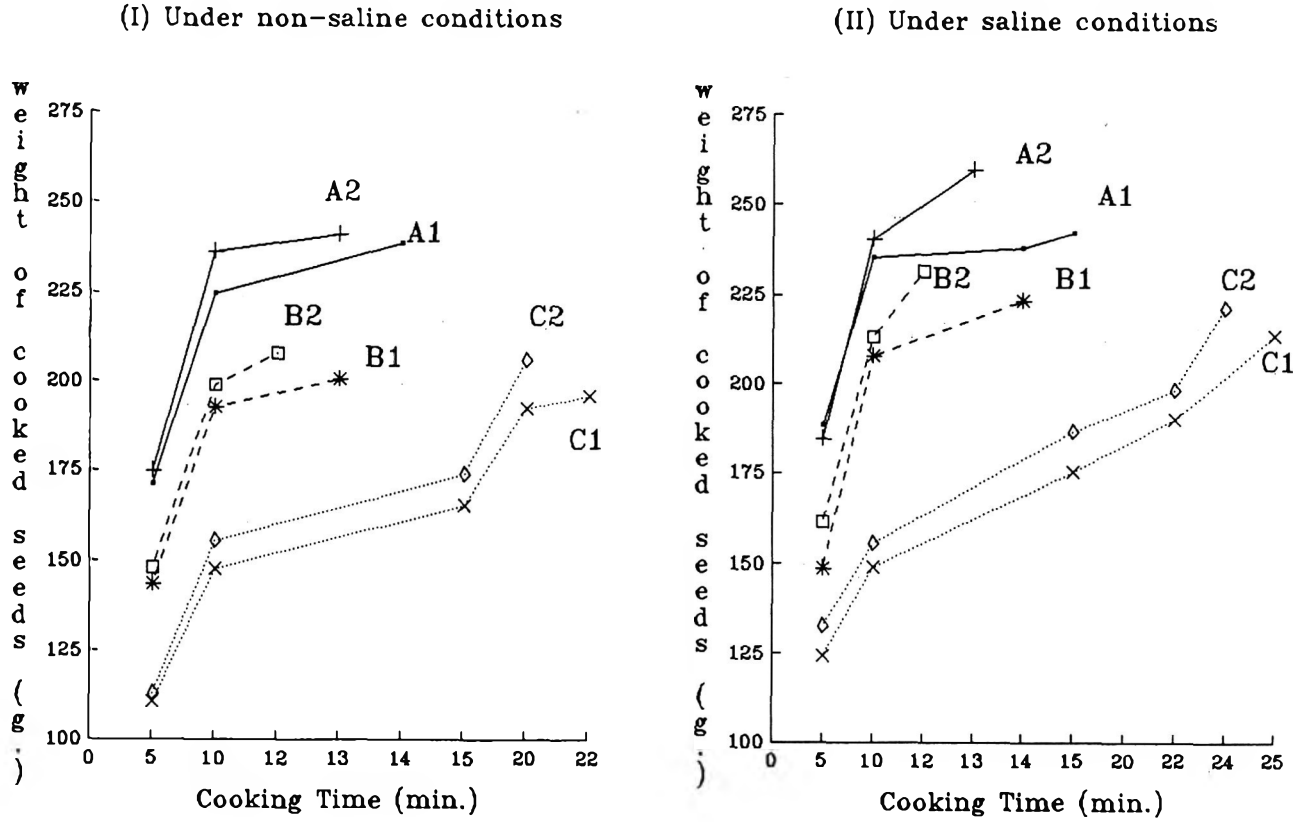

Fig 1. Effect of cooking time on the rate of imbibition of cooked cowpea seeds from plants grown under non-saline (I) and saline (II) conditions

chelation. Ions responsible for cellular firmness are either replaced by sodium ions or leached out, resulting in solubilization of pectic substances during soaking and cooking[10]. The results are in agreement with those obtained by El-Ashwah *et al*[11]. It could be noticed that the increase in weight of cooked seeds after soaking or without soaking was linear with the cooking time and the soaked seeds in distilled water gave the highest weight at any time of cooking (Fig. 1). Also, cooking in 2% NaCl solution led to a remarkable increase in water absorption i.e. weight of cooked seeds. This may be attributed to the ability of NaCl to dissociate or to alter the structure of the protein molecules to monomeric subunits which may have more water binding sites[12].

TABLE 2. EFFECT OF SALINITY AND FOLIAR APPLICATION SUBSTANCES ON THE LOSS OF SOLIDS IN COOKING MEDIA OF UNSOAKED AND SOAKED COOKED COWPEA SEEDS

Foliar application substances	Insoluble solids (%)						Soluble solids (%)					
	A_1*	A_2	B_1	B_2	C_1	C_2	A_1	A_2	B_1	B_2	C_1	C_2
					0 mM NaCl							
Unsalinized, control	0.28	0.45	0.36	0.63	0.74	0.88	0.98	1.45	0.60	1.10	2.25	2.60
GA_3 100 ppm	0.30	0.41	0.33	0.58	0.71	0.86	1.00	1.50	0.62	1.20	2.10	2.70
CCC 1500 ppm	0.29	0.38	0.31	0.60	0.69	0.85	1.05	1.60	0.69	1.05	2.15	2.50
White wash 6%	0.31	0.40	0.34	0.63	0.72	0.84	1.10	1.70	0.75	1.10	2.00	2.75
					60 mM NaCl							
Salinized, control	0.42	0.58	0 48	0.76	0.89	1.20	1.20	1.65	0.95	1.30	2.60	2.80
GA_3 100 ppm	0.53	0.56	0 44	0.72	0.87	1.17	1.30	1.75	1.05	1.50	2.40	2.95
CCC 1500 ppm	0.47	0.52	0 39	0.74	0.84	1.13	1.35	1.80	1.10	1.25	2.75	2.70
White wash 6%	0.46	0.53	0 46	0.78	0.87	1.19	1.35	1.85	1.25	1.40	2.30	2.90

*A_1, A_2, B_1, B_2, C_1, and C_2, as in Table 1

TABLE 3. EFFECT OF SALINITY AND FOLIAR APPLICATION SUBSTANCES ON SOME NUTRIENTS OF UNSOAKED AND SOAKED COOKED COWPEA SEEDS

NaCl mM	Foliar application substances	Raw	A_1*	A_2	B_1	B_2	C_1	C_2
			Starch content (%)					
0	Unsalinized control	43.9	30.2	29.3	36.2	33.9	21.6	18.4
0	GA_3 100 ppm	45.2	33.8	31.4	37.8	35.0	23.2	20.1
0	CCC 1500 ppm	41.7	28.4	25.8	34.5	32.4	20.9	16.4
0	White wash 6%	47.5	34.3	32.7	38.3	36.5	24.8	22.3
60	Salinized control	33.7	16.8	16.2	21.8	18.8	15.0	14.3
60	GA_3 100 ppm	35.6	19.6	18.9	23.9	21.3	16.9	15.5
60	CCC 1500 ppm	32.1	14.7	14.0	20.2	17.0	14.1	13.6
60	White wash 6%	36.8	20.4	19.3	24.7	22.9	17.9	16.7
			Reducing sugars (as glucose, %)					
0	Unsalinized control	8.3	8.2	8.4	7.5	7.8	8.6	8.8
0	GA_3 100 ppm	8.7	9.6	8.9	7.6	7.9	9.2	9.3
0	CCC 1500 ppm	8.0	7.7	8.0	7.1	7.4	8.3	8.5
0	White wash 6%	8.8	8.8	9.1	7.8	8.1	9.3	9.5
60	Salinized control	8.6	8.7	8.8	8.2	8.4	9.1	9.3
60	GA_3 100 ppm	9.2	9.1	9.3	8.5	8.7	9.5	9.7
60	CCC 1500 ppm	8.4	8.8	9.0	7.9	8.2	8.4	8.6
60	White wash 6%	9.4	9.0	9.4	8.3	8.8	9.8	9.8
			Available lysine content (g/16gN)					
0	Unsalinized control	6.4	5.4	5.3	5.8	5.6	5.0	4.8
0	GA_3 100 ppm	6.6	5.7	5.4	6.1	5.8	5.2	5.1
0	CCC 1500 ppm	6.1	5.1	5.0	5.6	5.3	4.8	4.8
0	White wash 6%	6.8	5.9	5.7	6.3	6.0	5.4	5.1
60	Salinized	6.1	4.9	4.8	5.3	5.0	4.7	4.5
60	GA_3 100 ppm	6.2	5.1	5.0	5.5	5.3	4.9	4.8
60	CCC 1500 ppm	6.0	4.8	4.6	5.1	4.9	4.4	4.2
60	White wash 6%	6.3	5.6	5.5	5.8	5.7	5.2	5.0
			Riboflavin (mg/100g DM)					
0	Unsalinized control	0.33	0.18	0.17	0.20	0.18	0.16	0.15
0	GA_3 100 ppm	0.39	0.20	0.18	0.23	0.21	0.17	0.15
0	CCC 1500 ppm	0.30	0.13	0.13	0.17	0.15	0.13	0.12
0	White wash 6%	0.42	0.22	0.20	0.24	0.22	0.18	0.18
60	Salinized control	0.27	0.12	0.10	0.18	0.16	0.11	0.10
60	GA_3 100 ppm	0.31	0.16	0.12	0.20	0.19	0.11	0.10
60	CCC 1500 ppm	0.25	0.11	0.10	0.14	0.12	0.10	0.11
60	White wash 6%	0.34	0.17	0.16	0.21	0.19	0.13	0.12
			Thiamin (mg/100g DM)					
0	Unsalinized control	1.05	0.58	0.55	0.68	0.62	0.50	0.47
0	GA_3 100 ppm	1.07	0.61	0.60	0.71	0.67	0.55	0.52
0	CCC 1500 ppm	1.09	0.54	0.51	0.59	0.53	0.47	0.45
0	White wash 6%	1.12	0.70	0.67	0.82	0.71	0.63	0.60
60	Salinized control	0.85	0.44	0.42	0.49	0.46	0.41	0.40
60	GA_3 100 ppm	0.92	0.50	0.48	0.52	0.50	0.46	0.44
60	CCC 1500 ppm	0.90	0.40	0.37	0.44	0.41	0.35	0.33
60	White wash 6%	0.99	0.53	0.51	0.59	0.55	0.48	0.47

* A_1, A_2, B_1, B_2, C_1 and C_2 as in Table 1 DM = Dry matter

Effect of soaking and cooking processes on the loss of solids in cooking media of treated and untreated cowpea seeds: The results in Table 2 show variations in the percentage loss of soluble and insoluble solids in different treated samples. The highest loss of solids occurred in samples cooked in salted water without soaking, especially in seeds from plants grown under saline conditions. Generally, the per cent losses of soluble solids were higher than those of insoluble solids for all samples. The increases in the losses in soluble solids during cooking of seeds may be due to the addition of salt in the cooking water. Salt can be omitted during boiling to be added later at the time of serving. This observation agrees with that mentioned by El-Ashwah *et al*[11]. No differences in the losses of solids in cooking media due to the effect of the three foliar substances were observed.

Effect of soaking and cooking processes on some constituents of treated and untreated cowpea seeds: Results

TABLE 4. EFFECT OF SALINITY AND FOLIAR APPLICATION SUBSTANCES ON THE SENSORY CHARACTERISTICS OF UNSOAKED AND SOAKED COOKED COWPEA*

NaCl mM	Foliar application substances	A_1**	A_2	B_1	B_2	C_1	C_2
			Appearance				
0	Unsalinized control	7.6	7.3	7.7	7.5	5.3	5.0
0	GA_3 100 p.p.m.	7.4	7.3	7.6	7.4	5.2	5.1
0	CCC 1500 p.p.m.	7.4	7.4	7.5	7.4	5.4	5.5
0	White wash 6%	7.6	7.3	7.6	7.3	5.3	5.2
60	Salinized control	7.4	7.1	7.4	7.2	5.0	4.8
60	GA_3 100 p.p.m.	7.3	7.0	7.5	7.2	4.9	5.0
60	CCC 1500 p.p.m.	7.2	7.2	7.3	7.0	5.2	4.6
60	White wash 6%	7.3	7.1	7.4	7.0	5.5	4.7
			Taste				
0	Unsalinized control	7.0	7.2	7.9	7.6	6.5	5.5
0	GA_3 100 p.p.m.	7.4	7.0	7.7	7.5	6.2	5.2
0	CCC 1500 p.p.m.	7.3	7.1	7.8	7.4	6.6	5.3
0	White wash 6%	7.2	7.2	7.9	7.3	6.0	5.1
60	Salinized control	6.8	7.0	7.3	7.4	5.8	5.2
60	GA_3 100 p.p.m.	6.7	6.8	7.2	7.3	5.5	5.0
60	CCC 1500 p.p.m.	6.5	6.6	7.3	7.2	5.3	4.7
60	White wash 6%	6.3	6.9	7.2	7.3	5.4	4.5
			Colour				
0	Unsalinized Control	7.9	7.6	7.6	7.3	5.9	5.5
0	GA_3 100 p.p.m.	7.7	7.4	7.2	7.0	5.8	5.2
0	CCC 1500 p.p.m.	7.3	7.2	7.3	7.1	5.3	5.0
0	White wash 6%	7.0	7.0	7.0	7.3	5.9	5.1
60	Salinized Control	7.3	7.3	6.8	7.7	5.6	5.3
60	GA_3 100 p.p.m.	7.2	7.2	6.3	7.9	5.3	5.2
60	CCC 1500 p.p.m.	6.7	7.0	6.1	7.7	5.6	5.1
60	White wash 6%	6.5	6.7	6.3	7.3	5.2	5.0
			Texture				
0	Unsalinized control	6.5	6.0	6.9	6.7	4.9	4.3
0	GA_3 100 p.p.m.	6.3	5.7	6.7	6.1	4.7	4.4
0	CCC 1500 p.p.m.	6.0	5.6	6.7	6.3	4.6	4.6
0	White wash 6%	6.7	5.5	6.3	6.1	4.7	4.5
60	Salinized control	6.2	6.1	6.6	6.0	4.7	4.0
60	GA_3 100 p.p.m.	6.0	5.7	6.3	5.3	4.4	3.8
60	CCC 1500 p.p.m.	5.8	5.6	6.7	5.9	4.3	4.4
60	White wash 6%	5.6	5.2	6.3	6.2	4.3	4.5
			Odour				
0	Unsalinized control	7.0	6.8	7.3	7.5	5.5	5.1
0	GA_3 100 p.p.m.	7.1	6.7	7.1	7.3	5.5	4.8
0	CCC 1500 p.p.m.	7.3	7.0	7.2	7.5	5.3	4.7
0	White Wash 6%	7.2	7.1	7.3	7.6	5.4	4.5
60	Salinized control	7.3	6.7	7.2	7.3	5.4	4.7
60	GA_3 100 p.p.m.	7.2	6.7	7.1	7.2	5.0	4.6
60	CCC 1500 p.p.m.	6.7	6.5	7.0	7.0	4.3	4.9
60	White wash 6%	6.5	6.3	7.2	6.8	4.9	5.0

* Each figure of quality characteristic number is on the scale range from 1-9

** A_1, A_2, B_1, B_2, C_1 and C_2 as in Table 1

in Table 3 indicate that salinization with 60 mM NaCl of plants decreased starch, available lysine, thiamin and riboflavin contents of raw seeds by 23.2, 4.9, 19.0 and 18.2% respectively against control. This could be related to the fact that salinity disturbed protein and vitamin synthesis[1]. However, salinity increased the reducing sugars in seeds. Foliar application with gibberellic acid and white wash brought about a favourable effect on the nutritive value of seeds because of their stimulative effect on protein, carbohydrates and vitamin synthesis by plants and their accumulation in seeds. The highest loss in the nutritive value was found in seeds cooked in 2% NaCl solution without pre-soaking, which can be attributed to the longer cooking time and consequent destruction of some amino acids and vitamins. Of interest is the relatively high reducing sugar content of these treatments which may be due

to the effect of heat during the longer cooking time on the hydrolysis of starch and its derivatives. For greater nutritive value, the optimum cooking time was found to be between 11-15 min in samples subjected to soaking periods of 12 h in hot distilled water. These results are partially in accordance with those obtained on cowpeas by Edijala[13] and Cabezas *et al*[14].

