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Volume 21 Number 5 Sept./Oct. 1984

CONTENTS

Research Papers

-2 ก.ย. 2528
ห้องสมุดกรมวิทยาศาสตร์บริการ

Presence of an Inhibitory Factor to Gas Production by *Clostridium perfringens* in Husks of Chick Pea *(Cicer arietinum)*, Cow Pea *(Vigna sinensis)* and Horse Gram *(Dolichos biflorus)*

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Endosperm carbohydrate fractions of chick pea, cow pea and horse gram cause flatulence, while the husk and the derived carbohydrate fractions were inhibitory to gas formation by *Clostridium perfringens*. Addition of chick pea husk (15%) to a fermentable substrate containing glucose, or chick pea endosperm resulted in complete inhibition of gas production. Similarly the water, 70% ethanol, alkali and acid extracts of the husk from chick pea were also inhibitory due to the retardation of growth of the bacteria. Addition of manganese chloride and ferric chloride to the medium enhanced the gas formation. This suggests that the inhibitory effect may be attributed to phytates and/or phenolic acids present in the seed coat or to some other unknown factor.

Whole seeds are consumed in the case of certain legumes but in the majority of cases split seeds (*dhal*) are utilized. This is practised to improve palatability and to reduce the cooking time. The husk does not contribute much to nutrition but recent studies have indicated that dietary fibre, a constituent of husk plays an important part in nutrition.[1]

Many people are of the opinion that *dhal* produces digestive discomfort while consumption of whole seed reduces the heaviness of stomach and flatulence. It was thought desirable to study this aspect and to compare the relative gas forming and/or inhibiting ability of whole and dehusked pulses.

Materials and Methods

Husk of the three legumes, chick pea (*Cicer arietinum*), cow pea (*Vigna sinensis*) and horse gram (*Dolichos biflorus*) was removed by mild heat conditioning followed by abrasive milling. The whole grain, the dehusked grain (*dhal*) and the husk were powdered to 60-mesh. Various carbohydrate fractions were isolated from the husk and purified as detailed elsewhere[2,3]. *Clostridium perfringens* of intestinal origin was used for *in vitro* gas production studies as per the procedure mentioned previously[4].

The effect of the isolates from the husk was determined by incorporating them at 0.5 per cent level into the medium containing glucose or endosperm. In the next experiment, endosperm powder containing the husk at different levels (5-50 per cent) was used as substrate and the gas produced after 24 hr of incubation was determined. Similar studies were carried out with aqueous, alcoholic, acid or alkali extracts of husk. These extracts were obtained by refluxing the husk with 20 times its weight of water, 70 per cent ethanol or 1N NaOH at 96°C for 2 hr. The extracts were neutralized, concentrated and lyophilized.

In order to study the inhibitory effect of the husk and its fractions on gas production through inhibition of bacterial growth or change of metabolic pathway, concentration of glucose in the basal medium was determined, both before and after incubation by the glucose oxidase method[5]. Acidity of the medium was determined titrimetrically using 0.05N NaOH and bromothymol blue as the indicator.

The effect of adding manganese chloride or ferric chloride at a level of 10 mg/100 ml of the media containing endosperm and husk was also studied.

Results and Discussion

In vitro studies using *Clostridium perfringens* showed that neither the husks from chick pea, cow pea and horse gram nor the carbohydrate isolates derived from them were capable of inducing gas formation when compared to the endosperm flours (Table 1). The quantity of gas produced when endosperm fractions of chick pea, cow pea and horse gram were incorporated into the media were 17.0 ml, 15.5 ml and 16.0 ml respectively, while there was no gas production with the husk or the derived carbohydrate fractions. These husks may contain carbohy-

TABLE 1. EFFECT OF ADDITION OF HUSK FRACTION ON GAS PRODUCTION *IN VITRO* BY *CLOSTRIDIUM PERFRINGENS*

Medium	Gas formed (ml/4 ml broth) by			
	Chick pea	Horse gram	Cow pea	Glucose*
Endosperm	17.0	15.5	16.0	22.0
Husk or isolated pectin, hemicellulose A or B or cellulose	Nil	Nil	Nil	Nil
Endosperm with husk or isolated pectin, hemicellulose A or B or cellulose	Nil			Nil+
Glucose+husk or isolated pectin, hemicellulose A or B or cellulose	Nil			
Endosperm+ 5% husk	16.5			
Endosperm+10% husk	6.5			
Endosperm+15% husk	Nil			
Endosperm+30% husk	Nil			
Endosperm+50% husk	Nil			
Endosperm+20% aqueous, 70% alcohol, alkali or acid extract of husk	Nil			
Endosperm+20% husk+$MnCl_2$	21.0			
Endosperm+20% husk+$FeCl_3$	23.0			
Whole chick pea flour	3.5			

*Only glucose was added to medium

+Husk or its isolate fraction added to glucose medium.

drate fractions not assimilable by *Clostridia* and may also be due to the presence of some inhibitory factors.

Chick pea husk and the carbohydrate fractions derived from it were added separately to the basal media containing either glucose or endosperm substrate. Incubation of these media with the bacterial cultures produced considerable volume of gas only in the case of controls but not when husk or its fractions were added. This confirmed the presence of an inhibitory factor both in the husk and isolates from it. Incorporation of seed coat at more than 15 per cent level, inhibited the gas formation. Whole chick pea flour produced 3.5 ml gas as compared to 17 ml with only the endosperm. The husk constitutes 12 per cent of the whole chick pea used in this study. Thus, the gas inhibition was only partial with the whole chick pea. The lyophilized material obtained by aqueous, 70 per cent ethanol, alkali and acid extraction of chick pea husk flour also inhibited gas formation indicating that chick pea husk contains a soluble fraction which is very effective in inhibiting the gas formation.

This inhibition could either occur by retardation of the growth of the bacteria or by changing the metabolic pathways of fermentation. To ascertain this, both the inoculated control (glucose medium) as well as the same containing 20 per cent husk were incubated and glucose determination was carried out. It was found that the organism utilized 60 per cent of the glucose in the medium which did not contain the husk. In the medium containing either glucose or endosperm there was a decrease in pH consequent to increase in acidity (equivalent to 3.3 and 2.8 ml, respectively of 0.05N sodium hydroxide/4 ml broth), whereas the pH of the medium containing the husk remained unaltered. These experiments showed that the inhibitory effect of the husk was due to inhibition of the growth of the bacteria and not due to change in the metabolic pathway, as reported in the case of curcumin[6].

Addition of manganese chloride or ferric chloride to chick pea endosperm medium produced considerable gas even in the presence of 20 per cent husk indicating that the inhibition may be due to binding of essential minerals in the medium by the husk fractions and rendering them unavilable to the bacteria. Phytates, which compete with bacteria for metallic ions, may also be the cause for the inhibitory effects shown by legume husk.

The results obtained with chick pea are contrary to the report on cereal bran (wheat and oat). Hickey *et al*[7] have shown that human intestinal gas production increased when wheat bran was consumed. Similar results were obtained by Mayer and Calloway[8] with wheat bran and oat bran. They suggested that at least two nondigestible fermentable components are present in wheat bran. Wheat bran contains about 6 per cent total free sugars of which about half is oligosaccharides such as raffinose, stachyose, verbascose and necketose[9,10], which are known to cause flatulence. It is also reported to contain α-amylase inhibitors, which are physiologically active and slow down the rate of starch digestion in humans[11]. Such studies have not been reported in the case of legume husks.