Effect of soaking and cooking processes on the sensory characteristics of cooked cowpea seeds: Data in Table 4 indicate that pre-treatments applied in the preparation of cowpeas have an effect on the appearance, texture, taste, odour and colour of cooked seeds. No noticeable differences were found in the sensory characteristics of cooked seeds obtained from plants with foliar treatment. Boiling in salted distilled water had a great effect on the appearance and colour of cooked seeds. Cooking with 2% NaCl under atmospheric pressure without soaking seemed to be inadequate for optimum preparation of cooked cowpeas, because this pre-treatment gave the lowest value in all sensory characteristics of cooked seeds, when compared with the effect of other pre-treatments.

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Suitability of Packaging Boxes for Tomatoes

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Textural properties of tomato were evaluated with different packaging conditions on vibration table under simulated transportation. The colour of tomatoes changed from greenish to pink and from pink to red. Firmness decreased and decay increased with longer duration of vibration and storage period.

Fresh fruits and vegetables are susceptible to spoilage due to their high moisture content (75-90%). Even 10-15% loss of moisture affects the textural quality and subsequently the price to a greater extent. Horticultural produce, by nature, is location specific because of their specific climatic requirements. Therefore, the distribution of these local surplus over a vast consumer horizon becomes necessary. Packaging is one of the major factors which influences the quality of fruit during transportation and distribution, till it reaches the consumer. This paper deals with quality of tomatoes as affected by different packaging materials.

Different types of packaging and cushioning materials used include; a) Deodar wooden box 50 × 33 × 14 cm size of 17 kg capacity and 53.5 × 33 × 47 cm size of 50 kg capacity, (b) rigid cardboard box 50.5 × 19.5 × 21 cm size of 12 kg capacity, (c) Eucalyptus wood 44 × 29.5 x 18 cm size of 17 kg capacity and (d) plastic crate 51 × 20.5 × 16 cm size of 18 kg capacity. Paddy straw and mat were used as cushioning materials at the bottom of the box with 15 cm thickness. The boxes were packed with tomatoes and were given controlled vibrations for 6, 12 and 20 h on vibration table powered by a 2.68 KW (2 HP) electric motor. A gear box with one reverse and three forward gear speeds was used in conjunction with pulleys for adjusting frequency of the vibration table.

Textural quality, firmness and decay rates were determined. Force per unit deformation of tomatoes was considered as the index of firmness and was measured on the Instron Universal Testing Machine. For subjective or sensory evaluation, a trained panel of six persons was constituted. Scoring or rating was done using a Standard 9-point hardness scale[1]. Vibration-free (control) tomato samples brought from field were sorted out into three categories such as green, pink and red tomatoes and stored. Firmness of these stored samples is given in Table 1 along with the correlation values between the two methods of firmness evaluation. High degree of correlation was noted[2].

TABLE 1. FIRMNESS (Kg) AT DIFFERENT LEVELS OF MATURITY

Storage period (days)	Green		Pink		Red	
	Instron value	Panel score	Instron value	Panel score	Instron value	Panel score
0	9.2	8	8.5	8	6.1	7
4	6.8	7	5.8	7	4.9	5
8	5.6	5	4.8	6	4.0	5
12	4.9	4	4.0	4	3.2	3
16	4.7	4	3.6	4	2.4	2
20	3.9	4	3.1	2	-	-
Corr.Coeff.(r)	0.96		0.91		0.97	

Data are average of 3-5 replicates; Average dimension of tomatoes: length 6.1 cm; diameter 3.1 cm
X Head speed - 5 cm/min.
Deformation 1 cm.

Effect of vibrations on tomato texture: Tomatoes at the bottom of the boxes were found to be totally deformed with flattened faces. Other tomatoes in the boxes lost 15 to 20% of their round shape. Colour also changed in boxes stored for 24 h. Few tomatoes of green colour were found and the number of pink tomatoes almost remained the same and those with red colour increased.

The changes in firmness values of the tomato samples with respect to storage period are presented in Table 2. During storage, maximum dry bulb temperature varied from 43-45°C and relative humidity from 45-50% Generally, firmness decreased as the storage period increased in all the treatments. Longer duration of vibrations resulted in decreased firmness. In general, every sample showed approximately 1.5 kg decrease in firmness over six days. It was found that samples from 47 cm deep boxes exhibited lower firmness compared to samples from 14 cm depth of tomato in the same box. These findings are in agreement with those of O'Brine and Guillon[3]. Further comparison shows that the firmness is not affected by the material

TABLE 2. AVERAGE VALUES OF FIRMNESS (Kg) AT VARIOUS STORAGE PERIODS

Type of box	Depth of fill (cm)	Duration (h)	Storage period (days) 0	2	4	6	8	10	12	14
Deodar wood	14	6	5.1	4.9	4.2	3.8	3.2	2.6	2.4	2.0
		12	4.6	4.2	3.9	3.4	2.9	2.4	2.1	1.7
		20	4.5	4.1	3.8	3.0	2.8	2.2	2.0	1.5
Plastic crate	16	6	5.0	4.7	4.4	3.8	3.5	3.1	2.8	2.2
		12	4.8	4.3	4.0	3.5	3.0	2.4	2.1	1.8
Eucalyptus wood	18	6	5.0	4.6	4.2	3.6	2.7	2.2	-	-
		12	4.5	4.3	3.8	3.3	2.2	1.8	1.4	-
		20	4.9	4.6	3.3	3.1	2.5	2.0	1.6	-
Rigid cardboard	21	6	5.1	4.7	4.2	3.7	3.1	3.0	2.7	2.5
		12	4.9	4.6	3.8	3.2	3.0	2.3	1.9	1.2
		10	4.8	4.5	3.5	3.2	2.8	2.3	1.7	1.3
Deodar wood	47	6	4.6	4.2	3.9	3.3	3.0	2.6	2.4	2.1
		12	4.4	4.3	3.9	3.1	2.8	2.8	2.4	1.8
		20	3.9	3.0	2.6	2.5	2.1	1.6	1.5	-

TABLE 3. CUMMULATIVE DECAY (%) FOR DIFFERENT STORAGE PERIODS

Type of box	Depth of fill (cm)	Duration (h)	Storage period (days) 0	2	4	6	8	10	12	14	16	18
Deodar wood	14	6	1.1	3.7	8.3	16.2	31.8	62.3	80.6	91.7	97.1	99.2
		12	1.7	4.8	10.4	18.0	33.2	64.4	82.3	92.0	97.6	99.3
		20	2.7	8.0	16.4	32.4	63.9	84.3	93.0	97.3	99.3	100
Plastic craft	16	6	1.1	4.8	11.2	22.5	38.0	72.2	93.1	99.5	100	-
		12	1.3	5.0	12.1	22.9	39.4	74.4	92.1	99.2	100	-
Eucalyptus wood	18	6	1.1	3.9	9.7	22.2	41.6	69.5	84.9	96.1	98.2	99.6
		12	2.9	7.8	14.6	22.4	35.9	65.1	81.5	92.2	98.2	99.6
		20	4.5	12.0	20.9	34.8	62.2	79.4	89.0	94.1	98.3	100
Rigid cardboard	21	6	0.5	2.4	9.0	21.3	40.3	73.4	88.6	96.1	99.5	100
		12	1.4	3.7	8.3	20.3	45.0	75.2	88.9	97.2	100	-
		20	2.9	9.1	17.7	32.7	56.5	76.1	89.5	97.0	100	-
Deodar wood	47	6	3.6	9.1	24.1	39.4	69.0	83.7	92.6	96.1	98.5	100
		12	4.9	11.9	27.0	43.3	72.0	83.1	93.5	96.7	99.1	100
		20	10.1	23.1	34.6	54.1	73.7	87.9	93.9	97.0	99.7	100

Decay was 100% at 20 days in Deodar wooden box

of construction of boxes. Firmness is also related to the maturity level of tomatoes. It is noted that with an increase in maturity level, firmness decreases (Table 2).

Decay of tomatoes: Decay % of tomatoes in all the treatments increased with an increase in storage period (Table 3). Longer duration of vibration resulted in enhanced decay. The decay rate is practically same for 6 and 12 h duration of vibration but increased rapidly in 20 h vibration. Approximately, 90-95% of tomatoes have decayed for all the treatments at the end of 14 days storage. The depth of fill of tomatoes in the boxes has a pronounced effect on the decay. It is observed that the samples from 47 cm depth show higher decay compared to 14 cm depth in Deodar wooden box (Fig 1.)

Cushioning material was found to influence the firmness to a greater extent[4]. Both paddy straw and mat lowered the damage and increased the firmness of tomatoes as compared to those without cushioning. Further, it was noted that with the same material box, lower firmness values were observed in case of mat as cushioning material as compared to loose paddy straw as cushioning material (Fig.1.)

From this study, it is concluded that the firmness of tomatoes decreases and decay rate increases with increase

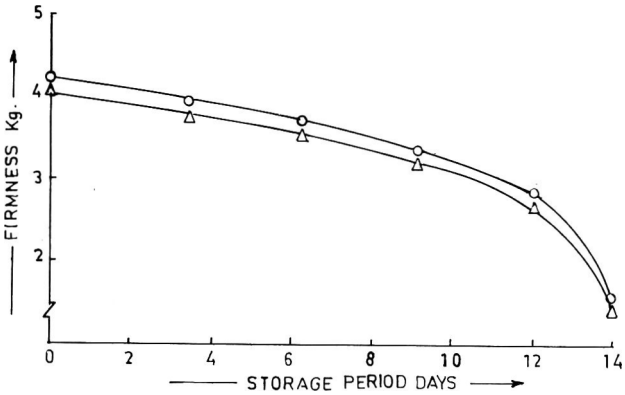

Fig.1 Variation of firmness with storage period for different cushioning materials in 47 cm. Deep Deodar box with 12 H. vibration duration
o-o Loose paddy straw as cushioning material
Δ-Δ Mat cushioning material

in depth of fill, duration of vibration and storage period. Material of construction of boxes does not affect the quality, whereas cushioning material helped in maintaining the quality. Loose paddy straw is more appropriate as compared to mat type cushioning material.

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Enterotoxin Production by *Staphylococci* Isolated from Pork *Kabab, Salami* and Other Sources by ELISA

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The studies on enterotoxin production by staphylococci isolated from pork, *kabab, salami* and other sources as detected with ELISA, indicated the production of toxin by both coagulase positive and negative isolates. Few of the isolates produced enterotoxins C and D, while others produced either B, C or D toxins. The quantity of enterotoxin C produced by the isolates was in the range of 20-45 μg/ml.

Enterotoxins A, B, C, D produced by staphylococci have been implicated in food poisoning outbreaks[1,2]. ELISA (Enzyme Linked Immuno – Sorbent Assay) has been used for their detection in foods[3]. The presence of staphylococci in food can be a possible risk of food poisoning, if they are enterotoxigenic.

Enterotoxin production by staphylococci isolated from *kabab, salami, salami* casing, air and working surface of chill rooms is reported in the present communication.

Staphylococci (157 Nos.) isolated from *kabab* (38), *salami* (64), *salami* casing (13), *salami* stuff (3), trekker (13), trekker casing (6), trekker stuff (1), coarsely chopped pork (4), minced pork (4), sample of water supply (2), surface of knife (1), air (4) and working surface of chill room (4) from Govt. Bacon Factory, Ranchi were studied. An overnight growth in Brain Heart Infusion Broth (BHIB) of each was centrifuged to obtain cell-free supernatant for use in ELISA. The method of Notermans and Duferenne[4] and as described in the literature supplied with standard staphylococcal enterotoxin test kit obtained from Labor Dr. W. Bommeli, Langasse-Strasse 7, CH-3012 Bern were followed.

Results of ELISA test showing the types of enterotoxins produced by staphylococci and their source of isolation are summarised in Table 1. Type D enterotoxin producing strains constituted the maximum (12/19), followed by type C (4) and B (3) producing strains. Two isolates produced both SEC and SED. The production of any one or more than one enterotoxins by staphylococci isolated from food poisoning outbreak has been reported[1-3]. It is interesting

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TABLE 1 RESULTS OF ELISA TEST SHOWING THE TYPES OF ENTEROTOXINS PRODUCED BY STAPHYLOCOCCI ISOLATED FROM PORK PRODUCTS AND ENVIRONMENT OF MEAT PROCESSING PLANT

Source	No. of organic isolated coagulase		Toxin production by isolated cultures			
	+ve	-ve	Coagulase +ve No. of isolates	type of toxin	Coagulase -ve No.of isolates	type of toxin
Kabab	9	29	1	C	1	B
					1	D
Salami	27	37	10	D	1	C
			1	C & D	1	C & D
Salami casing	0	13	-	-	1	B
Salami stuff	0	3	-	-	-	-
Working surface chill room air	1	7	1	D	1	B
Others	0	31	-	-	-	-
Total	37	120	13		6	

to note that all the 11 coagulase positive enterotoxigenic isolates from *salami* produced enterotoxin D and one produced C in addition. Against this, two coagulase negative cultures produced enterotoxin C or C and D. Thus, there were 19(12.1%) staphylococcal isolates which yielded various enterotoxins. Six of these enterotoxigenic cultures were coagulase negative. Omori and Kato[5] and Bergdoll *et al.*[6] also observed enterotoxin production by coagulase negative staphylococci. The enterotoxin C production by the isolated cultures ranged between 20 and 45 µg/ml.

It is of interest to note that none of the isolates from trekker, trekker casing, trekker stuff and pork was found to be enterotoxigenic. The presence of toxigenic staphylococci in *kabab*, *salami* and *salami* casing and their absence in *salami* stuff, coarsely chopped or minced pork suggest the role of environmental contamination. This is further supported by the presence of enterotoxigenic isolates in the environmental samples like air and working surface of chill room. Such environmental contamination with enterotoxigenic strains of staphylococci indicates possible risk of food poisoning.

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Effect of Addition of Soy Flour on the Quality Characteristics of Blackgram (*Phaseolus mungo* L.) *Papads*

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The effect of addition of soy flour (10-70%) to blackgram *papad* making, showed no change in the dough texture, though rolling property was affected. Greater amounts of water were needed for mixing the dough with an increase in soy flour concentration. Elasticity was reduced to a minimum at 40% and was completely absent at 50%. Quality characteristics remained the same for both the raw *papads*. However, the quality of fried *papads* decreased beyond 60% addition of soy flour. Acceptability decreased with increase in percent soy flour added. The results suggest that an addition of 30-40% of soy flour would not make a significant difference in the physical and sensory characteristics of blackgram *papad*.

Since centuries, *papad* has been a popular snack item of India and many varieties are available commercially[1-4]. Blackgram flour (BGF) is the principle constituent of the papads, as it contains mucilaginous principle necessary for obtaining a dough of desirable consistency and rolling property. The enhancement of nutritive value by the addition of soy flour (SF) in bread, biscuits, *chapati* and textured products has been demonstrated[5]. No attempt has been made so far to incorporate SF in papads. Hence, the present investigation was undertaken to study the effect of incorporating SF on the quality characteristics of blackgram *papads*.

Good quality blackgram *dhal* (*Phaseolus mungo* L.) from the local market was cleaned, ground into fine powder and passed through 60 mesh (British standard). Full fat soy flour (*Glycine max* L.) was obtained from the University of Agricultural Sciences, Hebbal, Bangalore and passed through 60 mesh. Common salt, spices (cumin seeds, black pepper, asafoetida) and refined groundnut oil were purchased locally. Sodium carbonate and bicarbonate of LR grade were used instead of papad *khar*.

Formulation of blends for the preparation of papads: Soy flour was incorporated to BGF in different ratios with BGF (100%) serving as the control. Hundred grams of each blend with common salt (7 g), sodium carbonate and bicarbonate (1 g), cumin seeds (1.2 g), asafoetida (0.2 g), refined groundnut oil (2-3 g) and black pepper (2.4 g) were kneaded with water to prepare dough, while the amount of water added varied from blend to blend, Manual kneading took 7-10 min. The dough was divided into small balls of 10 g each and pressed to 0.8 mm thickness using the *chapati* press to make 9-10 cm discs. The *papads* were dried at 50°C and sealed in polythene covers until used.