Rackis *et al*[12] using an *in vitro* technique have shown in soybean meal the presence of alcohol soluble growth promoting and growth inhibiting factors. Some of the phenolic acids such as syringic and ferulic acids present in the soybean are effective gas inhibitors *in vitro* and in intestinal segments of dogs. This gas inhibition is reported to be due to bacteriostatic action. Ferulic acid is also reported to be present in the water-soluble hemicelluloses of rice bran.[13]

From this study, it is however difficult to conclude about the exact nature of the inhibitory factor present in the legume husks. This inhibitory factor may be useful in preventing flatulence prevailing in legume based

foods, although the possibility of concomitant lowering of the nutritional quality cannot be excluded.

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Studies on the Growth of Pathogenic Bacteria at Different Temperatures on Mutton and Pork

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The growth of *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium* on goat meat and pork at 4-5, 20 and 37°C was studied by extract release volume (ERV) method. At 4-5°C there was no growth in goat meat till 72 hr. At 20°C, *B. cereus* and *S. aureus* showed considerable growth in goat meat within 48 hr, and *E. coli* and *S. typhimurium* within 36 hr. At 37°C, all the organisms showed sufficient growth within 20 hr in goat meat. In pork, *B. cereus* and *S. aureus* showed growth in 40 hr, and *E. coli* and *S. typhimurium* in 20 hr. With the decrease in ERV, there was corresponding increase in pH in both goat meat and pork. Sodium nitrite (0.02%) and sucrose (3%) inhibited the growth of *B. cereus*, *S. aureus* and *E. coli* in goat meat and pork at 37°C upto 48 hr; however, *S. typhimurium* was not inhibited by sodium nitrite but was inhibited to a lesser degree by sucrose. Sodium chloride (2%) inhibited the growth of *B. cereus* and *E. coli* in goat meat and pork upto 48 hr. *S. aureus* was not inhibited in goat meat while its inhibition was very limited in pork.

Meat and meat products get contaminated by a large number of pathogenic bacteria—*Bacillus cereus*[1-3], *Staphylococcus aureus*[4-6], enteropathogenic *Escherichia coli*[7,8] and *Salmonella typhimurium*[9-11]. Source of contamination may be environment or implements used in slaughtering the animals or through meat-handlers. These pathogenic bacteria also elaborate enterotoxin, which results in food posioning. In India, mutton and pork are stored at 4-5°C or at room temperatures varying from 20-40°C before consumption in raw or processed state.

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Pathogenic bacteria can grow well in meat under different storage conditions. Stored meats are generally consumed within 3 days and therefore it is important to assess the microbial spoilage by a method which is rapid and at the same time gives the correct status of these pathogenic bacteria. Extract Release Volume[12,13] is the method adopted in this study.

Sodium chloride, sodium nitrite and sucrose are known to preserve meat and meat products by controlling the growth of pathogenic bacteria.

Growth of *B. cereus*, *S. aureus*, *E. coli* and *S. typhimurium* in mutton and pork, at different temperatures of storage and the effect of chemicals like sodium nitrite, sodium chloride and sucrose in controlling them were studied and the results reported here.

Materials and Methods

Micro-organisms: Bacillus cereus B-57-1, *Staphylococcus aureus* B-43-3, *Escherichia coli* B-3-6 and *Salmonella typhimurium* B-35-1 were procured from Central Research Institute, Kasauli. Purity of the cultures was tested by colony characteristics, staining and biochemical tests.

Procurement and handling of meat samples: Samples of goat and pork meat were obtained in sterile containers from the local meat shops. Effort was made to procure the samples from the same region of the animals of same age group. The mutton and pork samples were taken from the thigh region. After trimming the adipose tissue, the lean meat was surface sterilized with alcohol. The meat was minced in a sanitized meat mincer and collected in sterile petri dishes.

Growth media: Mannitol-egg yolk-phenol red-plymyxin agar[14] was used for culturing *B. cereus*, *Staphylococcus* medium-110[15] for *S. aureus*, Brilliant green agar[15] for *E. coli* and *Salmonella-Shigella* agar[15] for *S. typhimurium*.

Preparation of inoculum: The inoculum was prepared by growing the organism in the nutrient broth for 18-24 hr at 37°C, centrifuging and washing the sediment twice with sterile distilled water. The suspension was finally made in the sterile distilled water. The optical density of the bacterial suspension was adjusted at 510 nm using Spectronic-20 (Bausch & Lomb, Rochester, N. Y.) containing 2.4×10^6 cells in case of *B. cereus* and about 5.0×10^5 cells in the case of *S. aureus*, *E. coli* and *S. typhimurium*.

Measurement of growth: Fifteen grams of minced meat, taken in the sterile petri-dish, was inoculated with 1 ml bacterial suspension and the samples were incubated at 4-5, 20 and 37°C. The samples were taken out at appropriate intervals and analyzed for extract release volume (ERV)[13,16]. The pH of ERV filtrate was recorded. In control samples, the inoculum was replaced by sterile distilled water.

Effect of preservatives on bacterial growth in meat: Sodium nitrite 0.02 per cent, sodium chloride 2 per cent and sucrose 3 per cent were added separately into the minced meat. The meat samples were then inoculated with each test organism and incubated at 37°C. The samples were taken at different intervals and analysed for ERV.

Results

Effect of temperature on growth of organisms in goat meat: The effect of temperature on the growth of *B. cereus* in goat meat is shown in Table 1. There was no growth of *B. cereus* at 4-5°C upto 72 hr. At 20°C there

TABLE 1. EFFECT OF TEMPERATURE ON THE GROWTH OF *B. CEREUS* AND *S. AUREUS* IN GOAT MEAT

Temp (°C)	Incubation period (hr)	Test		Control	
		ERV (ml)	pH	ERV (ml)	pH
		B. cereus			
4-5	0	39.0	6.00	39.0	6.00
	24	42.0	6.00	39.0	6.20
	48	38.0	6.10	38.0	6.05
	72	38.0	6.10	38.0	6.05
20	0	41.0	5.95	43.0	5.90
	24	43.0	6.00	42.0	6.05
	36	38.0	6.30	39.0	6.25
	48	25.0	6.50	36.0	6.35
	72	5.0	7.60	12.0	6.90
37	0	43.0	6.15	44.0	6.05
	8	40.0	6.10	44.0	6.10
	12	38.0	6.20	39.0	6.25
	20	20.0	6.70	28.0	6.40
	24	15.0	6.95	24.0	6.75
	26	8.0	7.50	18.0	6.85
		S. aureus			
4-5	0	39.0	6.00	39.0	6.00
	24	41.0	6.20	39.0	6.20
	48	38.0	6.15	38.0	6.05
	72	38.0	6.15	38.0	6.05
20	0	42.0	5.90	43.0	5.90
	24	43.0	6.05	42.0	6.05
	36	39.0	6.30	39.0	6.25
	48	35.0	6.45	36.0	6.35
	72	6.0	7.40	13.0	6.90
37	0	44.0	6.10	44.0	6.05
	8	44.0	6.30	44.0	6.10
	12	38.0	6.35	39.0	6.25
	20	22.0	6.50	28.0	6.40
	24	18.0	6.80	24.0	6.75
	26	6.0	7.45	18.0	6.85

was considerable growth of *B. cereus* in 48 hr. The pH increased from 5.95 to 6.50. At 37°C sufficient growth of the organism was noticed within 20 hr. Similar trend in growth was noted with *S. aureus*. Sufficient growth of *E. coli* was observed within 20 hr at 37°C and in 36 hr at 20°C; no growth was noticed upto 72 hr at 4-5°C (Table 2). Growth pattern of *S. typhimurium* (Table 2) was similar to that of *E. coli* which showed the fastest growth in goat meat at 37°C as compared to other organisms. There was always a corresponding increase in pH with the increase in ERV.