Physical and chemical characteristics: All *papad* samples ground to a fine powder were analysed for moisture, protein (N×6.25), crude fat and total ash by the standard AOAC procedure[6]. Carbohydrate content was derived by difference. Physical characteristics studied included colour of the dough and of the dry *papad*, thickness (using screw gauge) and percentage expansion after frying was calculated using the formula:

$$\frac{\text{diameter after frying - diameter of raw papad}}{\text{diameter after frying}} \times 100$$

Sensory properties: The raw *papads* fried in the refined groundnut oil at 190°C were served to a group of ten trained panelists for the evaluation of colour, aroma, taste, texture and overall quality as per the method of Govindarajan *et al*[3] on a 10 point scale. Quality attributes were also evaluated after storing the raw *papads* in sealed polythene bags for a period of three months at room temperature (28°C) kept in plastic containers with tightly fitted lids.

Physical characteristics and rolling properties of papads: The amount of water needed for the preparation of dough increased as the proportion of SF in *papads* increased [(47 to 74 ml (100% soy flour)]. Increased requirements of water for making satisfactory dough appeared to be due to higher water absorption by the full fat soy flour. The yield of *papads* for 100 g of blend was about 138.5 g for those containing soy flour as against 145.5 g for the control by weight. Though the texture of the dough remained soft to handfeel in all the blends, pliability

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TABLE 1. ACCEPTABILITY SCORES OF FRIED *PAPADS*

Blackgram dhal: soy blend	Acceptability scores* Colour	Appearance	Texture	Aroma	Taste	After taste	Overall quality	Total
100:0	8.2^{b}	6.8^{bc}	7.4^{bc}	7.2^{de}	7.6^{d}	7.6^{e}	7.8^{c}	52.0
70:30	8.0^{d}	7.4^{c}	8.2^{c}	7.6^{e}	7.6^{d}	7.4^{de}	7.8^{c}	54.0
60:40	7.4^{d}	8.2^{c}	7.2^{bc}	7.6^{de}	7.6^{d}	7.0^{de}	8.0^{c}	51.6
50:50	5.8^{c}	5.8^{ab}	6.4^{b}	5.6^{cd}	5.8^{c}	6.0^{cd}	6.2^{b}	41.6
40:60	4.2^{ab}	5.4^{ab}	4.6^{a}	4.8^{bc}	4.6^{bc}	5.2^{bc}	5.0^{b}	33.0
30:70	3.8^{ab}	4.6^{a}	4.2^{a}	3.8^{ab}	3.8^{ab}	3.4^{a}	3.2^{a}	26.2
10:90	4.8bc	5.6^{ab}	4.4^{a}	3.6^{ab}	3.6^{ab}	4.2^{ab}	3.0^{a}	29.3
0:100	3.6^{a}	5.0^{a}	4.2^{a}	3.2^{a}	3.4^{a}	3.2^{a}	3.0^{a}	26.8
± SEM (63 df)	0.42	0.57	0.47	0.46	0.47	0.56	0.47	

* Mean of scores by 10 panelists

Any two means in rows bearing different superscripts a, b, c..... differ significantly ($P \leq 0.05$)

TABLE 2. PROXIMATE COMPOSITION (g/100g) OF RAW *PAPADS* ON DRY BASIS

Blackgram *dhal:* soy blend	Moisture	Total ash	Protein	Fat	Carbohydrates (by difference)
100:0	2.5^{a}	8.5^{c}	25.71^{a}	4.8^{b}	57.49^{e}
70:30	2.5^{a}	9.0^{d}	31.27^{ab}	12.0^{c}	45.23^{d}
60:40	3.5^{b}	9.0^{d}	32.13^{ab}	14.0^{d}	42.37^{c}
50:50	5.0^{c}	9.5^{e}	33.32^{ab}	16.0^{e}	36.18^{b}
Blackgram *dhal* flour	5.5^{c}	3.5^{b}	26.07^{a}	1.3^{a}	63.63^{f}
Soy flour	5.5^{c}	3.0^{a}	39.57^{b}	23.5^{f}	28.43^{a}
± SEM (6 df)	0.16	0.14	2.16	0.15	0.03

Mean values carrying different superscripts a, b, c ..... in columns differ significantly ($P \leq 0.05$).

diminished as the proportion of the soy flour in *papads* increased upto 30% addition of soy flour. The pliability remained the same and above that level, the rolling properties of the *papads* were poor and more dusting material was required. However, it was possible to roll *papads* without having cracked edges.

The colour of the *papads* changed from yellow to dark brown as the proportion of soy flour increased. However, the *papads* did not vary in thickness, diameter and texture remained brittle to handfeel in all the blends. Blends having upto 50% SF were bright yellow with an appealing appearance. They were crisp and the taste was acceptable without any perceptible flavour of soy flour. With more than 50% SF in *papad*, the colour changed from yellow to brown with poor appearance. *Papads* with 100% soy flour were crisp but bitter in taste. The percentage of expansion for soy *papads* at all levels of incorporation remained 'nil', while 100% BGF *papads* showed an expansion of 38.5%. Based on the dough characteristics of raw and fried *papads*, it was evident that incorporation of SF upto 40% was acceptable for making *papads*. Even the *papads* prepared from a blend of 50:50 compared well with those of 100% BGF, except in pliability characteristics.

Sensory evaluation: The acceptability score was similar to the control upto 40% of SF and decreased with the increase in the level of SF in the product (Table 1). The quality of fried *papads* with SF upto 40% was very close to the control, in terms of colour, aroma, taste and texture. Though flavour of soy flour was not perceptible, the expansion percentage of *papads* with soy flour was negligible. Among the blends, *papads* of 70:30 (BGF:SF) were superior to the control and scored the highest. Further, *papads* stored upto 3 months at room temperature (28°C) did not show significant differences in the quality attributes of *papads* (raw and fried).

It was evident from the data on physical characteristics and acceptability scores that *papads* having SF upto 50% were acceptable. Hence, proximate composition was determined only in these products (Table 2). Moisture content ranged from 2.5 to 5% as compared to 15% (maximum) recommended by ISI[7]. The fat and ash contents increased while the carbohydrate content decreased with increased levels of SF in *papads*. The increase in protein and fat content of *papads* on incorporating soy flour amounted to 15-22% respectively. However, the increase in protein content due to the addition of soy flour was not significant. Perhaps, the protein value may further be increased and fat decreased with the use of defatted soy flour in place of full - fat soy flour. This may be essential in view of the probable risks associated with high fat foods.

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Crisp and Spicy Soybean Snack

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Use of soybeans as snack food of the type exemplified by roasted peanuts was explored by roasting hydrated beans, hull removal and splitting. The product had a crunchy texture, mild nutty flavour and was devoid of urease activity. Shelf life of the unspiced product was 4 months in ordinary storage and one year in air-tight containers.

Soybean, a unique crop, containing about 40% protein and 20% fat, has an undesirable beany flavour. The major off-flavours are of lipid origin and are produced by the action of the enzyme lipoxidase on polyunsaturated fatty acids, principally linoleic and linolenic[1]. Roasted peanuts have a high degree of acceptibility as snacks among all age-groups[2]. The possibility of utilising soybeans to make related types of crunchy snacks could open up large domestic markets if an acceptable product is available. In this communication, roasting has been explored as a means to destroy the enzyme associated with off-flavour.

Whole soybean (Bragg variety), procured from local market and containing 8.5% moisture, was graded and cleaned. Water uptake data were obtained by soaking 10g soybeans in 40 ml tap water at 28-29°C for $^1/_2$ - 4 h. The beans were removed from water, superficially dried with facial tissue, and weighed to calculate weight gain as the difference between the measured weight. The dehydrated soybeans were spread on a paper for $^1/_2$ h to remove adhering water to avoid sticking of beans during sand roasting. The dried beans were roasted using sand and an electrically operated heater at 150-250°C for 5-15 min. The roasted products were cooled immediately. Dehulling was carried out by pressing the beans between the palms. The cuticles, already loosened during roasting, disintegrated readily. Sensory evaluation by 8 members from the scientific staff was carried out using a 9 - point hedonic scale for appearance, odour, flavour, texture and overall acceptance of the product[3]. The average score of 4 tests was calculated. Proteins, fat and ash contents were determined by AOAC methods[4]. Moisture content of roasted beans was determined using an infra-red moisture balance. For determining the urease activity, the ISI method (SP:18, Part VI-1982)[5] was used.

The effect of soaking period on water absorption at room temperature (28 -29°C) is given in Fig. 1. As the soaking period increases, so does the amount of water imbibed. Alongside, the shape of the beans changes from round to an oval shape with a smooth surface by preferential elongation along the lengthwise axis[6]. After 4 h soaking, when the beans were almost saturated with water, the profile resembled that of a peanut (Fig. 2). Table 1 shows that as the time and temperature of roasting increase, the moisture contents of roasted soybeans decrease. A temperature above 150°C caused charring of the products. Roasting at 150°C for 10 min gave a product which was free from charring and beany flavour. It was also judged superior to those produced using other time-temperature combination and this was adopted as the optimum.

Loss of urease activity in soyabeans is an accepted measure of the inactivation of other anti-nutritional factors present, viz. trypsin inhibitor, haemagglutinin and goitrogenic factor[7]. It was found that urease activity of raw soybeans was 1.4 units and as the time and temperature

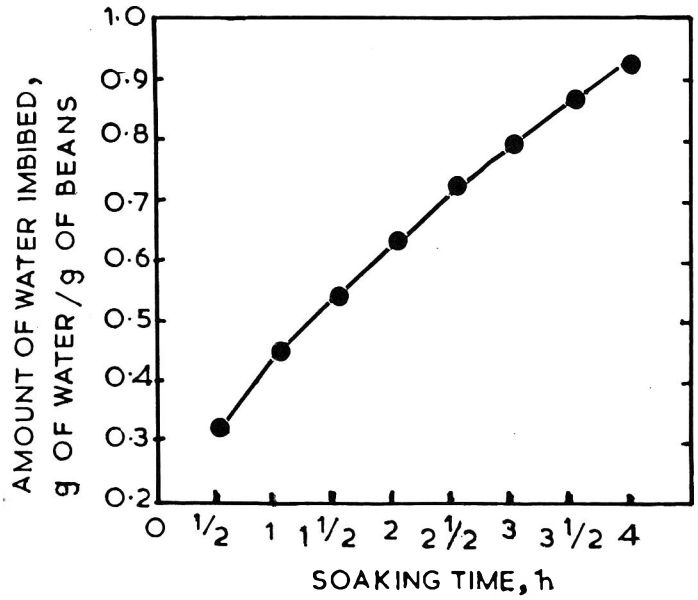

Fig. 1. Effect of soaking period on amount of water imbibed by soybeans soaked in water at room temperature (28-29°C).

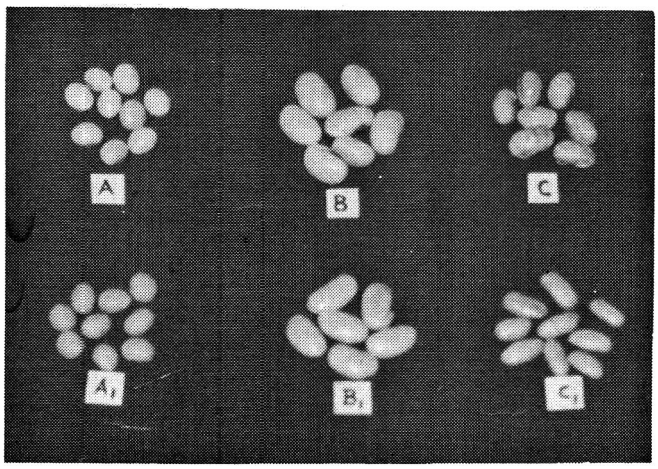

Fig. 2. Appearance of soybean at different stages of processing for preparation of a crisp snack food

Whole soybeans: A - Raw, B - Soaked for 4 h., C - Soaked for 4 h + roasted at 150°C for 10 min., Dehulled soy splits, A_1 - Raw, B_1 - Soaked for 4 h, C_1 - Soaked for 4 h + roasted at 150°C for 10 min..

of roasting increased, urease activity decreased. Soybeans roasted for 5 min at temperature ranging from 170°C to 250°C showed a significant declining trend in urease activity from 0.15 units to 0.02 units. Soybeans that had been roasted at 150°C for 10 min showed no urease activity, the time factor being more important than the temperature applied. Analysis of soybeans processed under the optimum conditions of soaking and roasting showed that it contains 19.4% fat, 4.5% total ash, 0.2% acid insoluble ash and

TABLE 1. EFFECT OF TIME AND TEMPERATURE OF ROASTING ON THE QUALITY AND MOISTURE CONTENT OF SOYBEANS

Time of roasting (min)	Temperature of roasting (°C)	Moisture content after roasting (%)	Colour and taste of product[a] (average assessment)
5	150	19.4	soft with poor flavour
	160	16.6	soft with poor flavour
	170	8.6	fair
	180	5.0	good
	200	3.0	good
	250	1.2	charred
10	150	2.8	very good
	160	2.2	fair
	170	1.6	fair
	180	1.2	fair
	200	0.6	charred
	250	-	charred even more
15	150	1.5	good
	160	0.9	fair
	170	0.8	good, but some charring.
	180	0.1	good, but even more charring
	200	-	yet more charring.

a. Evaluated by a 8-member panel.

8.6% crude fibre. Roasting reduced the moisture content to 2.8% A protein content of 43 to 45% would merit the term "high protein" for these snacks.

The protein-rich ready-to-eat crisp snack items were stored at room temperature. Samples were removed at intervals and evaluated for quality. Samples stored in LDPE bags (which are permeable to oxygen) at room temperature for 4 months were acceptable. Thereafter, the moisture content of the product increased considerably and as a result, its crispness was lost to a great extent. The quality of the product remained good for more than one year when stored in HDPE containers, PVC containers and aluminium pouches, all of which did not permit free access to the atmosphere.

On the basis of these results, it is concluded that soybeans soaked in water for 4 h and roasted at 150°C for 10 min are readily dehulled even by hand to yield a high protein ready-to-eat snack for a type exemplified by roasted peanuts or cashewnuts. Mixing with salt or *masala* enhanced the spiciness, and could widen popular appeal. A *masala*-soy snack made by the above method would, at the present seed prices, cost less than roasted and salted peanuts.

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Chemical Composition of Isabgol (*Plantago ovata* Forsk) Seed

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Dehusked isabgol seed had low oil content with a lipid profile high in free fatty acids, sterols and hydrocarbons making the oil a non-edible type. The defatted meal had high proportion of protein with high albumin, high lysine, medium methionine and digestible protein comparable to red gram *dhal*.

India holds a near monopoly in the production and export of isabgol seed husk in the world market. It is now cultivated in 40,000 ha land in Gujarat and Rajasthan producing about 39,000 tons of seed which, in turn, yield over 11,600 tons of processed seed husk for export. The dehusked seed, about 70% by weight of annual seed crop, does not find an effective use other than animal feed. Lack of scientific information about seed composition precludes its proper exploitation. Recent interest in the Western countries for isabgol as cholesterol reducing agent has led to renewed interest in its detailed composition. Kanitkar and Pendse[1] found the seed oil possessing property of reducing cholesterol level of serum in rabbit. Proximate composition of isabgol seed is known for a long time. The mucilage constitutes over 30% of the whole seed. Pendse *et al*[2]. reported about 5% pale yellow oil, 8.3% moisture content, 6.8% ash content in isabgol seed of Gujarat region. In the present communication, the protein and lipid compositions of these dehusked seeds, commercially known as *gola*, are reported.

Dehusked seeds were collected from market, ground in a Sumeet grinder and sieved through 60 mesh sieve. The powder was analysed for oil content by Soxhlet extraction in n-hexane. Free fatty acid contents of the extracted oil were determined by titrimetry[3] and fatty acid composition by gas chromatography[4]. Total lipids were extracted by chloroform: methanol (2:1), purified[5] and separated into lipid components by TLC using petroleum ether: diethyl ether : acetic acid (80:20:1) as developing solvent and treatment with sulphuric acid for visualization[6] and then quantitated by densitometry. Triolein, stearic acid and stigmasterol were used as standards. Polar lipids were separated from the total lipid by solvent partitioning in 90% methanol from hexane solution. Total phospholipid contents of these polar lipids were determined spectrophotometrically[7].

Crude protein content was determined by microkjehldahl method and proteins were fractionated into five categories as per Landry and Moureaux[8]. Lysine and tryptophan were estimated according to Villigas and Merty[9] and methionine by the method of McCurthy and Pailly[10]. *In vitro* protein digestibility of isabgol seed protein was determined by pepsin-pancreatin digestion method of Akeson and Stahman[11].

Oil, FFA, phospholipid, MFA composition of isabgol seed oil, lipid classes, crude protein and protein classes, essential amino acids generally regarded as limiting in cereals and pulses (lysine, tryptophan and methionine) and

TABLE 1: ANALYTICAL COMPOSITION OF DEHUSKED ISABGOL SEED

Total oil (%)	8.6 ± 0.014
Total free fatty acids (%)	1.9 ± 0.04
Phospholipids (%)	0.2 ± 0.003
Crude protein % in seed	20.0 ± 0.85
Crude protein % in defatted meal	29.3 ± 0.50
Mixed Fatty Acid Profile (Relative %)	
Palmitic acid	10.9 ± 0.61
Stearic acid	1.7 ± 0.13
Oleic acid	48.0 ± 0.63
Linoleic acid	37.7 ± 0.54
Linolenic acid	2.10 ± 0.57
Polar lipids	4.7
Monoglycerides	3.1
Sterols	12.9
Diglycerides	20.0
FFA	17.7
Triglycerides	34.5
Hydrocarbons and sterol esters	7.1
Protein fractions (% Protein)	
Albumin	42.3 ± 2.4
Globulin	19.2 ± 1.8
Prolamine	7.8 ± 1.0
Gluteline	16.2 ± 1.6
NPN	9.4 ± 1.3
Essential amino acids (% Protein)	
Lysine	4.1 ± 0.04
Tryptophan	1.8 ± 0.01
Methionine	1.7 ± 0.04
FAO Pattern	
Lysine	5.5
Tryptophan	1.0
Methionine	3.5
***In vitro* Protein digestibility (%)**	63.1-64.6

digestible protein content of dehusked seed are presented in Table 1. Electrophoretogram of albumin fraction of seed protein revealed one dark, two light and three diffused bands, the first one being the major and less mobile.

Results (Table 1) show that the relatively low oil content, high FFA, sterol and hydrocarbon fractions do not make isabgol seed oil very promising as edible oil, although in fatty acid composition, oleic/linoleic ratio is in the desirable range with low content of easily oxidizable linolenic acid.

Protein content of isabgol seed was found to be high (defatted meal 29.3%) with very high proportions of albumin and low content of prolamine which is generally a good indicator of easily digestible edible grade protein. Lysine content of seed protein (4.14%) was relatively higher than cereal proteins like wheat, maize, sorghum and pearl-millet, somewhat comparable to rice, but less than in pulse protein. Tryptophan content in isabgol seed protein was found adequate as per FAO pattern. Methionine content was much higher than pulse protein like pigeonpea and chickpea and comparable to cereals, but less than the FAO provisional pattern which is for total sulphur amino acids. The data reported here are, however, only for methionine and do not include cysteine and cysteic acid. *In vitro* protein digestibility studies showed that digestible protein in isabgol was 63.1-64.6% which is comparable to the reported value for pigeonpea *dhal*[12]. Tannin content of seeds were determined by AOAC method[13] and was found slightly higher (1.50%) than redgram (0.61-1.14%) as reported by Makkar[14].

The results indicate that defatted isabgol seed has the potential to be used as edible grade protein which stands in between cereals and pulses in its status of nutritional quality. However, long term animal feeding trials are necessary to ascertain its biological effect, if any, before recommending its use as additional source of edible grade protein.

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Physical and Chemical Characteristics of Chinese Sarson (*Brassica chinensis*) Seeds and Oil

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Comparison of physical and chemical characteristics of Chinese sarson (*Brassica chinensis*) seeds and oil with other mustard varieties indicated that the Chinese sarson seeds are low in oil but high in protein content. Specific gravity and saponification value of Chinese sarson oil are slightly higher compared to other Brassicae seed oils. Oil contains more erucic acid and less linolenic acid than other varieties.

Rapeseed-mustard constitutes an important source of edible oil in India next only to groundnut. Different varieties are being cultivated as oilseed crops in Punjab, Haryana, Rajasthan, Uttar Pradesh, West Bengal and Bihar with a total production of about 4.5 million tons[1]. Recently, a new variety of mustard, Chinese sarson has been introduced in Punjab especially as a leafy vegetable to replace Raya variety for making *saag*[2]. The plant is non-heading type, high foliage nature and with good seed setting ability in the plains, yielding about 41800 kg of green leaves in eight pickings per hectare[3]. A seed yield of about 1000 kg/ha is obtained[3]. The present paper reports the physical and chemical characteristics of seeds and oil of Chinese sarson in comparison to other sarson varieties.

The seeds of the four *Brassicae* viz., 'Toria', 'TL-15' (*B. compestris*), 'Gobbi sarson', 'GSL-1' (*B. compestris*), 'Raya', 'RL-1359' (*B. juncea*), 'Chinese sarson', Sel-1 (*B. chinensis*), and 'Taramira, ITSA' (*Eruca sativa*) were procured from Punjab Agricultural University, Ludhiana. These were examined for physical characteristics namely, colour (visual), size and shape (visual), weight per 100 seeds and pungency of crushed and moistened seeds (odour). Moisture, protein and oil contents in the seeds were determined according to AOAC methods[4]. Volatile oil (as allylisothiocyanate) content in seeds was determined according to ISI methods[5]. The oil was examined for its specific gravity, refractive index, acid value, saponification value, and iodine value using AOCS official methods[6]. The fatty acid composition of the oil was determined by gas chromatography on a BDS column as described by Batta *et al*[7].

The physical and chemical characteristics of Chinese sarson and other *Brassicae* seeds are given in Table 1. The Chinese sarson seeds, as compared to other *Brassicae* seeds, are low in oil content and high in protein. The oil is of light brown colour and has light pungent odour. Specific gravity and saponification value of the oil are slightly higher than for other *Brassicae* seeds oils (Table 2). The fatty acid composition of the oil (Table 3) shows that the oil is composed of over 95% unsaturated fatty

TABLE 1. PHYSICAL AND CHEMICAL CHARACTERISTICS OF DIFFERENT *BRASSICAE* AND *TARAMIRA* SEED VARIETIES

'Seed variety'	Colour	Size/ Shape	Wt. per 100 seeds (g)	Pungency	Volatile oil (% by wt.)	Moisture (%)	Protein (%)	Oil (%)
'Chinese sarson'	Light to reddish brown	Small, round	0.2	Light pungent	2.8	6.2	27.4	25.8
'Raya'	Dark amber	Bold, round	0.5	Very pungent	3.0	7.5	22.2	35.0
'Toria'	Dark amber	Medium, round	0.3	Pungent	2.9	5.2	18.8	37.0
'Gobbi sarson'	Amber	small, round	0.2	Light pungent	2.8	7.2	22.1	36.7
'Taramira'	Earthy yellow	Medium, oval	0.3	Strong pungent	3.1	7.5	24.2	27.2

TABLE 2. CHARACTERISTICS OF THE OIL FROM DIFFERENT *BRASSICAE* AND *TARAMIRA* SEED VARIETIES

Seed variety	Specific gravity (25°C/25)	Refractive index (25°C)	Saponification value	Iodine value
'Chinese sarson'	0.9102	1.4697	172	102
'Raya'	0.9098	1.4700	167	103
'Toria'	0.9088	1.4698	168	103
'Gobbi sarson'	0.9089	1.4692	171	99
'Taramira'	0.9095	1.4691	163	100

+ Average of two determinations

TABLE 3. FATTY ACID COMPOSITION (PER CENT OF TOTAL METHYL ESTERS) OF THE OIL OF DIFFERENT *BRASSICAE* AND *TARAMIRA* SEED VARIETIES

Fatty acid	'Chinese sarson'	'Raya'	'Toria'	'Gobbi sarson'	'Taramira'
$>C_{16:0}$	0.1	0.1	0.0	0.1	0.2
$C_{16:0}$	2.8	2.4	2.5	3.5	4.3
$C_{16:1}$	0.2	0.1	0.1	0.1	0.1
$C_{18:0}$	0.9	0.8	0.8	0.9	1.0
$C_{18:1}$	13.2	9.6	14.4	17.5	15.0
$C_{18:2}$	14.4	16.0	13.9	13.9	9.5
$C_{18:3}$	8.4	11.0	8.8	8.3	11.5
$C_{20:1}$	7.0	6.6	9.6	12.7	10.8
$C_{22:0}$	0.4	0.9	0.4	0.4	0.2
$C_{22:1}$	52.5	52.4	49.4	42.4	47.3
$C_{24:0}$	0.1	0.1	0.1	0.2	0.1
Total Unsaturated	95.7	95.7	96.2	94.9	94.2

+ Average of two determinations

acids. The ratio of oleic acid: linoleic acid which plays an important role in nutrition, was 1:1. The erucic acid (22:1), an undesirable fatty acid, was highest in the seed oils of 'Raya' and 'Chinese sarson'. The oils of 'Gobbi sarson' and 'Chinese sarson' contained least amounts of linolenic acid, a desirable property from storage point of view as oils with high linolenic acid content turn rancid rapidly. The quality of the oil from Chinese sarson seeds is thus comparable with that of the oils from other *Brassicae* varieties.

The oilseed cake may have better animal acceptance because of the low pungency compared to other sarson varieties. The Chinese sarson crop will, thus, provide higher income to the farmers through green leaves as vegetables and seeds as source of edible oil.

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Effect of Frozen Storage on Protease and Lipase Activities of Oil Sardine and Ribbon Fish

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Highest protease activity was observed in whole gut of oil sardine and ribbon fish. On the contrary, lipase activity was found to be the highest in liver of both the species. Statistically, significant decreases in the activities of proteases and lipases were found to occur in various tissues of oil sardine and ribbon fish during frozen storage.

Degradation of muscle protein by endogenous proteases especially cathepsin, bacterial activity or both is a major reaction in spoilage of fish[1]. The measurement of enzyme activities could help in predicting early changes in quality of frozen stored fish[2]. Most fish enzymes exhibit significant losses following freezing and thawing and the enzymes have significant activity in partially frozen systems[3-5]. The present study was undertaken to assess the proteolytic and lipolytic activities in Indian oil sardine (*Sardinella longiceps*) and Ribbon fish (*Trichiurus* sp.) during frozen storage. These fishes form two commercially important species caught along South West coast of India.

Oil sardine (50 kg) of 0⁺ age group from a single shoal, was directly brailed out of the purse-seine net into an insulated box with sufficient crushed ice. Ribbon fish (40 kg) of 2⁺ age group, caught by night trawling, was transported to the processing hall in iced condition. These were washed with chilled water so as to remove extraneous material. Lots of oil sardine (500 g each) were arranged in galvanised trays lined with polyethylene sheets, while ribbon fish (10 numbers) were covered individually with polyethylene sheets and arranged neatly on long trays. After adding sufficient chilled water, the fishes were frozen in a coil freezer (-28 ± 2°C) for 48 h, were packaged in master cartons and stored at -18° ± 2°C. Whole gut (intestine with stomach), liver and muscle of the fish were used for assay. The samples were drawn from fresh fish, immediately after freezing and at 30 day interval during frozen storage. The tissue was extracted with chilled glass distilled water (20 ml/g) and centrifuged at 6000 × g for 45 min at + 4°C (JANETZKI Cooling Centrifuge, Model K 24). The resultant supernatant was employed for enzyme assay.

Proteolytic activity was determined by the method of Laskowski[6], using casein as substrate at pH 8.0. Protein concentration was measured by the method of Lowry *et al*[7]. Protease activity (PU) was defined as the amount of crude enzyme which, under defined conditions, (30 min incubation at 37°C; 2 ml final volume of the mixture) increases OD by 1.0 at 720 nm in one min. Lipase activity was based on the titrimetric determination of fatty acids liberated from esters by hydrolysis of Tween - 80[8], after incubating at 37°C for 10 min. and 8.3 pH. One ml of 0.01 N NaOH was considered equivalent to 100 lipase units.

Among the various tissues analysed for the protease and lipase activities, the highest protease and lipase activities were observed in the gut and the liver, respectively of both the fishes. The protease in whole gut of oil sardine showed steep decline during storage upto 120 days in contrast to the increase in liver and muscle till 30 and 90 days, respectively (Fig. 1). During frozen storage, protease activity in ribbon fish decreased in all the tissues analysed, after a moderate increase upto 30 days in case of gut and liver (Fig. 2). However, in muscle tissue, peak

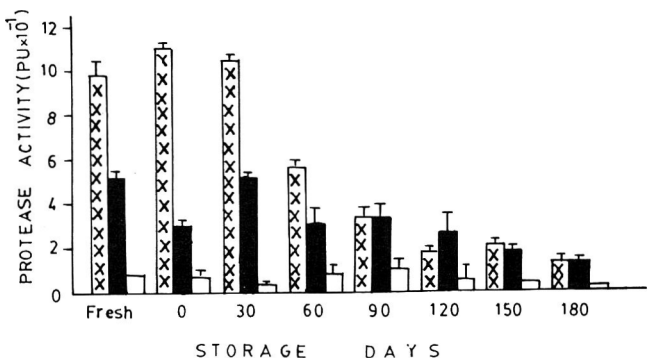

Fig.1. Protease activity at pH 8.0 in frozen stored oil sardine
☒ Gut ■ Liver □ Muscle

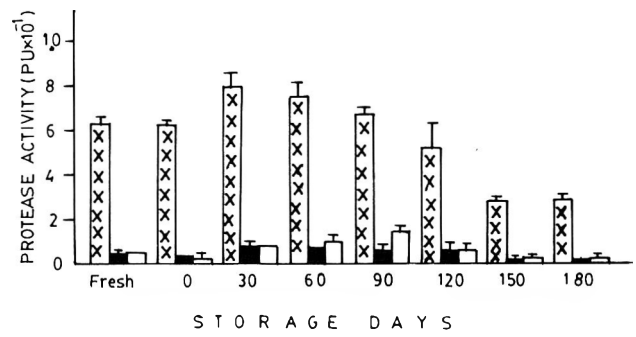

Fig.2. Protease activity at pH 8.0 in ribbon fish during frozen storage.
☒ Gut ■ Liver □ Muscle

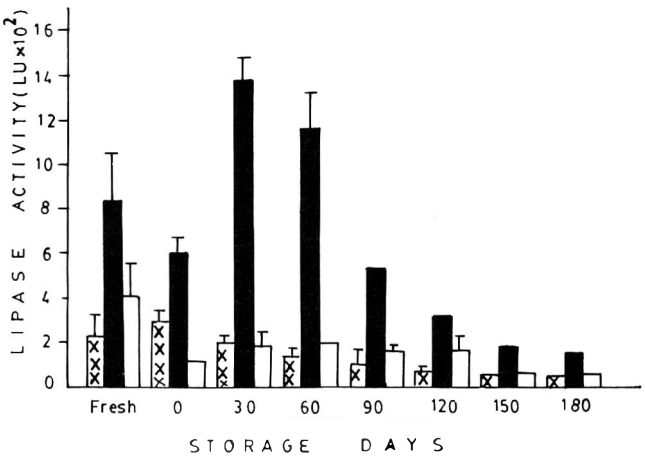

Fig.3. Lipase activity at pH 8.0 in frozen stored oil sardines.
☒ Gut ■ Liver □ Muscle

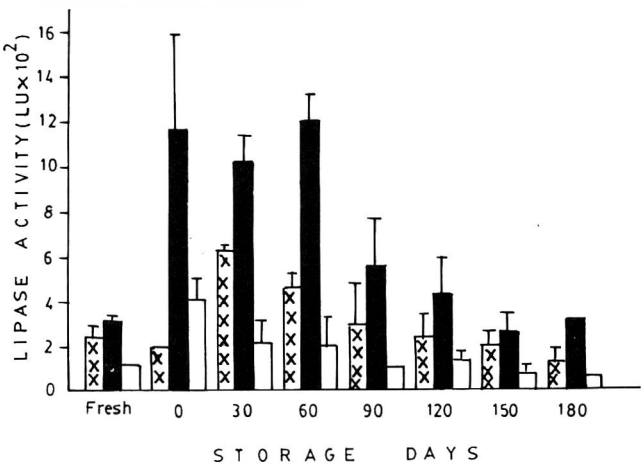

Fig.4. Lipase activity at pH 8.0 in ribbon fish during frozen storage.
☒ Gut ■ Liver □ Muscle

activity was observed at 90 days and showed a gradual decrease thereafter. Changes in lipase activity of oil sardine and ribbon fish during frozen storage at pH 8.0 are depicted in Fig. 3 and 4. In general, lipase activity decreased steeply after 60 days of storage at low temperature.