Effect of temperature on growth of organisms in pork meat: Growth studies with pork meat at 37°C (Table 3) indicate that *E. coli* showed maximum growth (ERV 7.0 ml) in the shortest time (20 hr), followed by *S. typhimurium* (ERV 13.0 ml at 20 hr), while *B. cereus* took 40 hr and *S. aureus* 32 hr. The pH also increased considerably.

TABLE 2. EFFECT OF TEMPERATURE ON THE GROWTH OF *E. COLI* AND *S. TYPHIMURIUM* IN GOAT MEAT

Temp. (°C)	Incubation period (hr)	Test ERV (ml)	Test pH	Control ERV (ml)	Control pH
		E. coli			
4-5	0	39.0	6.20	38.5	6.10
	24	39.0	6.10	41.0	6.05
	48	43.0	6.10	41.0	6.10
	72	42.0	6.10	40.0	6.15
20	0	40.0	6.00	41.0	6.05
	24	41.0	6.10	41.0	6.15
	48	16.0	7.15	36.0	6.30
	72	12.0	7.65	20.0	6.65
37	0	42.0	6.15	42.0	6.15
	8	42.0	6.20	41.0	6.15
	12	41.0	6.25	42.0	6.15
	20	13.0	7.30	34.0	6.55
	24	11.0	7.60	28.0	6.50
	26	5.0	7.85	20.0	6.90
		S. typhimurium			
4-5	0	39.0	6.00	39.0	6.10
	24	41.0	6.10	41.0	6.05
	48	42.0	6.05	40.0	6.10
	72	40.0	6.10	39.0	6.10
20	0	41.0	6.05	41.0	6.05
	24	42.0	6.10	41.0	6.15
	36	20.0	6.70	38.0	6.20
	48	7.0	7.50	36.0	6.30
	72	—	—	20.0	6.65
37	0	43.0	6.10	42.0	6.15
	8	42.0	6.15	41.0	6.15
	12	41.0	6.15	42.0	6.15
	20	25.0	6.40	34.0	6.25
	24	14.0	7.00	28.0	6.50
	26	8.0	7.70	20.0	6.90

TABLE 3. GROWTH OF DIFFERENT ORGANISMS IN PORK MEAT AT 37°C

Organism	Incubation period (hr)	Test ERV (ml)	Test pH	Control ERV (ml)	Control pH
S. cereus	0	47.0	5.50	47.0	5.50
	24	44.5	5.85	46.0	5.90
	32	39.5	6.20	42.0	5.95
	40	31.0	6.30	40.0	6.00
	48	17.0	6.45	35.0	6.20
S. aureus	0	47.0	5.50	47.0	5.50
	24	46.0	5.80	46.0	5.85
	32	30.0	6.15	42.0	5.95
	40	25.0	6.35	40.0	6.00
	48	9.5	6.65	35.0	6.20
E. coli	0	47.0	5.50	47.5	5.55
	8	45.0	5.60	46.0	5.65
	20	7.0	6.20	25.0	6.10
	24	5.0	6.30	20.0	6.15
S. typhimurium	0	47.0	5.50	47.5	5.55
	8	46.0	5.65	46.0	5.65
	20	13.0	6.15	25.0	6.15
	24	7.0	6.25	20.0	6.15

Effect of preservative on growth of organisms in goat meat: In goat meat, growth of *B. cereus*, *S. aureus* and *E. coli* was inhibited in 48 hr at 37°C, when 0.02 per cent sodium nitrite was used, as indicated by ERV and pH, whereas sufficient growth was observed within 24 hr in the control (Table 4). Sodium nitrite did not inhibit the growth of *S. typhimurium* till 48 hr, whereas some inhibition was noticed in 48 hr.

At 37°C the growth of *B. cereus* and *E. coli* in goat meat was inhibited by 2 per cent sodium chloride upto 48 hr. *S. aureus* was not inhibited while there was slight inhibition of *S. typhimurium* in 24 hr. But there was no effect of 2 per cent sodium chloride on the growth of *S. aureus* and *S. typhimurium* at 48 hr.

Sucrose at 3 per cent concentration inhibited *B. cereus*, *S. aureus* and *E. coli* upto 48 hr. But the pH decreased from 5.8 to 5.1 in 24 hr and to 4.3 in 48 hr in case of *B. cereus*, from 5.8 to 4.8 in 48 hr in *S. aureus*, from 5.8 to 5.3 in 48 hr in *E. coli*. There was no change pH in 24 hr by these organisms. Sucrose did not inhibit the growth of *S. typhimurium* in goat meat at 37°C. The

TABLE 4. EFFECT OF VARIOUS PRESERVATIVES ON THE GROWTH OF DIFFERENT ORGANISMS IN GOAT MEAT AT 37°C

Preservative	Level (%)	Organism	0 hr ERV (ml)	0 hr pH	24 hr ERV (ml)	24 hr pH	48 hr ERV (ml)	48 hr pH
Control	—	*B. cereus*	43.0	6.10	15.0	7.00	—	—
		S. aureus	44.0	6.15	17.0	6.90	—	—
		E. coli	43.0	6.10	13.0	7.55	—	—
		S. typhimurium	44.0	6.20	12.0	7.30	—	—
Sodium nitrite	0.02	*B. cereus*	43.0	6.10	43.5	6.10	43.0	6.20
		B. aureus	44.5	6.10	44.0	6.10	44.5	6.10
		E. coli	43.5	6.15	43.0	6.10	42.0	6.15
		S. typhimurium	43.0	6.15	22.0	7.05	—	—
Sodium chloride	2.0	*B. cereus*	43.0	6.05	43.0	5.85	44.0	6.05
		S. aureus	44.0	6.00	20.0	6.70	—	—
		E. coli	43.0	6.00	44.0	5.95	42.0	6.15
		S. typhimurium	43.5	6.00	20.0	6.95	—	—
Sucrose	3.0	*B. cereus*	44.0	5.80	43.5	5.15	40.0	4.30
		S. aureus	43.0	5.80	43.0	5.80	43.5	4.80
		E. coli	44.0	5.85	43.0	5.90	43.0	5.30
		S. typhimurium	43.0	5.90	43.0	5.85	12.0	6.60

pH increased from 5.9 at 0 hr to 6.6 in 48 hr in the presence of 3 per cent sucrose.

Effect of preservatives on growth of organisms in pork meat: Sodium nitrite (0.02 per cent) inhibited the growth of *B. cereus*, *S. aureus* and *E. coli* upto 48 hr in pork meat at 37°C as there was no change in ERV and pH (Table 5). But 0.02 per cent sodium nitrite did not inhibit the growth of *S. typhimurium* as the reduction

TABLE 5. EFFECT OF VARIOUS PRESERVATIVES ON THE GROWTH OF DIFFERENT ORGANISMS IN PORK MEAT AT 37°C

Preservative	Level (%)	Organism	0 hr ERV (ml)	0 hr pH	24 hr ERV (ml)	24 hr pH	48 hr ERV (ml)	48 hr pH
Control	—	*B. cereus*	48.0	5.65	45.0	5.90	20.0	6.35
		S. aureus	48.0	5.65	47.0	5.75	10.0	6.60
		E. coli	48.0	5.50	47.0	5.70	7.0	6.35
		S. typhimurium	48.0	5.60	48.5	5.75	10.0	6.15
Sodium nitrite	0.02	*B. cereus*	47.5	5.65	47.0	5.85	45.0	5.70
		S. aureus	48.0	5.60	45.0	5.65	45.0	5.70
		E. coli	47.5	5.60	47.0	5.65	47.0	5.75
		S. typhimurium	47.5	5.55	48.0	5.60	15.0	6.00
Sodium chloride	2.0	*B. cereus*	48.0	5.65	48.5	5.80	47.0	5.90
		S. aureus	47.5	5.65	46.0	5.70	22.0	6.50
		E. coli	48.5	5.55	48.5	5.65	46.0	5.75
Sucrose	3.0	*B. cereus*	47.0	5.55	47.0	5.30	42.0	5.10
		S. aureus	47.5	5.65	46.5	5.40	47.0	5.20
		E. coli	48.0	5.60	47.0	5.60	47.0	5.15
		S. typhimurium	48.0	5.60	48.0	5.60	30.0	5.90

in ERV at 48 hr was almost the same as that of control samples.