Even though protease and lipase activities of various tissues of oil sardine and ribbon fish decreased during frozen storage reaching the least activity at the end of 180 days, statistically significant decreases in the activities of both proteases and lipases were observed in oil sardine gut ($P< 0.01$), and liver ($P<0.05$) in contrast to only proteolytic activity in case of ribbon fish gut ($P<0.05$) and lipolytic activity of ribbon fish liver and muscle ($P<0.01$). The decreases in the activities of proteases and lipases during frozen storage of oil sardine and ribbon fish may be due to loss of enzyme configuration, formation of catalytically inactive enzymes, more tight folding of enzyme proteins, aggregation reactions, and enzyme denaturation and formation of low molecular weight compounds with ability to inhibit the enzymes[5,9,10]. The role of autooxidation of lipids as well as formation of FFA and their possible role in enzyme denaturation may also be involved.

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Composition Characteristics and Potential Uses of South Indian Tea Seeds

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The analytical data on tea seed and the seed oil indicate the possible use of tea seed oil for edible purposes.

All species of the genus *Camellia* produce large oleaginous seeds[1]. Some tea plantations, especially those in China and Japan, grow the ornamental shrubs, *Camellia japonica*, *Camellia sasanqua* and *Camellia oleifera*, mainly for seed and subsequent oil production[2]. Though the beverage tea plant, *Camellia sinensis*, is mainly cultivated for its prolific vegetative growth, it also produces oil-rich seeds[1]. Numerous tea plantations in different parts of the world are shifting from seed *bari* cultivation to clonal propagation techniques and India is no exception. While much research and development have taken place in the agricultural and production aspects of tea, only sporadic attempts have been made regarding the utilization of tea seeds, especially in India. It is reported that, China produced 1,80,000 tons of tea seeds during 1958, with an annual production of 28,000 tons of tea seed oil[3].

It is claimed that 50,000 ha of China hybrid-type tea can produce 14,000 tons of seeds and 3,000 tons of tea seed oils, besides its beverage value[1]. Cloughley[4] reported that 4,000 ha under local jat in Malawi could produce 350 tons of tea seed and 50 tons of seed oil. India with tea cultivation of over 4,00,000 ha, goes through biennial cycles of seed production and no official seed estimates are available on the Indian tea gardens[1].

The oil contents of *Camellia* seeds show wide variations, ranging from 20% in *C.sinensis* to 45% in *C. sasanqua*[1]. Tea seed oil was reported to resemble groundnut and olive oils in chemical composition[1,2]. The residue after

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the extraction of oil and saponin, possesses a high content of protein (12.6%) with all essential amino acids, indicating its usefulness as cattle feed. The present study examines the composition of three varieties of South Indian tea seeds and characterization of the oil derived from them.

Biclonal mature tea seeds were collected over the main seed-drop period (April-May) from three genetically diverse, commercial cultivars, viz. 'China clone', 'Assam clone' and 'Combod clone', which are available in a breeding plot of an estate in Valparai (Anaimalai Hills), one of the important tea growing districts in South India. Seed samples were sun-dried for two days and the hulls were removed manually. The kernels and hulls were oven-dried at 105°C to constant weight and ground well (separately) for chemical investigations.

The hull:kernel weight ratio was determined by taking their respective weights from 100 seeds. Moisture, nitrogen, total ash and crude fibre contents were determined by AOAC methods[5]. The total carbohydrates were estimated by phenol-sulphuric acid method[6] and saponins by the method of de Silva[7]. The ground seeds were defatted by extracting with petroleum ether (40-60°C) and the recovered oil was analysed[8]. Fatty acids were analysed by GLC[9] using a Pye-Unicam 204 series gas chromatograph, and polyester columns were used at 205°C with flow rates of 60-80 ml of helium (pre-heated) per min. The polyester dissolved in acetone was added to the solid support (Celite 60-90 mesh) in the ratio of 1:4 (w/w) and was packed in a coiled copper column (6 ft long with 0.22 inch inner diam). The detection system employed two filament Gow-Mac thermal conductivity detectors in the reference channel and two in the sample channel. The sample injected into the gas stream was 5 µl of a petroleum ether solution containing 500-1000 γ of the esters.

The hull:kernel ratio in China, Assam and Combod clonal seeds was 1:2 (Table 1). The moisture content ranged from 6.6 to 7.1% in the hull and 3.9 to 4.1% in the kernel. The protein content of the kernel ranged from 20 to 23%, which is higher than the values reported so far[1,4]. The contents of carbohydrates ranged from 18 to 20% in seed kernel. The values of ash and crude fibre contents agree with the reported figures[4]. The kernel possessed high contents of saponins (11%).

The kernels yielded oil in the range of 30 to 32% and it was clear, free-flowing and had acceptable organoleptic properties. Differences among the oils in chemical characteristics and composition can be seen in Tables 2 and 3.

TABLE 1. COMPOSITION OF SOUTH INDIAN TEA SEED

Components	China clone	Assam clone	Combod clone
Hull:kernel (weight ratio)	31:69	33:67	32:68
Moisture (%)	7.1*	6.6*	6.9*
	3.9	4.1	4.0
Nitrogen (%)	0.9	0.8	0.6
	3.5	3.4	3.1
Protein (N × 6.25 %)	5.8	5.2	3.9
	22.7	22.1	20.1
Carbohydrate (%)	10.1	11.8	10.9
	18.5	18.5	19.8
Total ash (%)	1.1	1.4	1.0
	4.0	4.2	4.6
Crude fibre (%)	70.3	74.5	70.7
	10.2	7.9	9.8
Saponins (%)	0.5	0.8	0.9
	9.9	11.3	11.0
Oil (%)	2.8	2.1	2.3
	31.0	30.2	31.5

*The values in the two rows down refer to hull and kernel, respectively,
Values are average of three trials.

TABLE 2. CHARACTERISTICS OF TEA SEED OIL AND SOME EDIBLE OILS

Oil	Refractive Index (27°C)	Iodine value	Thio cyanogen value	Saponification value	Acid value
China	1.46469	91	67	194	2.3
Assam	1.46471	93	68	197	2.5
Combod	1.46870	86	75	190	3.1
Groundnut*	1.41061	92	64	191	1.2
Olive*	1.46701	84	79	189	2.5

* Values taken from CRC Handbook of Biochemistry (1970), 2nd Edn.

TABLE 3. FATTY ACID COMPOSITION OF TEA SEED OIL AND SOME EDIBLE OILS

Oil	Saturated fatty acids		Unsaturated fatty acids		
	16:0	18:0	18:1	18:2	18:3
China	14.8±0.2	3.1±0.1	57.1±0.3	22.5±0.3	1.5±0.1
Assam	12.0±0.2	2.7±0.2	58.7±0.2	24.3±0.2	1.4±0.1
Combod	8.6±0.1	1.2±0.1	78.7±0.2	9.8±0.1	1.2±0.1
Groundnut*	8.3±0.2	3.1±0.1	56.0±0.3	26.0±0.1	–
Olive*	6.9±0.1	2.3±0.2	84.4±0.2	4.6±0.1	–

* As given in Table 2
@ 14:0 was present only in trace amounts.
Mean ± SD

It is of interest to note that South Indian tea seeds had about three times the protein content compared to other tea seeds in the world[1]. Data regarding the carbohydrates and crude fibre indicate that tea seed flour can be used in animal and human nutrition. Saponins constituting 11% of the kernel are another valuable by-product that can be obtained from tea seeds. They are plant glycosides, with good emulsifying properties and are widely used in industries[7]. Tea saponins exhibit anti-exudative and anti-inflammatory properties[7]. Saponins may also be toxic[1].

The oil content of tea seeds was about 31%. It is to be noted that the oil obtained from China and Assam clonal seeds, closely resemble groundnut oil and would, probably, be equally acceptable. Cloughley[2] reported that, tea seed oil is edible and foods fried in tea seed oil were indistinguishable from those fried in groundnut oil. At the same time, the keeping quality of tea seed oil was found to be excellent[4]. The oil would also seem acceptable from medicinal and nutritional considerations, because of the high unsaturated fatty acid content, particularly of linoleic acid. The oil can be readily hydrogenated to give *vanaspathi*-like product[1]. In India, where tea production is plentiful and where there is an acute shortage of edible oils, tea seed oil could be considered as a potential substitute for the traditional edible oils.

The oil obtained from Combad variety resembled olive oil in its chemical composition and characteristics. In fact, oil from *Camellia sasanqua* has been used to adulterate the costly olive oil, due to their close similarity[2]. The Combod oil can be a possible substitute for olive oil in the cosmetic and pharmaceutical industries.

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Glucosinolate Contents of Commonly Grown and Consumed Vegetables of Himachal Pradesh

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On fresh matter basis, high glucosinolates were found in mustard seeds (3.1%) and mustard leaves (2.7%) followed by fenugreek leaves (1.9%). The values for cauliflower, turnip, cabbage and knol-khol were 1.7, 1.2, 0.7 and 0.6%, respectively. When expressed on dry matter basis, the glucosinolate contents were highest in mustard leaves (26.3%) followed by cauliflower (18.2%) but lowest in knol-khol (8.7%) and cabbage (8.3%).

Goitrogens, the sulphur containing organic compounds, viz, isothiocyanate, thiocyanate and glucosinolates, occur in plant foods and cause thyroid enlargement and other disorders such as reduced body weight and brain size due to inadequate uptake of iodine[1,2]. Keeping in view the interference of goitrogens with iodine metabolism and Kangra valley of Himachal Pradesh noted as goitrous belt, the present study was undertaken to determine the contents of glucosinolates in commonly consumed vegetables.

A dietary survey was conducted in Ghuggar, Lona-chowki and Arla villages of Kangra district, Himachal Pradesh with respect to frequently consumed vegetables. The vegetable samples were thoroughly washed, cut into small pieces, dried, ground (1 mm) and defatted using petroleum ether. A comparison was made for the glucosinolate contents in fresh and dried vegetables and between fresh and defatted samples. Glucosinolate contents were determined[3] with some modifications. The method is based upon the reaction of excess silver with glucosinolates and the determination of unreacted silver volumetrically with a standard thiocyanate solution using ferric ammonium sulphate as indicator[4]. During standardisation of the method, it was found that the volume of alcohol (during refluxing) had an insignificant effect on the end point.

Dietary survey revealed that the mustard and fenugreek leaves and cabbage, cauliflower and turnip were frequently used. Production and consumption of knol-khol was sparse, while mustard seeds were used in small quantities, but quite often. Glucosinolate contents of commonly grown and consumed vegetables in Kangra valley, on fresh and

TABLE 1. GLUCOSINOLATE CONTENTS OF LOCALLY GROWN AND CONSUMED VEGETABLES OF KANGRA VALLEY, HIMACHAL PRADESH

	Moisture content (%)	% Glucosinolate content	
		Fresh basis	DM basis
Brassica oleracea (Var. Capitata), Cabbage	91.9	0.7	8.3
Brassica oleracea (Var. Botrytis), Cauliflower	90.8	1.7	18.2
Brassica oleracea (Var. Caulorapa), Knol-khol	92.7	0.6	8.7
Trigonella foenumgraecum, Methi	86.1	1.9	13.9
Brassica compestris (Var. Sarson), Mustard leaves	89.8	2.7	26.3
Brassica nnapa Turnip	91.6	1.2	13.9
Brassica nigra Mustard seeds	8.5	3.1	3.4

The glucosinolate contents of dried and defatted turnip, cauliflower and knol-khol were 18.2, 5.7 and 8.7%, respectively.

dry matter basis are presented in Table 1. The moisture content of all the vegetables ranged from 86.1 to 91.9%. On fresh matter basis, glucosinolate content was found to be highest in mustard seeds (3.1%), followed by mustard leaves (2.7%) and fenugreek leaves (1.9%). In cauliflower, turnip, cabbage and knol-khol the values were 1.7, 1.2, 0.7 and 0.6% respectively. On dry matter basis, glucosinolate content was highest in mustard leaves (26.3%) followed by cauliflower (18.2%). Concentrations of glucosinolates in turnip and fenugreek were similar. For knol-khol and cabbage, the values were 8.7 and 8.2% respectively.

In 22 varieties of Brussels, glucosinolate contents were reported to range between 90 and 390 mg/100g DM[5]. The reported glucosinolate contents in cabbage (0.03-0.13%)[6] and in turnip (0.08-0.20%)[7] are on the higher side than observed in the present study which could be due to the genetic and/or agro-climatic differences. Direct comparisons for other vegetables were not possible for want of literature.

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Efficacy of Food Preservatives in the Control of Penitrem-B Production by *Penicillium aurantiogriseum*

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Different food preservatives were screened for their efficacy in the control of penitrem-B production by *Penicillium aurantiogriseum*. Acetone, sodium metabisulphate and propionic acid were found to be effective in the control of penitrem-B production by *P. aurantiogriseum*. Rest of food preservatives were ineffective in checking the production of penitrem-B

In recent times, use of food additives to prevent spoilage of food and feedstuffs has increased considerably. The efficacy of certain food preservatives in the control of *Aspergillus flavus* proliferation and aflatoxin production in different substances has been reported by various workers[1-2]. Similarly, different food preservatives have been used in preventing mould infestation during storage[3-5]. The utility of different food preservatives in checking the growth of *Penicillium citrinum*, *Aspergillus terreus* and *Penicillium griseofulvum* and citrinin, patulin and cyclopiazonic acid (CPA) production, respectively, have been reported[6-8]. Penitrems which are reported to have a wide range of tremorgenic activity are responsible for various neurological disorders in man and animal[9-10]. Hence,

in the present investigation, efficacy of different food preservatives in preventing growth and penitrem-B production by *P.aurantiogriseum* was studied.

Monosporic cultures of *Penicillium aurantiogriseum* isolated from stored rice fodder maintained on PDA (potato dextrose agar) medium were employed in the present investigation. Fifty ml of Richard's Medium (KNO_3 10 g; KH_2PO_4 1 g; $MgSO_4$ $7H_2O$ 2.5 g; Sucrose 35 g; $FeCl_2$ traces, distilled water 1000 ml, pH 5.5) contained in 250 ml Erlenmayer conical flasks was sterilized at 1 kg/cm^2 pressure for 30 min. Different food preservatives (Table 1) were prepared freshly and added aseptically to the flasks before inoculation of the fungus and incubated at 27-29°C for 15 days. At the end of incubation period, cultures were harvested on previously dried and weighed Whatman filter paper No.42 for determining the growth of the fungus. The pH of culture filtrates was also recorded. For the extraction of penitrem-B, mycelial mat was employed. Known quantity of dry mycelium was extracted with diethyl ether in Soxhlet for penitrem-B and estimated as suggested by Hou *et al*[11].

Table 1 reveals that the food preservatives inhibited the growth and penitrem-B production by *P. aurantiogriseum* which, however, varied with the compound. Propionic acid, acetone and sodium metabisulphate were also responsible for total inhibition of penitrem-B production and growth. Similarly, Krishna Reddy and Reddy[8] have reported the inhibitory effect of propionic acid and sodium metabisulphate on the production of CPA by *P. griseofulvum*. The use of boric acid (50-200 mg), benzoic acid (5-40 mg), crystal violet (0.1 to 1.0 ml), sodium acetate (50-200 mg) and P-aminobenzoic acid (25-100 mg) in 50 ml medium resulted in the production of about 4 mg penitrem-B per gm of dry biomass and growth to the extent of 8 mg dry biomass per ml. Potassium metabisulphate and citric acid were effective only at higher concentrations. pH changes were minimum wherever mycelial growth was less or absent, while in the rest of the media, the final pH was near neutral.