Sodium chloride (2 per cent) inhibited the growth of *B. cereus* and *E. coli* while *S. aureus* and *S. typhimurium* were inhibited to a lesser degree in 48 hr. This was reflected by less decrease in ERV than that of control.

B. cereus, *S. aureus* and *E. coli* were inhibited by 3 per cent sucrose in pork meat. The pH also decreased in these cases in 48 hr. *S. typhimurium* was inhibited to a very limited extent and pH also increased in 48 hr.

Discussion

The reduction in ERV may not be due only to the large number of bacteria *per se* but may also be due to the activity of the bacterial proteases. At 4-5°C, the enzyme activity may be low[17].

The substrate on which the organism was grown also played an important role for enzyme production. It was found that pork was slightly more favourable for the growth of various organisms than the goat meat as was revealed by larger reduction in ERV by the organisms in pork. The decrease in ERV in control samples occurred possibly due to the growth of these micro-organisms which survived alcohol sterilization or which were introduced subsequently through handling. This inhibitory effect of nitrite was due to the undissociated nitrous acid[18]. The greater inhibition of *B. cereus*, *S. aureus* and *E. coli* as compared to that of *S. typhimurium* by sodium nitrite in goat and pork meat at 37°C might be due to the fact that *Salmonella* was more resistant to nitrite[19]. But *S. typhimurium* was inhibited less in goat meat than in pork. This was basically due to the higher pH of the goat meat as compared to that of pork, and the effect of nitrite was more under acidic conditions[20,21]. *Staphylococci* has been reported to grow faster on nitrite-free frankfurters[22].

No inhibition in the growth of *S. aureus* upto 48 hr in goat meat and to a sufficient extent in pork was due to the fact that this organisms was known to grow even in 10 per cent sodium chloride. Inhibition of *S. typhimurium* by sodium chloride to a limited extent in both goat meat and pork was due to the decreased water activity (a_w). With the decreased a_w, the lag phase of the organisms was increased and the rate of growth decreased.

Inhibitory effect of 3 per cent sucrose on the growth of *B. cereus*, *S. aureus* and *E. coli* in goat meat and pork might be due to the decrease in pH caused by the fermentation products of sucrose (mainly acids). At this decreased pH, the organisms might have not been able to grow and multiply fully. The growth of *S. typhimurium* in goat meat and pork was not much inhibited by 3 per cent sucrose because there was no decrease in pH as sucrose was not fermented by *S. typhimurium*. The minor decrease in growth might be due to the increased osmotic pressure and decreased water activity produced by the addition of sucrose.

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Spectrophotometric Determination of Quinine in Soft Drinks

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A method has been developed for the determination of quinine in soft drinks based on the extraction of the 1:2 complex formed between quinine and alizarin brilliant violet R into chloroform at pH 1.0 The bluish violet complex in chloroform showed maximum absorbance at 578 nm. Beer's law is obeyed over a concentration range of 1.0 to 16 μg/ml and the percentage recovery in soft drinks ranged from 98.6 to 100.2 with a standard deviation of $\pm$0.35. The method is sensitive and can be applied in the presence of other food additives.

Quinine ($C_{20}H_{24}N_2O_2$, mol. wt. 324.41), an important alkaloid of *Cinchona officianalis* L. is permitted in soft drinks such as synthetic ready-to-serve beverages and tonic waters. Since excessive use of quinine may cause some allergic reactions, a maximum limit of 100 ppm (expressed as quinine sulphate) has been laid down under the provisions of Prevention of Food Adulteration Act, 1954, and Rules 1955 thereof[1] for its use in soft drinks. Several spectrophotometric procedures are described for its determination in soft drinks and medicinals[2–16]. The spectrophotometric methods developed so far are either not very sensitive or having interferences from other additives, which has necessitated a search for an alternative method.

A sensitive spectrophotometric method is described in this paper by extracting the 1:2 complex formed between quinine and alizarin brilliant violet R (ABV R), C.I. No. 60730, an amino anthraquinone dye, into chloroform at pH 1.0. The method developed does not suffer from the disadvantages mentioned above and can be used for determination of microgram quantities of quinine.

Materials and Method

Instrumentation: Spectral and absorbance measurements are carried out using Perkin Elmer, Coleman 575 model double beam digital spectrophotometer. Toshniwal pH meter was used for making pH measurements.

Reagents: Quinine sulphate solution (1 mg/ml) was prepared from quinine sulphate (G.R. grade; Loba-Chemie, Wien Fischamend, Austranal-Praparate) in 0.1N sulphuric aicd. The stock solution so prepared was diluted to give a working standard solution of 40 μg/ml of quinine. A 1×10^{-3}M solution of ABV R (M/s Chroma-Gessllscaft. Stuttgart) was prepared in distilled water. pH 1.0 buffer was prepared by adding 97.0 ml of 1.0N hydrochloric acid to 50 ml of 1.0 M potassium chloride and made upto one litre. All other reagents used were of A.R. grade.

Preparation of standard curve: To a 30 ml buffer solution in a 100 ml clean separating funnel were added an aliquot of quinine solution ranging from 10 to 200 μg and 5 ml of dye solution. The total volume was adjusted to 50 ml with distilled water and extracted with 10 ml of chloroform. The absorbance was measured at 578 nm against reagent blank after drying the chloroform layer over anhydrous sodium sulphate. The absorbance against the concentration of quinine were plotted to get standard curve.

Determination in soft drinks by Method A: An aliquot of 50 ml of the soft drink solution was taken in a separating funnel. Ten ml of 10 per cent sodium hydroxide solution was added and extracted with three 30 ml portions of chloroform. The combined chloroform extract was shaken with three 30 ml portions of 1.0 N sulphuric acid solution. The combined sul-

phuric acid extract was transferred quantitatively to a 100 ml volumetric flask and made upto volume. An aliquot of this solution (1 to 5 ml) was taken and the procedure given under preparation of standard curve was followed for its determination.

Determination in soft drinks by Method B: An aliquot (1 to 5 ml) of the soft drink was taken directly and the procedure for its determination as given under preparation of standard curve was followed.