TABLE 1. EFFECT OF FOOD PRESERVATIVES ON GROWTH AND PENITREM-B PRODUCTION BY *P. AURANTIOGRISEUM*

Name of the compound	Concentration (mg/ml)	Final pH	Dry Wt. (mg/ml)	Penitrem-B (mg/gm)
Potassium meta-	25	6.0	6.1	3.8
bisulphate	50	7.5	5.8	3.5
	100	7.5	5.0	2.6
Sodium chloride	50	7.0	7.9	4.0
	100	7.0	7.0	3.9
	200	7.0	6.5	3.1
Citric acid	200	4.5	7.1	2.9
	400	4.5	6.0	2.3
	800	4.5	4.8	1.0
Control	-	7.0	8.8	4.1

From the present investigations, it can be concluded that propionic acid, acetone and sodium metabisulphate are effective inhibitors of growth and penitrem-B production by *P. aurantiogriseum* and can be used for preventing growth of *P.aurantiogriseum* in fodders.

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Studies on Hydroxy Methyl Furfural Formation During Storage of Honey

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The changes in hydroxy methyl furfural (HMF) during honey storage and utility of Fiehe and aniline chloride tests to detect low levels of HMF were studied. Usefulness of parameters like fructose/dextrose ratio, acidity and organoleptic quality as possible indices of honey deterioration has been reported. Formation of HMF appears to be auto-catalytic.

Adulteration of honey by acid hydrolysed sucrose is detectable by aniline chloride and Fiehe tests[1]. However, sugar and saccharine are known to develop HMF on heating, storage and treatment with acids[2-4]. Hence, even a genuine sample of honey can contain HMF which, in requisite amounts, may respond to the above tests. The Codex Alimentarius Commission of FAO/WHO has recommended that the concentration of HMF in honey should be below 40 ppm[5] to check old, badly stored and adulterated (with invert sugar) honey. In the present study, formation of HMF on storage and limit of detection of HMF - to serve as limit tests - in honey have been investigated.

The samples received from the Central Bee Research Institute, Pune, in sealed tins were thoroughly mixed, divided into two parts in glass stoppered bottles and stored at room temperature (20-31°C) and at 37°C. Aniline chloride and Fiehe tests[1], analyses of HMF[6,7] and other parameters[1] were done.

The composition of honey samples at the beginning of the storage is given in Table 1. A progressive increase of HMF on storage was noticed (Table 2), the rate of increase being more at higher temperature. Initial HMF was found to be appreciably correlated with those after 12 months storage (corr. coefficients being 0.78 and 0.74 at room temperature and 37°C, respectively). The correlation and progressively higher and higher rates of formation imply that formation of HMF is auto-catalytic.

Positive Fiehe and aniline chloride tests indicate that HMF concentration is not less than 70 ppm and 210 or more, respectively (Table 3). Thus, these can serve as semi-quantitative limit tests. It may be possible to reduce the limit to 40 ppm, the CAC recommended limit, by performing Fiehe test in the form of rapid TLC (microscope slide or mini column).

The factors responsible for varying degrees of colour and viscosity could not be identified on enquiry from the supplier of the samples. The colour and viscosity were presumably contributed to some extent by the flowers and some sort of heat treatment during manufacture. The effect of heat treatment on HMF promotion in honey is positively correlated. The fructose/dextrose ratio does change during storage and thus, has limited or corroborative

TABLE 1. INITIAL CHARACTERISTICS OF HONEY SAMPLES

Sample No.	Description	Moisture %	Total** ash %	Total reducing sugars %	Sucrose %	Fructose/ Dextrose ratio	Acidity as formic acid %	HMF ppm
1	Straw yellow colour, low viscosity	19.0	0.3	75.1	0.9	1.2	0.1	Nil
2	"	20.6	0.1	72.4	1.2	1.1	0.1	2.5
3	"	19.4	0.1	73.3	1.2	1.2	0.1	8.8
4	Reddish brown colour, medium viscosity	19.4	0.3	74.7	0.8	1.1	0.1	Nil
5	"	16.0	0.2	73.3	1.9	1.1	0.1	2.5
6	"	18.0	0.3	72.2	2.3	1.3	0.1	3.7
7	Dark brown colour, high viscosity	17.4	0.5	73.9	1.4	1.1	0.2	1.5
8	"	23.4	0.4	65.5	6.2	1.0	0.2	4.1
9	"	18.6	0.6	68.6	1.1	1.2	0.3	12.0

* Fiehe and aniline chloride tests were found negative in all the samples.
** Ash insoluble in dilute HCl was nil in all cases except 0.02% in sample No.9

TABLE 2. HMF (ppm) IN HONEY SAMPLES UNDER STORAGE AT ROOM TEMPERATURE (20-31)°C AND AT 37°C**

Sample No.	Storage period months				
	0	3	6	9	12
1	Nil	0.8 (12.0)	1.0 (34.0)	2.5 (61.0)	4.6 (136.0)
2	2.5	9.8 (49.0)	12.2 (123.0)	25.0 (252.0)	35.0 (430.0)
3	8.8	13.5 (212.0)	17.0 (355.0)	27.5 (450.0)	46.0 (685.0)
4	Nil	0.9 (13.0)	1.1 (43.0)	2.8 (93.0)	6.4 (165.0)
5	2.5	12.8 (66.0)	18.0 (135.0)	28.0 (300.0)	37.0 (507.5)
6	3.7	15.0 (256.0)	22.0 (427.0)	36.0 (465.0)	47.5 (690.0)
7	1.5	4.2 (88.0)	7.1 (140.0)	9.9 (220.0)	16.5 (320.0)
8	4.1	13.0 (116.0)	22.5 (306.0)	58.0 (640.0)	70.0 (1010.0)
9	12.0	46.0 (598.0)	70.5 (775.0)	100.0 (835.0)	120.0 (1150.0)

* Description of the sample 1-9 is as in Table 1.
** Values at 37°C are in parenthesis.

TABLE 3. HMF (ppm) FIEHE AND ANILINE CHLORIDE TESTS VALUES OF HONEY SAMPLES

Samples analysed	HMF, ppm	Fiehe test	Aniline chloride test
13	0-66.0	All (-)ve	All (-)ve
1	68.0	Trace (+)ve	(-)ve
6	70-200	All (+)ve	(-)ve
1	205	Highly (+)ve	Trace (+)ve
5	210-775	Highly (+)ve	(+)ve
2	1010 & 1150	Highly (+)ve	Highly (+)ve

evidence for the age of honey. Similarly, acidity and organoleptic properties (smell, taste and colour) do not change perceptibly or appreciably (Results not shown).

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BOOK REVIEWS

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Microbiology Applications in Food Biotechnology: Ed. by B.H. Nga, and Y.K. Lee : Elsevier Science Publishers Ltd, Crown House, Linton Road, Barking, Essex IG11 8JU, England; 1990; pp 232; Price: £ 31.00

The book published under the broad category of microbiology applications in food biotechnology records the proceedings of the Second Congress of the Singapore Society for Microbiology, held for four days during October - November 1989 in Singapore. The timeliness of the holding of this congress and the publication of the proceedings speak for the thoughtfulness and the efforts put up by the Congress organisers, editors and publisher, especially at the time when the food biotechnology is forging ahead with rapid developments of both scientific and industrial significances. The book consists of sixteen papers dealing with current advances in molecular genetics, production of useful biomolecules and biotechnology of microbial enzymes. It also provides details of various aspects such as food standards, food safety, rapid methods of characterizing microorganisms, microbial criteria, etc. All these papers are not strictly related to each other; but this non-cohesiveness appears to be intentional for covering novel and exciting ideas as well as the new development in the field of food biotechnology. Afterall, the subject itself is multidisciplinary and of the magnitude without boundaries.

The first five papers deal with the successful genetic improvements of microorganisms such as *Saccharomyces cerevisiae, Bacillus pumilus* IPO, *Corynebacteria* and *Yarrowia lipolytica,* all of which have definite roles in food biotechnology. These improvements were made to enhance the production of metabolites such as xylanase, B-xylosidase, proteases and threonine. Various principles such as mitotic segregations in heterogenous hybrids, molecular cloning, chromosome engineering, sub-cloning, deletion, inversion, reciprocal recombination, promoter-probe vectors, multiple chromosomal integrations, autonomous replicating plasmids, protoplasm fusion, intergenic crossing and sequencing have been covered systematically with aspects such as expression of the foreign gene. The authors have given critical accounts from scientific and practical points of view. These papers collectively would serve as an excellent guide and base for researchers and students.

The paper on mass cultivation of microalgae, their genetic manipulations and the improvements in the technology by Y.K. Lee, gives an excellent and concise compilation of the products from these unique biotechnological tools. The details on bioreactor design, diurnal light cycle and light energy conservation efficiency are useful for technological exploitation. The paper on biotechnology of enzymes and pigments is a master-piece, a quality apparent in most other works of A.L. Demain and coworkers. It reports the unusual properties of cellulase elaborated by *Clostridium thermocellum* under submerged fermentation. The parameters for optimal production of the pigment by *Monascus* spp. in chemically defined media have been dealt with critically. This paper is a mirror of the trends in production and utilization of new enzymes and natural colours in food biotechnology.

The topic on microbial production of acrylamide, an industrially important chemical produced from nitriles through the action of nitrile hydratase of *Pseudomonas chlororaphis* B23, has been presented critically by yet another excellent team of researchers from Japan, H. Yamada and T. Nagasawa. In addition, the aspects on nicotinamide synthesis by *Rhodococcus rhodochrous* J1 are cited. This chapter highlights the role of bioconversion processes in food biotechnology and also indicates as to how these technologies can find applications in totally unrelated industries such as petrochemical one. It stresses the future promises of biotechnology for producing fine and commodity chemicals.

Javelot and co-workers have critically discussed the theoretical and practical aspects of the biosynthesis of monoterpenes by ergosterol auxotrophs of *Saccharomyces cerevisiae* for production of geraniol and linalool. Another mutant with ability to practically suppress ergosterol auxotrophy and prevention of reversion as well as the above auxotrophs were shown to produce a strong muscatel-like aroma during the fermentation and thus, show promises for imparting novel flavours to fermented beverages.

The theme that the new drugs of the space-age will emerge from the seas and the oceans is substantiated by Halevy by giving a number of examples in his critical review, thereby stressing the value of this richest known source which covers about 70% of the land surfaces. It is stressed that the marine bioactive agents are more compatible with terrestrial animals, than those from terrestrial plants and microbes due to phylogenetic relation existing between marine animals and their terrestrial offsprings. An elaborate description of the origin of marine animals and marine algae and their use in the synthesis of variety of bioactive molecules with antibiotic, antiviral, antifungal, antipyral, antitumour and immunomodulating activities have been cited. The objective for inclusion of this paper is probably to stress the microbiological applications in health-care of animals which form the source of meat and meat products. It may further stimulate interest for developing processes for production of biomolecules useful in food processing. The role of intestinal flora in health with

emphasis on their control by dietary components forms a subject matter of the paper by T. Mitsuoka. The aspects on the role of vitamins C and E, dietary fibre, bifidobacteria and fermented milk in preventing various harmful diseases, liver and kidney disorders, atherosclerosis etc., are discussed vividly. Improvements in intestinal flora due to dietary intake of oligosaccharides like raffinose and soybean oligosaccharides has been suggested. The whole paper is interesting to microbiologists/immunologists and opens an exciting field of research and development.

The critical review on food regulation and safety by J.H.B. Christian as well as on microbiological criteria in regulatory standards by R.G. Bell and C.O. Gill define the substances that make foods unsafe and give the methods to prevent these hazards. The reasons and rhetoric for formulating microbiological criteria make a mind - provoking reading. The paper on safety assessment of genetic manipulations of microorganisms and plants, as applied to foods, by J.E. Smith gives an account which can be adopted by all those engaged in food biotechnology. The foodborne outbreaks of Salmonellosis are recurrent phenomena in many countries. An exhaustive and critical account on the organism, its occurrence and prevention in foods by W. Budnik will be highly useful to those dealing with these aspects. The last paper on the application of DNA probes in food microbiology by K.H. Schleifer reports the details of this rapid approach for detecting microbial contamination of foods, especially by the foodborne pathogens. A number of examples are given along with the discussion on the non-isotopic test for detection of *Listeria* and the account of future developments.

Except for a few typographical errors, this book certainly would serve as an excellent source of information to researchers and students of like - interests, since the book covers a wide range of topics of interests to microbiologists, molecular biologists, nutritionists, environmentalists and food processers. It will be an added asset to any library catering to the demands of advanced information by these specialists dealing with food biotechnology.

B.K. LONSANE AND
SRIRAM PADMANABHAN
C.F.T.R.I., MYSORE

Principles and Practices of Solar Energy, by C.P. Ananta Krishnan and D. Sethu Rao, Sri Lakshmi Publications, Madras; 1991; pp:221; Rs. 125/-

Usefulness of solar energy in various fields has been well recognised and development of this subject in different fields of application is enormous and fast. Presenting these vast developments in a concise form is undoubtedly a hard task, if not impossible.

The book on "Principles and Practices of Solar Energy" has been basically written for beginners to make them aware and interested in this area. It deals with different aspects of solar energy utilisation including some basic definition and explanation of the terminologies. The chapter on solar radiation discusses the solar physics, some relevant solar radiation data and the measuring instruments including some worked out examples which would be very helpful in designing the solar devices. Next chapter on solar collectors encompasses a concise discussion on the application, important constructional features of different types of collectors, comparison of principle types of collectors and possible trouble-shooting which are essentially good guidelines. The chapter on solar thermal storage briefly discusses the different terms of storage systems where non-convective solar pond storage has been elaborately explained. The next chapter on physics of optical surfaces, however, lacks thoroughness as properties of optical surfaces in a Solar collector are very important in designing and these should have been appropriately dealt with. The chapter on solar energy into electricity is altogether an another aspect of application, wherein solar cell technology has been briefly incorporated. Some fundamentals on semi-conductors and photovoltaic effects necessitate in understanding the solar cell functioning and efficiency have been included. Section on factors affecting solar cell efficiency may be a helpful topic for the beginners. The chapter on applications of solar energy is too concise which should have been dealt otherwise, giving more about the designs of some specific systems. Nevertheless, to begin with, it would be obviously beneficial to understand the potentiality of this renewable energy sources. The chapter on indirect solar energy describes other forms of energy namely, wind, tidal, ocean, thermal and biomass which individually is a big subject. The authors could devote discussion on the solar energy in somewhat more detail which have not been or insufficiently covered in this book. In the last chapter on solar energy and environment, the authors have envisaged to highlight the relationship among utilisation of different types of energy and the environmental pollution which have not been properly organised to focus appropriately.

In general, the present book may be well suited for any person desiring to know some preliminary about solar energy. This entire volume, however, does not contain any reference. The figures presented in the book are illegible and far from the standard. The price of the book is within the reach of the students.

S.K. DAS
I.I.T., KHARAGPUR

Food Technology in the Year 2000 (Bibliotheca Nutritio et Dieta, No. 47, (Series Editor J.C. Somogyi), Volume Editors: S. Lindroth and S.S.I. Ryynanen, Copy right 1990 by Karger, A G., P.O.Box, CH-4009 Basel (Switzerland), pp. 122, Price: £ 5740 or US $ 105.75

It was a pleasure for me to go through the book which is the outcome of the International minisymposium "Food Technology in the year 2000" held on November 14, 1989 at Helsinki, Finland. This report comprises of nine invited papers on various aspects of food technology in Western Europe, USA and Japan.

This book begins with an introduction of welcome speech by J. Kuusi of Finland, which briefly illustrates the need for national and international R & D activities in the field of food technology.

Papers 1,2 and 3 describe various developments in food technology in Finland. Paper 1 presented by E.H. Koshinen deals with the technological innovations taking place in Finland and future needs for the possible research priorities in food technology by collaboration between universities and research institutes as well as with industry. It was also pointed out that priority should be given for international cooperation. Paper 2 presented by T.M. Enari deals with the topic "New opportunities in food technology created by biotechnology". It covers the role of biotechnology in the development of agricultural products used as feed stock in the food industry, industrial production methods, analytical procedure, quality control and new food products. A comprehensive review of the various recently developed biological methods in terms of technological advances has been illustrated.

Paper 3 presented by E. Nurmi and J. Him on the topic "Integration of Hygiene into Food Technology" provides, in detail, the work carried out on various aspects of hygiene and sanitation in raw as well as processed food products at National Veterinary Institute, Helsinki. Some predictions on the changes in food processing methods and consumers, acceptability for food products in the year 2000, have been projected. Some recommendations for the food processing industry have also been made by the authors to avoid any microbiological spoilage.

Fourth paper presented by T. Sasaki, concerns the role of Japanese Agriculture, Forestry and Fisheries Research Council Secretariat in assisting the food industry to maintain and improve its competitive edge in the year 2000. This paper gives in detail the changing scenario of various aspects of food technological research in Japan. Information on the joint activities of different research organisations (Government - Industry - University) engaged in food technological research in Japan, has been given. It also gives future plan of research work in these sectors to be carried out in the 21st century in Japan.

Fifth paper presented by M. Solberg and J.L. Rossen, of the Centre for Advanced Food Technology, Rutgers, USA, reviews the importance of coordinated efforts of University, Industry and Government in the development of food technology in the year 2000. It deals with the CAFT model which has been successful in achieving the ultimate goal of all the three organisations by way of cooperation.

In sixth paper, attempts have been made by D. Arthey to review the subject matter "The Challenge of the Year 2000 for Food Technological Research in an Industry-based Research Association in UK". It covers, in detail, the activities of Campden Food and Drink Research Association in UK. It is stated that the primary aims of the Research Association are to carry out research in cooperation with government and industry and provide services including training in processing efficiency, product safety and product quality by a team of trained personnel.

Eighth paper presented by J. Olkku of Finland on the topic "The Capability of the Food Industry in a small Country to Utilize Technological Innovations" enlightens the problems of food industry in a small country. Various ways and means have also been suggested to improve the existing food industries in the country. It is indicated that international cooperation can partly solve the problems faced by food industry in a small country like Finland.

Ninth paper presented by W.E.L. Spiess, of FRG on "Technological Development in the Food Industry in the Outgoing 20th Century", covers the important factors (social changes, ecological changes, new approach towards nutrition and changes in raw materials) which may, in one way or other, contribute to changes in the food industry in the year to come in Germany. Various possibiltics towards changing the food industry pattern in Germany, have been explored, keeping in view the technological innovations in the related scientific fields.

The summary remarks on the above papers have been presented by S. Lindroth of Helsinki, Finland, in the last chaper of the book. The outcome of each paper has been briefly indicated in a concluding manner.

The editors have done a commendable job in compiling these different papers and bringing out this volume. The subject matter discussed here is pertinent to all food scientists and technologists, and industrialists too. The contents are well organised, easy to read and are supplemented with 18 figures and 17 tables, indicating the future possibilities of food technological research in the years to come. The new ideas projected in the text will definitely stimulate much research in the field. It will be a good addition to libraries of research institutes and universities. The editors and the publishers are thanked for bringing us a very resourceful book.

L.P. RAJPUT
JNKVV, JABALPUR, (M.P.)

Bioinstrumentation and Biosensors: by Donald L. Wise (Ed) published by Marcel Dekker, Inc., New York, 1991, pp 824, bound, illustrated. Price $ 165 (U.S. and Canada) and $ 189.75 (all other countries).

The book is envisaged to be an important reference text in the newly emerging field of instrumentation, coupling modern biotechnology and advanced electronics. There are 25 contributed chapters by experts directly carrying out research and development in this new field of bioinstrumentations and biosensors. The chapters can be broadly divided into three types (1) biosensors based on immobilised enzymes and microbes (2) new medical diagnostic instruments based on magnetic, ultrasonic and electro-chemical methods and (3) computer modelling.

The use of microbes as sensors for online measurements of acetic acid, alcohol, glutamic acid, ammonia etc. has been described in chapter 1. The assimilation of the substrate by specific microorganisms immobilised in membranes results in decrease of dissolved oxygen reflecting in the current decrease of the oxygen electrode till it reaches steady state. The time taken for the analysis is of the order of 15-20 minutes. Determination of cell number by potentiometric method with sufficient sensitivity for practical applications has also been described.

The combination of the action of enzymes immobilised physically or covalently on a membrane or gel matrix with polarographic, potentiometric or conductometric electrodes for the analysis of a host of substrates is surveyed in detail in chapters 2,9,12,19 and 22 with ample illustrations. The developments which have been taken place in different methods of immobilizations, configurations of bio-catalytic electrodes, selectivity and sensitivity of these electrodes have been dealt with in these chapters extensively.

The chapter on fish freshness with a biosensor system is based on the analysis of substrates on decomposition of ATP to ADP, AMP and related compounds in fish meat. Inosine and hypoxanthine are accumulated with increased storage of time. The freshness indicator is governed by the ratio of inosine + hypoxanthine to total amount of ATP related products, the individual constituents determined by sequential analysis using specific bio-catalytic electrodes. Mention has also been made to determine freshness based on amines and glucose content of fish meat and blood. There is also a mention about the development of an enzyme strip test for the hypoxanthine assay which made the estimation of hypoxanthine in fish rapid and simple on a visual basis.

The human skeletal motor system functions normally to control posture and movement. Understanding the mechanisms of a muscle-load oscillations is aided by study of the parts of the system producing oscillation. A particular tremor of a limb may be mechanical resonance oscillation, it may arise from the rhythmical bursting of neurons, it may arise from within the muscle itself or it may involve an interaction between either the muscle or the muscle load system and CNS. Chapter 4 reviews the findings and hypothesis of many workers regarding both voluntary and involuntary muscle-load oscillations, including the authors' work in the field. The subject has been dealt with clarity and forethought.

The development of rare earth magnets with large magnetic force has made it possible to use tiny magnets in dental prosthesis. Chapter 6 describes the materials, configurations, magnetic forces and use of magnetic devices in dentistry and some clinical cases in which the devices are used, are given.

Increased intracranial pressure (ICP) is a life threatening situation in which early intervention can be of great importance if detected in time. Raised ICP may be due to head trauma, hydrocephalus, brain tumour, intracerebral haemorrhage or cerebral oedema. A variety of non-invasive techniques have been used to measure ICP, like changes in skull diameter, changes in anterior fontanelle pressure and changes in tymphanic membrane tension. Changes in evoked potentials using standard electroencephalogram recording electrodes by signal averaging techniques is non-invasive. Chapter 7 describes the use of sensory stimuli like auditory, visual and somatosensory evoked potentials in relation to changes in the wave form latency or amplitude with elevations in ICP. Visual evoked potential offers good promise as a non-invasive technique giving reliable estimates of ICP and be of use in diagnosis and management of raised ICP as a consequence of a variety of etiologies.

Chapters 23 and 24 also deal with systems for monitoring brain functions, effect of visual stimulation or body equilibrium and automated monitoring and interpretation of sensory evoked potentials. The development of new type of EEG with evoked potentials, processing and display techniques is highly developed, yielding information on the brain functions hitherto not available. The opto-kinetic stimulation reflects the brainstem oculomotor region and reveals the integration function of eye movement and of body sway reaction.

In chapter 10, the author presents the basic methods developed for continuous blood gas monitoring. The principles of invasive and non-invasive monitoring of mixed venous oxygen saturation, arterial oxygen saturation, oxygen tension, pH and carbon dioxide tension using electrochemistry, spectrophotometry, gas chromatography and mass-spectrometry techniques are briefly reviewed.

The chapter on electronically simulated hearing loss and the perception of degraded speech describes the potential usefulness of a realistic simulation of hearing loss for an increased understanding of the origin of perceptual problems of the hearing impaired.

Chapters in parallel information processing biological system, from photo transactions to neural networks; modelling and identifications of lung parameters; and characterisation of conduction properties of nerves with the distribution of fibre conductions velocities deal with mathematical modelling of the different biosystems for information and understanding.

The chapter on simultaneous analysis of serum uremic toxins and ionic compounds by chromatography using an immobilised enzyme column combined with a column switching method has made it difficult to attain simultaneous determination of these uremic toxins and electrolytes possible.

The application of radiation pressure and force in an ultrasonic field has found application in separation of blood and it is described in detail in chapter 15.

Magnetic resonance imaging (MRI) is rapidly becoming accepted as a valuable diagnostic tool in modern radiology. Chapter 14 deals exhaustively with the use of this technique in atheroscoerosis and breast lesions.

Chapter 17 deals with non-contact temperature measurements in medicine. The human body is a very efficient thermal radiator emitting about 6 to 8 mW from every square centimeter of exposed skin under normal conditions. That lost energy can be utilized to determine the surface temperature without touching the body. The chapter gives a basic understanding of the principles of thermal radiation, definitions, theory, interrelations, estimation and methods of instrumentation design and deals exhaustively on the latest developments in the field.

All the chapters in the book have given extensive references which will be very useful for the researcher in the emerging fields described in the book.

It has been a formidable task to review a book of this nature as it touches a broad spectrum of specialised fields. I completely agree with the editor of the book that the text will be extremely valuable for persons working in the areas of modern biotechnology, advanced electronics and instrumentation. The publishers, Marcel Dekker, Inc., should be complimented to bring out a book of this nature.

C.S. NARAYANAN
R.R.L. TRIVANDRUM

Protein Immobilization: Fundamentals and Applications. by R.F. Taylor, (Ed) Marcel Dekker, Inc., New York, 1991, pp. 392, Price: $ 100 (US and Canada), $ 126.50 (all other countries). This is the 14th volume of a series of publications on Bioprocess Technology. Series editor is W.C. McGreger.

A decade or two ago, protein immobilization almost meant immobilization of enzymes. However, now, in addition to enzymes, a wide variety of proteins including antibodies, structural proteins, binding proteins, receptors, etc., are immobilized. Immobilization of proteins may be considered as a good example of application-oriented basic research which has resulted in a number of successful commercial products useful in food and drug industries, environment monitoring, and diagnostic and therapeutic agents in medicine. Appropriately, this book deals with both these basic problems of protein immobilization and the development of technologies for application.

In all, the book contains 11 chapters. The introductory chapter (chapter 1) outlined by the editor spells out the importance of protein immobilization technology at the present context. The remaining 10 chapters are grouped under two parts. Part I consists of four chapters, each one dealing with the basic aspect of protein immobilization. The immobilization techniques are conveniently classified into non-covalent immobilization techniques which include adsorption, aggregation, entrapment and micro-encapsulation (chapter 2) and covalent methods (chapter 3). Almost all the immobilization methods have been clearly described with specific examples. However, new-comers to the field should be cautioned that these are not standard procedures applicable to any protein. Chapter 4 gives information on commercially available support materials for protein immobilization, which many books of similar type either fail to provide or hide it somewhere in the middle of text. Characterization of immobilized proteins, which again in many books on immobilization is not adequately covered, is well described in chapter 5. Chapters under Part II mainly deal with the application aspects of immobilized proteins. Chapter 6 on immobilized enzyme reactions in organic solvent medium, an apparently new application of enzyme for catalysis is very well presented. Yet another new field wherein immobilized proteins find wider application is that of biosensor. The use of immobilized enzyme in the biosensor field is elegantly described by G.G. Guilbault and colleagues in chapter 7, while immobilized antibodies and receptors for biosensor is dealt separately in chapter 8. Therapeutic use of immobilized protein in the medical field is described in chapter 9. Immobilization of microbial and animal cells are discussed in chapter 10. Lastly industrial applications of immobilized proteins are presented in chapter 11.

There is a fine mixture of industrial and academic contributors to the book. The contributors, in general, are distinguished groups in their respective specialized fields. In general, each chapter has a concise introduction to the subject and has a well surveyed up-to-date reference list, should the reader wish to pursue any topic further. On the negative side, a small book of this size should have restricted itself to the immobilization of proteins only (true to its title) instead of attempting to cover immobilization of whole cells (chapter 10) which, by itself, is a vast field. Immobilization and application of plant cells and subcellular organelles are not covered in this book.

Also, a repetition of immobilization techniques in chapters 7,8 and 10 should have been avoided as all immobilization techniques are elaborately dealt under chapters 3 and 4.

I found very few print-mistakes in the book. In a remarkably little space, this book manages to cover a lot of ground. It does represent a very up-to-date account of a commercially important field of research and technology development. Overall, the book is to be recommended for both beginners and people already working in the field. However, the price of $ 126 for pp. 392 might defer few individual buyers, but should be a worthy addition to libraries.

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Fish Processing Technology: by G.M. Hall, (Ed) Published by Blackie Academic & Professional, An imprint of Chapman & Hall, 1992, pp. 309. (Price £ 65/-)

Technological advances particularly in relation to preservation and processing have played a significant role in rapid development and utilisation of fishery resources. In recent years, there have been many exciting and innovative developments in the utilisation of economically unviable or the so called 'Trash fish' for the production of value-added products.

This book, edited by George M. Hall is an attempt in updating the knowledge of traditional areas of fish processing viz. curing, freezing, canning and fishery by-products and highlights important new areas such as membrane filtration. Besides providing an account of recent innovations such as mined fish, *surimi,* etc., efforts have been made to highlight the biochemical aspects of fish processing, rapid microbial methods and assessment of fresh fish quality.

The first chapter is an authoritative review on biochemical dynamics and quality of fresh and frozen fish and offers an updated information on biological condition and the role of lipids, proteins, pH, pigmentation, flavour compounds and minerals in governing fish quality and processability.

Preservation of fish by curing, including drying, salting, and smoking, is dealt in detail in chapter 2. Limiting the water activity for the growth of microorganisms is very important from the point of view of long term preservation of fish. Fundamentals of drying, salting and smoking processes have been discussed with mathematical expressions wherever necessary.

Chapter 3 deals with *surimi* and fish mince products. The success of *surimi* products depends on the process control and gel forming ability of proteins. An excellent overview of *surimi* and mince products is provided by the authors. The essential difference between *surimi* and fish mince, exploration of new species and consideration of mixed species for *surimi* production, the role of myofibrillar proteins, problems with high lipid *fishes,* application of cryoprotectants for preventing protein denaturation have been well presented.

The next three chapters are devoted to conventional areas of processing. Attention is focussed on modified atmospheric packaging and freezing. Mathematical models have been worked out for heat transfer during freezing process. A review of different methods of freezing and the changes in quality of chilled and frozen stored fish has been furnished. Quality criteria due to thermal processing and mathematical aspects of process lethality, D,L,Z and F values have been described lucidly. Many developments such as rigid plastic fishiness and their advantages and disadvantages have been clearly brought out. Production methods of various by-products have been covered with the aid of flow charts. However, the classification of *surimi,* as by-product, is debatable considering the enormous developments in this area. The details of *surimi* production process would have been more appropriate in chapter 3.

Lactic acid bacteria (LAB) are widely used in preservation and processing of foods. Marine LAB, their isolation, production of seafood fermented products using LAB cultures, inhibitory effects of LAB by production of bacteriocins and antibiotics, technological, biochemical and microbiological aspects of fish fermentation have been dealt in the chapter on application of lactic acid fermentations.

The chapter on membrane filtration for waste water protein recovery is a significant contribution towards our understanding the principles and application of membrane filtration in the treatment of waste waters from fish processing industries. Apart from recovery of proteins, the most likely application of membrane filtration is in the treatment of effluents. An insight to the theory and practice of this technique is furnished in chapter 8.

Functional properties are the overall physico-chemical behaviour of proteins in food systems during processing, storage and consumption. Chapter 9 is restricted to functional properties of fish protein hydrolysates and other byproducts. It would have been more appropriate to have dealt with the functional properties of products by different processing methods considering the nature of the book.

The last chapter deals with rapid microbial methods and fresh fish quality assessment. Assessment of quality of fresh fish by sensory, chemical and microbiological methods have been outlined. Alternative microbiological techniques such as impedimetric and conductimetric techniques have been extensively reviewed and compared with organoleptic quality.