Results and Discussion

The absorption maximum of the bluish violet coloured complex in chloroform was found to be at 578 nm and the optimum pH for the extraction was found to be 1.0. A minimum of 9 fold molar excess of the dye was found required for the maximum extraction of the complex. Among the solvents like $CHCl_3$, CH_2Cl_2, CCl_4, n-butanol and methyl isobutyl ketone tried for extraction, $CHCl_3$ and CH_2Cl_2 were found to be useful for the maximum extraction of the complex. $CHCl_3$ was preferred to CH_2Cl_2 due to its low volatality. The ratio of the ion-pair association complex was determined according to the slope analysis method[17] and was found to be 1:2 for quinine: dye. Under the experimental conditions Beer's law was obeyed over a concentration range of 1.0 to 16 μg/ml of quinine. The molar extinction coefficient and Sandell's sensitivity were found to be $2.106 \pm 0.065 \times 10^4$ moles/l and 0.015 $\mu g/cm^2$ respectively. The complex in chloroform was found stable over 24 hr.

To check the recovery of quinine by the proposed method, quinine was added to soft drink samples at 20, 50 and 100 ppm levels and analysed by methods A and B. The recoveries ranged from 98.6 to 100.2 per cent by method A and from 98.9 to 104.1 per cent by method B (Table 1) which are comparable with the values obtained by fluorimetric method after extraction of quinine[18].

The other dyes of the same class like alizarin brilliant blue and alizarin varidin were also tried, but were not found satisfactory for determination in micro levels. The normally present food additives in soft drinks such as sugar, citric acid, phosphoric acid, benzoic acid, saccharin, dulcin, caramel and food colours were found not to interfere even if they were present in 100 fold molar excess to quinine. Caffeine, strychnine and nicotine showed no interference upto two fold excess, but large excess of their presence resulted in a positive error. The interference due to caffeine, normally present in cola type beverages ranging from 70 to 140 ppm can be avoided by extracting quinine into sulphuric acid solution leaving caffeine in organic phase during extraction (method A). The higher recovery ranging from 101.5 to 104.1 per cent in the direct estimation (method B) in cola type beverages may be due to the presence of caffeine in varying amounts. However, for routine analysis, direct estimation from the beverage (method B) can be followed as a rapid method.

The proposed method is more rapid and sensitive among the spectrophotometric methods available and can be used for the determination of quinine in soft drinks such as aerated waters, cola type beverages and tonic waters.

TABLE 1. RECOVERY OF QUININE FROM SOFT DRINKS

Type of soft drink	Quinine added (ppm)	Recovery* (%) Method A Mean±S.D.	Method B Mean±S.D.	Fluorimetric method
Orange flavoured	20	98.6±0.42	98.9±0.35	98.40
	50	99.0±0.39	99.2±0.22	98.80
	100	99.7±0.39	99.8±0.18	99.80
Lime flavoured	20	100.1±0.41	100.2±0.41	99.65
	50	99.4±0.39	99.8±0.36	100.20
	100	98.9±0.32	99.2±0.24	99.00
Cola type(I)	20	100.2±0.41	104.1±1.12	99.60
	50	99.3±0.32	102.7±0.75	99.10
	100	99.4±0.32	101.5±0.54	98.90
Cola type(II)	20	99.7±0.22	103.8±0.91	99.40
	50	98.8±0.35	102.3±0.64	100.45
	100	99.4±0.27	101.5±0.39	98.70

*Values are mean of 4 analyses

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Glyceroglycolipids and Glycerophosphatides in Finger Millet Seeds *(Eleusine coracana)*

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Glycolipids (0.25 and 0.26%) and phospholipids (0.10 and 0.11%) were isolated from the total lipids of two varieties ('Indaf I' and 'Hamsa') of finger millet seeds (*Eleusine coracana*). Two glyceroglycolipids and four glycerophosphatides were further isolated from the glycolipids and phospholipids by column and preparative thin-layer chromatography. Monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) were the major glycolipids whereas, phosphatidyl chloine (PC) and phosphatidyl ethanolamine (PE) were the major glycerophosphatides, comprising 57.0 and 60.7 mole % of the total phospholipids in 'Indaf I' and 18.9 and 16.9% in 'Hamsa'. Oleic, linoleic and palmitic acids totalled 94-97% in all compounds though the proportions varied. The MGDG and DGDG from 'Indaf' contained more palmitic acid (30.4 and 29.4%) than did those from 'Hamsa' (25.6 and 24.8%). The ratio of unsaturated to saturated fatty acids was also different in the various phosphatides present in these varieties ('Indaf' 2.6-2.8, 'Hamsa' 1.7-1.9). The sugar component of MGDG and DGDG was identified as D-galactose.

Glyceroglycolipids and glycerophosphatides are known to be widely distributed in the plant kingdom. The unique chemical composition of various lipid classes from many cereals such as wheat[1,2], rice[3,4], pearl millet[5], Italian millet[6] and other plant sources[7] has been reported. In all, more than a dozen different lipid classes, each containing several major fatty acid constituents are now known. The present paper descri-

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bes the isolation and characterization of glyceroglycolipids and glycerophosphatides from finger millet seeds, an important food material in several regions of India.

Materials and Methods

Two varieties of seeds of finger millet ('Indaf I', brown; 'Hamsa', white of 1976 crop) were obtained from the University of Agricultural Science, Hebbal, India. Reagents and chemicals used were of analytical grade. Standard fatty acid methyl esters and standard glycerophosphatides were obtained from Sigma Chemicals, USA, silicic acid from the V.P. Chest Institute, New Delhi, silica gel G from S.M. Chemicals, Baroda, and Trisil reagent from Pierce Chemical Co., USA.

Extraction and fractionation of lipids: Millet seeds were finely ground in a mill and extracted three times with chloroform: methanol (2:1 v/v). Following solvent removal in a rotary flash evaporator under nitrogen, the lipids were passed through a Sephadex G-25 column to remove non-lipid contaminants[8]. The lipids were further resolved into neutral lipids, glycolipids and phospholipids by silicic acid column chromatography[9]. The glycolipids and phospholipids recovered in the eluate accounted for 10 and 12 per cent and 5 and 6 per cent respectively, of the total eluted lipids of 'Indaf' and 'Hamsa'[10].

Isolation of glyceroglycolipids (MGDG and DGDG): The crude glycolipid fraction was subjected to separation on silicic acid column using chloroform: acetone mixtures. An 8:2 (v/v) mixture eluted monogalactosyl diglyceride (MGDG), and a 2:8 (v/v) mixture eluted digalactosyl diglyceride (DGDG), both being contaminated with small percentages of sterol glycosides and cerebrosides[11]. Each of these fractions was repeatedly chromatographed on silicic acid to reduce the extent of contamination. Final purification was done on preparative thin layer plates developed with chloroform: methanol 95:12 (v/v).

Isolation of glycerophosphatides: The crude phospholipid fraction obtained in the methanol eluate from silicic acid column chromatography was separated on TLC plates. Identification of each class was through Rf-values, specific colour reagents and reference standards. These were further quantitated on preparative one mm thin layer plates using chloroform: methanol: water (65:25:4 v/v). The plates after drying were sprayed with Rhodamine 6G, visualised under UV and portions containing different compounds scraped off. Each fraction was extracted with chloroform: methanol: formic acid: water (94:94:2:4, v/v). The amount of each class was determined by phosphorus analysis[12].

Infrared spectrophotometry: Infrared spectral characteristics of the isolated glyceroglycolipids (MGDG and DGDG) were obtained as a KBr pellet on a Hilger Watts Infragraph.

Degradation of glyceroglycolipids and glycerophosphatides: Isolated MGDG, DGDG, PC, PE, PG and PI (5-10 mg each) was refluxed with 4-5 ml of 5 per cent methanolic HCl in a hydrolysis flask with a side arm for 2 hr. Water (0.5 ml) was added and fatty methyl esters were extracted 3-4 times with 5 ml portions of light petroleum, concentrated under nitrogen and stored at −20°C until analysed. The methanol-water phase from MGDG, DGDG, PC and PE was concentrated in a stream of nitrogen at 45°C almost to dryness. HCl (2 ml of 1.0N) was added and the solution refluxed on a boiling water bath for 2 hr. The hydrolysate was passed through a column of Amberlite IR-4 B (OH^-) to remove chloride ions.

Paper chromatography: Methyl glycosides obtained from MGDG and DGDG were subjected to descending paper chromatography on Whatman filter paper using n-butanol: pyridine: water 6:4:3 for 18 hr at room temperature. Spots were located with silver nitrate-sodium hydroxide reagent.

Choline and ethanolamine obtained from PC and PE were chromatographed by developing with phenol saturated with water: ethanol: acetic acid 50:5:6. The spots were located with ninhydrin and Dragendorff's reagent[13].

Gas chromatography: Fatty acid methyl esters from MGDG, DGDG, PC, PE, PG and PI were analysed by gas chromatography using a Varian Aerograph, 1400 [FID, carrier gas nitrogen, 8 ft×1/8 in. column with 15 per cent (w/w) DEGS polyester on Chromosorb W, 185°C]. For resolution as trimethylsilyl ether derivatives[14] of the methyl glycosides from MGDG and DGDG, an SE-30 column [5 per cent w/w on Chromosorb W, 230°C] was employed. Unknown peaks were identified by comparison with standards. Unsaturated fatty acid methyl esters were confirmed when occasion demanded by bromination and subsequent disappearance on gas chromatograms.

Results and Discussion

Glyceroglycolipids: The two glyceroglycolipids (MGDG and DGDG) isolated from two varieties of finger millet seeds each gave a single spot corresponding to reference compounds. IR spectra were likewise in agreement with published characteristics[15], suggesting that the compounds were pure. The glycolipid fraction from silicic acid column which includes sterol containing glycolipids showed MGDG and DGDG as the major components. These glyceroglycolipids have likewise been reported to occur in other cereals such as wheat, rice and bajra though in varying proportions. In pearl millet[5] and wheat[1,2] DGDG has been reported as the major specie.

TABLE 1. FATTY ACID COMPOSITION OF INDIVIDUAL GLYCERO-GLYCOLIPIDS (% WT.)

Fatty acid	MGDG		DGDG	
	'Indaf I'	'Hamsa'	'Indaf I'	'Hamsa'
12:0	1.0	0.8	1.4	1.0
14:0	1.0	1.0	0.8	1.2
16:0	30.4	25.6	29.4	24.8
18:0	0.8	0.6	0.6	0.8
18:1	56.9	58.7	60.7	59.6
18:2	8.5	11.2	6.3	11.0
18:3	1.4	2.1	0.8	1.6
Unsat/sat. acid ratio	2.03	2.57	2.11	2.60
Iodine value (calculated)	70.5	78.6	68.3	78.0

TABLE 2. COMPOSITION OF PHOSPHOLIPID FRACTION FROM FINGER MILLET SEEDS

Phospholipid	Mole %	
	'Indaf'	'Hamsa'
Phosphatidic acid	2.0	2.2
Phosphatidyl glycerol	8.0	7.5
Phosphatidyl ethanolamine	18.9	16.9
Phosphatidyl choline	57.0	60.7
Phosphatidyl inositol	9.8	7.0
Un-recovered	4.3	5.7

Table 1 shows that MGDG and DGDG from 'Indaf I' were considerably less unsaturated than those from 'Hamsa'. This is evident from the ratio of unsaturated to saturated fatty acids, and the calculated iodine values. In both compounds, palmitic, oleic and linoleic acids together comprised 94-97 per cent of the total fatty acids, only the relative proportions varying. According to the earlier reports, reviewed recently by Harwood[16], the MGDG and DGDG fractions from some of the plant sources are enriched with linoleic constituting 80-90 per cent of the total. The above compounds obtained in the present investigation contain predominantly palmitic acid and oleic acid which may be expected to reflect in the fatty acid composition of 1, 2 diglyceride precursor. Both MGDG and DGDG from 'Indaf I' contain about 5 per cent more palmitic acid than that of 'Hamsa', with only small differences in the proportions of oleic acid. The percentage of linoleic acid in MGDG and DGDG from 'Indaf' was 3-5 per cent less than that in 'Hamsa'. Such varietal dissimilarity in the fatty acid distribution pattern of the same lipid classes is evident from earlier reports[7].

Paper chromatograms of the methyl glycosides obtained from MGDG and DGDG indicated that D-galactose is the major sugar component in both. Gas chromatographic resolution of the trimethylsilyl ether derivatives of these methyl glycosides yielded three peaks corresponding to γ-, β- and α-galactose, confirming galactose as the component sugar moiety. An additional small hump which appeared on the gas chromatograms in both instances, corresponding to α-and β-glucose comprised only 1-2 per cent. From these results, and the available literature data[7], the molecular species in the two glycero-glycolipids have been deduced to be 3-0-β-D-galactopyranosyl-Sn-1,2-diacyl-glycerol, where acyl represents the two major fatty acids palmitic and oleic, and 3-0-[-α-D-galactopyranosyl-(1'-6')-0-β-D-galactopyranosyl] -Sn-1, 2-diacyl-glycerol where acyl again represents mostly palmitic and oleic acids.

Glycerophosphatides: The various glycerophosphatides identified and quantified by phosphorus value are shown in Table 2. The major phosphatides in 'Indaf' and 'Hamsa' varieties are PC and PE which constitute 57.0 and 60.7 per cent mole and 16.9 and 18.9 per cent mole respectively of the total phospholipids. No lyso-compounds of PC and PE were detected in the phospholipid fraction of this millet. Likewise in wheat and bajra, PC and PE have been reported as principal phosphatides[2,5].

The fatty acid distribution pattern in the total phospholipids and its various phosphatide constitutents is shown in Table 3. Palmitic acid ranges from 25.8 to 37.5 per cent and is often the only saturated fatty acid in all phosphatides, infrequently accompanied by traces of lauric, myristic and stearic acid. Oleic and linoleic are the predominant unsaturated fatty acids constituting 41.2 and 47.1 per cent and 19.5 and 26.5 per cent respectively. Linolenic acid only accounted for about 1 per cent of the total fatty acids. These results are in agreement with the earlier reports[16]. The ratio of unsaturated to saturated fatty acid is strikingly different in the two varieties of the same millet, and this difference runs through all the phospholipid classes. Thus the ratio is between 2.56 and 2.82 for all the five phosphatides present in 'Indaf I', while for the 'Hamsa' variety the ratio lies between 1.67 and 1.92. This would strongly suggest that a common fatty acid pool is drawn upon in assembly of these phospholipids.

The isolated and purified triglycerides of 'Indaf I' showed a ratio of unsaturated: saturated acids of 2.8, similar to that in the various phospholipid classes. In

TABLE 3. FATTY ACID COMPOSITION OF INDIVIDUAL GLYCEROPHOSPHATIDES (% WT.)