A special feature of the book is a fair coverage of carefully chosen research literature at the end of every

chapter. This book provides a valuable and updated overview on various aspects of fish processing technology and will serve as a useful reference for students and academicians of fishery science, fish processing technology and those involved in the fish processing industry.

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Food Extrusion Science and Technology, by Jozef L. Kokini, Chi-Tang Ho and Mukund V. Karwe, (Eds) Marcel Dekker, Inc, 270, Madison Avenue, New York, 1992 pp: 760, Price $ 175.00 (US and Canada), $ 201.25 (all other countries)

Food extrusion is receiving utmost attention and their use dates back from 1930s. Single-screw extruders were first used to form and shape food products such as macaroni and ready-to-eat cereals. In these operations, relatively little heating or cooking was accomplished with the extruder. However, single-screw cooking extruders, having large electrical drive motors, were later developed in the 1940s for making puffed snacks from cereals. This extrusion consists of a thermo-mechanical processing operation in which the raw material is fed into a hopper and forced through the passage between a rotating screw and a stationary barrel. The processed material comes out through a die of specific shape. The high shear and temperature environment inside the screw channel resulted in mixing the material which lead to chemical reactions constituting the cooking process.

The book is divided into five sections. Part I covers transport phenomena during extrusion both for single and twin screw extrusions, effect of different extrusion parameters and the flow of both Newtonian and Non-Newtonian fluids under isothermal and non-isothermal conditions. The operations under partially filled conditions were also investigated. Transport phenomena in extruders were simulated to overcome the emperical approach and accordingly, it optimized the existing processes and also the development of new technologies. Product properties, which depend mostly on molecular transformations that are generated in various parts of extruders by temperature, pressure, shearing and residence time were studied. Residence time distribution, mass flow, mixing and numerical simulation of fluid flow and heat transfer in twin screw extruder were presented at length. The rates of reactions in extrusion processes, which are typically fast, were affected both by thermal and shear energy inputs. Good kinetic models are valuable for ensuring optimum product quality. Starch conversion kinetic model as well as water diffusivity in the extrusion cooking of starch materials were dealt with extensively. With investigations on moisture diffusion and gelatinization in extruded rice noodles, this part came to a close.

Part II deals with rheological properties of cereals during extrusion. It is difficult to characterize the rheological behaviour of food materials in an extruder because of the fact that the melt is typically Non-Newtonian. Its apparent viscosity is mostly a function comprising shear rate, temperature and moisture level. Various models based on integral and differential constitutive relations such as Convective Maxwell model, Leonov model, BKZ constitutive model and Bird-Carreau model, were discussed. On-line rheological measurements of food dough and experimental investigation of the rheological behaviour of rice flour water system with an extruder mounted with slit die viscometer were extensively presented. Rheology of starch-based materials and factors affecting the viscosity and structure of extrusion-cooked wheat starch were discussed in detail. It is also a common knowledge that pre-gelatinized starches are useful in a variety of prepared and convenience foods because of their relatively high viscosities at fairly low concentrations in products. The chapter ends with numerical models of cooking process with a reasonable hint that it is not yet possible to predict accurately the performance of a single-screw cooking extruder. However, the system of non-linear dimensional equations were solved using finite-element method and two/three - dimensional models were also presented to illustrate the potentiality of a numerical simulation of cooking extrusion.

Chemical and physico-chemical transformations during extrusion are presented in chapter III. The combination of sheer, temperature and pressure during extrusion processing created many opportunities for molecular transformations. The fragmentation of starch, is, however, fairly documented in this chapter. Changes in non-covalent bonding between carbohydrates, proteins and lipids have proved much more difficult to quantify. Much of the emphasis, therefore, will continue to blend methodology-development with the application of existing chemical properties. The twin screw extrusion cooker, as a bioreactor for starch processing, has demonstrated that cooking could be used in the thermo-mechanical gelatinisation and liquefaction of cereal starches and grains for the subsequent production of various starch-based syrups and ethanol. Other advantages for the production of syrups of high maltose content and simultaneous saccharification and fermentation for ethanol production were also highlighted. The other major topics discussed at length are: structural changes in biopolymers, denaturation and texturization, and application of extrusion cooking to dairy ingredients. Extensive coverage was given to lipid-protein, lipid-carbohydrate interactions, lipid binding during extrusion cooking and lipid oxidation in extruded products during storage as affected by extrusion temperature and selected anti-oxidants.

Chapter IV deals with the scale-up and control of extruders. Scale-up of food extruders deals with three distinct methods, such as common scale-up factors, scale-up for heat transfer and scale-up for mixing. In the first article, the author provided the data by actually performing the scale-up experiments. This topic is of paramount importance for food scientists/technologists to translate their laboratory data into commercial scale and for technology transfer. Other important articles include barrel and screw wear in Twin-screw cooker extruders when manufacturing breakfast cereals, effect of screw speed on the process dynamics and product properties taking a most specific example of corn meal and constant product quality by electronic process control through closed loop.

The concluding part bridges the gap between functionality and physical properties, chemistry and technology. Articles deal with, among others, systems approach to extrusion, rheological and process properties, operation variables on functionality, properties of expanded rice products, application of extrusion cooking to simulate traditional Korean foods, soybean texturization and processing parameters for various rice-based products.

This compilation of articles, contributed by specialists in the field of food extrusion, covers all aspects of extrusion cooking and is a useful compilation for graduate students, fundamental scientists and application-oriented R&D and industry personnel. Figures comprising sketches, drawings, illustration of profiles, operating principles, graphs are well presented and the get-up of the book is excellent. The editors of this compilation should be complimented for their painstaking efforts to bring the entire subject of extrusion cooking in the form of a book. The book will be of immense use to food/feed research institutions, universities and others interested, in general, food science and technology.

M.M. KRISHNAIAH
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Surimi Technology: (Food Science & Tech. Series 150) by Tyre C. Lanier and Chong M. Lee, (Eds) Marcel Dekker, Inc., New York, N.Y., 10016, 1992; pp. 554, Price $ 150 (U.S. and Canada), $ 172 (All other countries)

The word *surimi* was virtually unknown to the world outside Japan a decade ago. However, *surimi*, which is a minced form of fish meat, has been an important intermediate for several traditional Japanese products, especially 'Kamaboko' and was quietly undergoing changes in production methods and improvements in product quality during the past several hundred years. Two factors must have prompted the stormy entry of *surimi* into the Western world, especially U.S.A. There was a mounting need for profitable utilization of the abundant trawler by-catch, and also the marketable surplus of ever-increasing pelagic fish landings. Secondly, the advent of a new genre of processing machinery, the large-scale meat-bone separator in particular, and developments in newer processing techniques like the coating technology, have opened up avenues for fabrication of several novel products from minced fish meat. The crab analogue, for instance, exemplifies some of the popular delicacies manufactured from *surimi*.

Despite its close resemblance to meat sausage emulsion, *surimi* differs from meat mince, in that it is free from water solubles and lipids and subsequently dewatered to form a concentrate of the principal myofibrillar proteins, specifically myosin (or actomyosin). The chief quality attributes of *surimi* are dependent on the emulsifying and gel-forming properties of these proteins. These properties being largely species - dependent, and prone to changes during post-harvest handling and freezing, large-scale manufacture of *surimi* has become inbred with a multiplicity of scientific and technological hurdles, opening up of a new R&D area during the eighties.

The book under review is a joint effort of over 25 experts from both U.S.A. and Japan and covers the science and technology of *surimi* and its products in erudite detail. The authors have drawn information from nearly 750 references, a fact which bears testimony to the growing importance of this commodity on the one hand and to the thoroughness of the effort on the other. The volume contains 18 chapters grouped in 5 parts, covering the following aspects: (1) historical perspective and resources; (2) conventional technology using white fish; (3) newer technologies; (4) *surimi* - based products and (5) the science of *surimi*. The technology sections cover essential manufacturing details, complete with information on material handling, equipment and machinery, processing schedules, product testing methods and packaging. A chapter on the labelling requirements in the U.S. for *surimi* products indirectly tells the reader the various control measures needed in the manufacture and marketing of these products. Since freezing of *surimi* has become an essential pre-requisite to ensure uninterrupted supply of raw material, a full chapter is devoted to cryostabilization and the choice and application of cryoprotectants. New approaches like utilization of dark-fleshed species and the feasibility of dehydrating *surimi* have been described, so as to widen the scope for product development. Interestingly, the removal of water solubles and consequently the natural flavours have paved the way for incorporating exotic flavours of choice to the finished products. There is a detailed treatment on the interlink between rheological characteristics of *surimi* and the texture of the products made from it and how they are reflected in the respective microstructures of both. The molecular basis of conversion of sol to gel, the forces (bonds) which influence attributes like viscosity, elasticity and stability of the gel, the water-binding mechanisms

and the positive role played by additives, have been discussed using mathematical models. There is also a chapter on the microbiological aspects of *surimi*, and the application of H.A.C.C.P. concept in its production. It is however, surprising that, in such an exhaustive treatment on this commodity, this section appears to be disproportionately small. The source of information derived specifically on *surimi* microbiology is limited to just three publications. Perhaps, this is a case to be taken note of in future editions.

This is probably the first publication in book form fully devoted to the theory and practice of *surimi* and its products. It is gratifying to note that the editors and authors have carried out the task with diligence and objectivity. This volume is certainly a major contribution to the literature on aquatic foods.

R.B. NAIR,
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MYSORE

AFST (I) News

World Food Day was celebrated on 16th October, 1992 under the auspices of the AFST(I) Headquarters in the IFTTC Auditorium of CFTRI, Mysore. Dr. P. Narasimham, Vice-President, AFST(I) welcomed the gathering. Dr. S.R. Bhowmik, Director, CFTRI, presided. Dr. Rao N. Maturu, Former Food Standards Officer, Food Policy and Nutrition Division, FAO, Rome was the chief guest and delivered a talk on "Role of Food Quality and Standards in Food Security, Trade and Health".

Dr. M.N. Krishnamurthy, Hon. Exec. Secretary proposed a vote of thanks.

OBITUARY

We record with deep regret the passing away of Dr. S.P. Manjrekar at his residence in Srirangapatna near Mysore on 4th December 1992, following cardiac failure. Born on 28th July 1931, Srikant P Manjrekar had his early education in Bombay, leading to B.Sc(Tech) of Bombay university. He joined the faculty of the Department of Food Technology at the University of Bombay in the early fifties. Subsequently, he obtained his Ph.D. in Food Technology from the University of Kiel, West Germany. On his return, he continued his career in University of Bombay. Later, he became Professor and the Head of the Department of Food Technology at the Punjab Agricultural University, Ludhiana.

Dr. Manjrekar joined CFTRI in the year 1965 and became the Chairman of the International Food Technology Training Centre (IFTTC). Later, he was the Head of the Department of Microbiology and Sanitation, CFTRI, Mysore till his retirement in July 1991.

He was the Secretary of the Association of Food Scientists and Technologists(India) in the year 1970 and later its vice-president during the year 1989-90. He was also the Editor of Indian Journal of Microbiology, Published by the Association of Microbiologists of India during 1984-86. He was loved by all his colleagues and friends. His death is a great loss to the Scientific Community. AFST(I) joins the bereaved family in mourning his death.

SUBJECT INDEX
Vol. 29, 1992

AUTHOR INDEX
Vol. 29, 1992

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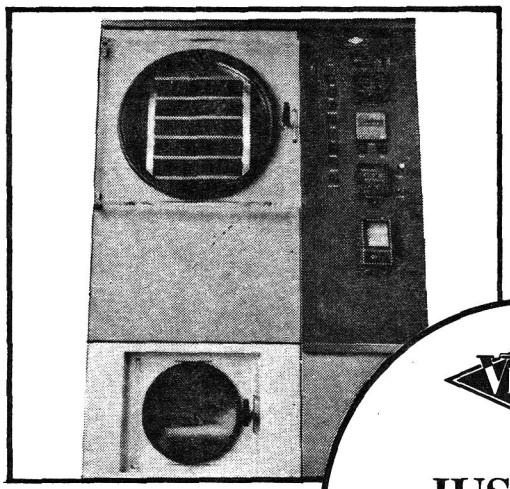

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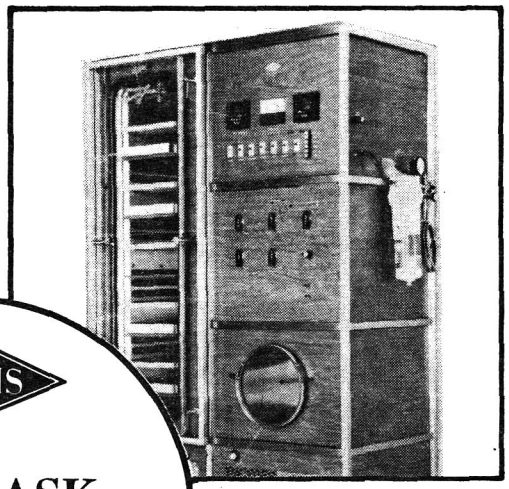

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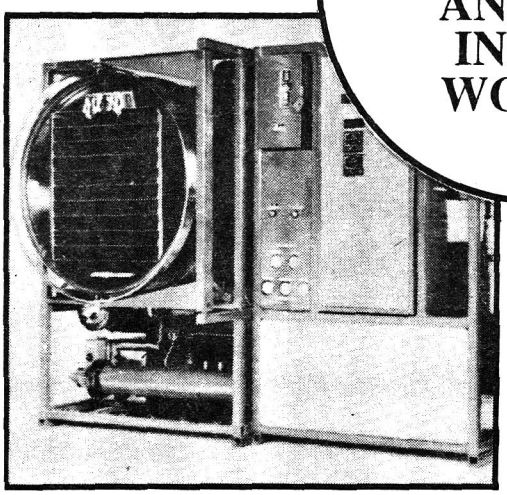

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Agnihothrudu, V. (1)
Alur, M.D. (3)
Ambekar, S.Y. (1)
Appu Rao, A.G. (2)
Arya, S.S. (3)
Azeemoddin, G. (1)

Balachandran R. (1)
Balagopalan, C. (2)
Balasubrahmanyam, N. (3)
Balasundaram, R. (1)
Basappa, S.C (4)
Bawa, A.S. (4)
Bhakare. H.A. (1)
Bhat, G.S. (2)
Bhat, S.G. (2)
Bhattacharya, D.K. (1)
Bhavanishankar, T.N. (2)

Chakraborthi, S.R. (3)
Chandrasekhara, N. (4)

Dalal, V.B. (1)
Deodhar, A.D. (3)
Deosthala, Y.G. (3)
Desai, B.B. (1)
Desikachar, H.S.R. (4)
Dhanaraj, S. (2)
Dhillon, B.S. (2)
Dhindsa, K.S. (3)
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Eipeson, W.E. (1)

Gangadhara, D.N. (1)
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Krishnakanth, T.P. (3)
Krishnamurthy, M.N. (2)

Laad, U.D. (2)
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Lodh, S.B. (2)

Mahadeviah, M. (1)
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Narang, A.S. (1)
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Narasimha Rao, D. (1)
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Nerkar, D.P. (3)
Nirankar Nath, (1)
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Ojha, T.P. (2)

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Patel, G.R. (1)
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Rajpoot, L.P. (6)
Ramakrishna, S.V. (1)
Ramamurthy, M.K. (3)
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Varadarajulu P. (3)
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Vasuki, H.K. (1)
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Venkataram, K. (1)
Venugopal, V. (1)
Vijaya Khader (1)
Vijaya Rao, D. (3)
Vijay Sethi (1)
Virupaksha, T.K. (1)
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Warrier, S.B. (1)

Zakia Banu (1)

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Bhagwat, A.S.
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Burma, D.P.

Chakrabarti, S.R.
Chattopadhaya, D.

Das, H.

Krishnaiah, M.M.
Krishnakumari, M.K.

Nair, R.B.
Narasimha Rao, D.

Padwal Desai, S.R.

Rajpoot, L.P.
Ramesh, A.
Rangaswamy, J.R.

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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 α–β–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.

(Continued on Page 400)

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Review

Research Papers

Research Notes

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