	Total phospholipids		Pnosphatidyl glycerol		Phosphatidyl ethanolamine		Phospnatidyl choline		Phospnatidyl inositol	
	'Indaf'	'Hamsa'	'Indaf'	'Hamsa'	'Indaf'	'Hamsa'	'Indaf'	'Hamsa'	'Indaf'	'Hamsa'
12 : 0	Tr.	Tr.	0.6	0.8	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.
14 : 0	Tr.	Tr.	0.8	0.8	Tr.	0.8	0.6	Tr.	Tr.	Tr.
16 : 0	26.6	37.5	26.0	32.0	26.5	34.0	25.8	36.0	26.2	34.2
18 : 0	Tr.	Tr.	0.7	1.0	0.8	0.8	Tr.	Tr.	Tr.	Tr.
18 : 1	46.0	41.2	46.4	43.0	45.7	43.4	46.1	43.4	47.1	43.7
18 : 2	26.3	20.5	24.5	21.0	26.0	20.0	26.5	19.5	25.4	21.0
18 : 3	1.1	0.8	1.0	1.4	1.0	1.0	1.0	1.1	1.3	1.1
Unsat./sat. acids	2.76	1.67	2.56	1.89	2.66	1.80	2.80	1.78	2.82	1.92
Iodine value (cal)	92.1	96.5	89.0	91.1	78.1	92.3	77.5	92.1	92.1	80.5

*Tr: Trace$<$0.5%

'Hamsa' however the ratio for triglycerides was 3.1, considerably greater than that (1.67-1.92) for the various phospholipids.

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Physico-chemical Studies in Relation to Cracking Properties in Rice Using Isogenic Lines

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Physico-chemical properties of one crack resistant variety and 3 pairs of isogenic lines differing in crack resistant properties were studied. It was found that crack resistance was associated with lower equilibrium moisture content on soaking in water and alkali score while gelatinization temperature, pentosan content and Brabender peak viscosity of milled rice were higher. It is also indicated that higher pentosan content in crack resistant lines may alter the cell wall properties and cause higher resistance to cleavage. Existence of a wide variation in the crack resistant property among the individuals of the original population ensured success in the intravarietal selection. Such wider variation was found to be associated with higher heteorogeneity among the grains to alkali degradation and could be initially identified by an alkali degradation test (Heterogeneity test). The factors contributing to crack resistance can be broadly grouped into four, namely, (i) hydration, (ii) starch, (iii) cell wall, and (iv) cellular structure and orientation.

The influence of grain cracking on milling breakage was reported by Rhind[1] and Angladette[2]. Later, Srinivas *et al.*[3-6] showed that varieties with high tolerance to cracking suffered minimum breakage during milling, and such varieties were more suitable for late harvesting. They also reported that intravarietal selection for crack resistance could be made in varieties in which the variation in crack resistance among the individuals was large.

Studies have shown that the structure and orientation of endosperm cells influence the characteristic transverse crack formation[7]. Kunze and Hall[8] have shown that low cracking was related to high gelatinization temperature(GT). In rice, the GT was known to vary inversely with alkali score[9] and alkali score bearing an inverse relation with pentosan content[10]. Nagato and Kono[11] have shown that rices with high GT have a harder endosperm.

Cracking in rice grain is thus influenced by a large number of factors. Results of the evaluation of these factors are presented in this paper.

Materials and Methods

The varieties used in the studies are listed in Table 1. Samples were collected from the field as described earlier[5,6]. The degree of crack resistance was measured by an index method[6], in which the values were computed

TABLE 1. THE RELATIONSHIP OF SELECTION INDEX WITH ALKALI SCORE IN DIFFERENT VARIETIES AND ISOGENIC LINES OF RICE

Variety	Description	Selection indices		Alkali score
		Range	Difference	
Jaya	Dwarf *indica*	5 to 31	26	7
Mangala	,,	1 to 19	18	3
Pusa-150	,,	2 to 15	13	4
Halubbulu	Tall *indica*	0 to 2	2	2
Intan	Introduction from Indonesia	5 to 18	13	2
Pushpa	Dwarf *indica*	18 to 55	37	2 & 7
Vani	,,	2 to 50	48	2 & 7
ES 18	,,	3 to 66	63	2 & 7
FT 1	CR line from Pushpa	—	—	2
FT 2	CS line from Pushpa	—	—	7
FT 12	CR line from Vani	—	—	2
FT 19	CR line from Vani	—	—	2
FT 14	CS line from Vani	—	—	7
FT 28	CS line from Vani	—	—	7

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as the arithmetic mean of the percentages of sun-cracked, stress-cracked and green grains. For studying the association of breakage with cracking, paddy was harvested at about 18 per cent average grain moisture. For other studies, harvesting was made prior to formation of cracks in the grain which generally coincided with 24 per cent average grain moisture.

Evaluation of morphological and physical characteristics: The percentages of sun-cracked and stress-cracked grains were estimated as per the method of Srinivas *et al.*[4] Shelling and milling of paddy were done by McGill laboratory sheller and miller respectively. Paddy (160 g) in duplicate were shelled and the brokens were separated in a grader and the percentage of shelling breakage was calculated. The brown rice samples were milled to 10 per cent degree of polish.

Measurement of husk thickness was made in a Mitutoyo Dial Caliper, length (L), breadth (B) and L/B ratio of brown rice were determined as per the method described earlier[12].

Evaluation of physico-chemical characteristics: Ten replicate samples of whole milled rice were prepared for determining the EMC-S per cent[13] and cooking time[14]. For estimating EMC-S per cent, rice samples were soaked in water at 30°C. After 24 hr, water was drained out and the grains were freed of adhering moisture by filter paper (Whatman No. 1) and weighed. The increase in weight was calculated and expressed as EMC-S per cent on wet basis. Data on EMC-S per cent and cooking time were analysed statistically using Duncon's multiple test for comparison. Alkali degradation of milled rice was scored as per the method of Bhattacharya and Sowbhagya[15]. Gelatinization temperature (GT), peak viscosity (PV) and set-back value (SBV) were determined in a Brabender Viscoamylograph[16]. For this, a 10 per cent slurry prepared from a flour of 80 mesh of BSS grade obtained from the milled rice was used.

For determining the total amylose content in milled rice, the colorimetric method of Sowbhagya and Bhattacharya[17] was adopted. Defatted milled rice (100 mg) flour of 45 mesh of BSS grade was treated with 1N NaOH at 30°C for about 12 hr. The dispersion was neutralised with acid and treated with 0.2 per cent potasium iodide and iodine solution. Spekol spectro colorimeter at 630 nm was used for measuring the colour. A standard curve, prepared from iodine treated potato amylose solution, was used for calculating the amylose content in the rice samples (dry basis).

Insoluble amylose content in milled rice was determined as per the method of Bhattacharya *et al.*[18] Defatted milled rice flour (100 mg) of 45 mesh of BSS grade was boiled with distilled water (50 ml) for 20 min over a water bath. The suspension was filtered through Whatman No. 4 filter paper and the soluble amylose present in the filtrate was estimated as above and the insoluble amylose fraction was calculated by difference (dry basis).

For the determination of alkali soluble pentosans in milled rice, the colorimetric method of Cracknell and Moye[19] was followed with a little modification. Defatted milled rice flour (2 g) of 45 mesh of BSS grade was treated with 1.4 per cent KOH solution (20 ml) in a 50 ml stoppered volumetric flask. After 20 hr, the volume was made up with distilled water and centrifuged for 15 min at 2500 rpm. The supernatant was transferred to 50 ml beaker, 0.4 ml of this supernatant was taken in a test tube and 5 ml of the reaction mixture (110 ml AR grade acetic acid + 1 ml 0.8 per cent D glucose+2 ml conc. HCl+5 ml 20 per cent phloroglucinol in absolute ethyl alcohol) was added to it. This mixture was boiled in a water bath for 16 min and cooled. The colour was measured at 552 and 510 nm in a Spekol spectro colorimeter.

A blank was run by using 0.4 ml water+5 ml reaction mixture and boiling for 16 min over a water bath.

A standard curve was prepared from D (+) Xylose by following the above procedure.

The difference in absorbance of samples at 552 and 510 nm was calculated and the pentosan content in the samples was estimated with the help of the standard curve (dry basis).

Bulk volume and consistency of cooked rice[20] were estimated as per the standard laboratory method. Protein content in brown rice was estimated by Kjeldahl method (N×5.95).

Results and Discussion

Establishment of isogenic lines: The versatility of the isogenic lines (i.e., lines of the same variety differing in a single character) as tools in the critical investigations and improvement of the plant characters has been indicated by several workers[21,22]. Srinivas *et al.*[5,6,23] have shown that such isogenic lines could be utilised for the precise study of a single character. In the present study isogenic lines for crack resistant (CR) and crack susceptible (CS) properties were evaluated. Data presented in Table 2 show that the morphological characters such as the husk thickness, length, breadth and L/B ratio of the grains were identical in the isogenic lines of each variety. In CR lines, the percentage of sun-cracked grains varied from 0 to 15 and in CS lines it varied from 48 to 75. Similarly, shelling breakage varied from 1.8 to 17 per cent in CR lines and from 60 to 66 per cent in CS lines. These results show that the pairs of lines described above were identical in all respect except in cracking and breakage properties confirming their isogenic nature.

Hydration and cracking: It is well established that

TABLE 2. PHYSICAL AND CULINARY PROPERTIES ASSOCIATED WITH CRACK RESISTANCE IN RICE VARIETIES[a]

Variety/ Isogenic lines	Cracking property	Sun-cracked grains (%)	Shelling breakage (%)	Brown rice			Husk thickness (μm)	Rice cooking time (min)	Cooked rice	
				Length (L) (mm)	Breadth (B) (mm)	L/B ratio			Bulk vol. (ml/100 g raw rice)	Consistency
Vani										
FT12	CR	5	8	7.00	2.10	3.33	95	17.5±0.52*	420	F
FT19	CR	9	17	6.95	2.05	3.39	95	18.1±0.73*	360	SP
FT14	CS	75	66	6.80	2.20	3.09	95	15.7±0.68	420	VF
FT28	CS	60	64	6.90	2.20	3.14	93	15.4±0.75	400	VF
Pushpa										
FT1	CR	15	17	6.90	2.00	3.45	90	16.6±0.57*	400	F
FT2	CS	48	60	7.00	1.95	3.59	94	13.1±0.61	420	F
Halubbulu										
	CR	0	1.8	6.25	2.40	2.60	90	18.2±0.59	385	SP

CR=Crack resistant; CS=Crack susceptible; F=Fluffy; VF=Very fluffy; SP=Slightly pasty.

[a]Varieties harvested at about 18% (w.b.) moisture were used for cracking and breakage studies and those harvested at about 24% (w.b) moisture were used for other studies.

*Significant at P=0.01 level.

the cracking in cereal grains is influenced by hydration properties and the rate of hydration is known to be influenced by several morphological barriers, such as husk, pericarp, seed coat, nucellus, aleurone layers, etc.[4,13,24]. The influence of protein content on hydration, kernel hardness and milling quality are known[25,26], although its effect on cracking has not been reported. The effect of differences in protein content on cracking could not be studied in the isogenic lines since they did not differ widely in protein content (Table 3).

TABLE 3. CHEMICAL PROPERTIES ASSOCIATED WITH CRACK RESISTANCE IN RICE VARIETIES[a]

Variety/ Isogenic lines	Sun-cracked grains (%)	EMC-S % (w.b.)	Total amylose % (d.b.)	Water-insoluble amylose % (d.b.)	Insoluble amylose as % of total amylose (%)	GT (°C)	Peak-viscosity (PV) BU	Set-back (SBV) BU	Pentosans (%) (d.b.)	Protein (%) (w.b.)
Vani										
FT12	5	28.3±0.32	28.0	16.8	60.0	72	320	300	3.9	6.5
FT19	9	27.8±0.55	27.6	11.8	42.7	68	200	195	4.5	6.4
FT14	75	30.9±0.49*	26.8	14.1	52.6	65	230	395	1.9	6.8
FT28	60	30.6±0.58*	26.8	13.7	51.1	62	175	370	2.0	6.8
Pushpa										
FT1	15	27.1±0.30	30.4	16.7	54.9	66	355	340	4.5	6.2
FT2	48	28.6±0.44*	28.6	15.5	54.1	61	300	460	2.3	6.6
Halubbulu										
	0	26.4±0.55	30.3	14.3	47.1	74	450	450	3.8	7.5

[a]Varieties used in the study were harvested at about 24% (w.b.) grain moisture.

*Significant at P=0.01 level. w.b.=wet basis; d.b.=dry basis;

Starch properties are known to have relation with EMC-S per cent[13]. The differences in the EMC-S per cent of isogenic lines were therefore estimated as an index of starch properties. The EMC-S per cent of CR lines varied between 27.1 and 28.3 per cent and those of CS lines ranged from 28.6 to 30.9 per cent. The difference in the EMC-S per cent was significant between any corresponding CR and CS sister lines (Table 3). The EMC-S per cent of CR variety 'Halubbulu' was the lowest (26.4 per cent) compared to other lines tested. These results indicate that with an increase in the crack resistance of a rice variety, the EMC-S per cent of the grains shows a tendency to get reduced.

Gelatinization temperature and cracking: Data presented in Table 3 show that GT varied from 66 to 74°C in CR lines and 61 to 65°C in CS lines, indicating that crack resistance was associated with higher GT. The association of fissuring resistance with higher GT was also observed by Kunze and Hall[8].

Culinary and textural properties: The milled rices of CR lines took 16.6 to 18.2 min and those of CS lines 13.1 to 15.7 min to cook. The differences were statistically significant (Table 2). This difference could be due to the higher GT of CR lines than of CS lines, since it is reported earlier that higher GT was associated with longer cooking time[7].

Data presented in Table 3 show that in general the PV value of CR lines were higher than the corresponding CS lines. These results by and large are in conformity with those of Juliano *et al.*[27] who have found an association of higher GT with higher PV values.

Alkali score and selection index: An inverse relation of GT with alkali score has been observed[9]. The association of higher crack resistance with lower alkali score recorded in the present experiment (Table 1 and Fig. 1) indicated that the factors causing resistance to cracking as well as alkali degradation may be common.

Juliano *et al.*[27] suggested that alkali score may be a simple and rapid method for estimating GT in rice varieties. They also suggested that alkali test could be an index of stability to a heritable property in lines and crosses since segregating populations will give varied reactions to this test.

Data presented in Table 1 indicate that selection for crack resistance was possible only when the differences in index values among the individuals of the same variety exceeded 37 as in the case of 'Pushpa', 'Vani' and 'ES-18'. When the differences in the index values were 26 or below among the individuals of the same variety, selection could not be done, as in the case of 'Jaya', 'Mangala', 'Pusa-150', 'Halubbulu' and 'Intan' varieties, indicating that the environmental factors under different conditions may bring about a variation in crack resistance amounting upto an index value of about 26.

The index method of selection for crack resistance described above, although based on a mathematical principle, was somewhat time consuming, and the success or failure of selecting a CR line in any particular variety will be known only at an advanced stage of selection. Therefore, an initial screening test was standardised by utilizing the alkali degradation properties of the kernel. Data summarised in Table 1 indicate that in varieties like 'Jaya', 'Mangala', 'Pusa 150', 'Halubbulu' and 'Intan' in which alkali reaction was homogeneous (Fig. 2), intra-varietal selection for crack resistance was not successful whereas in the varieties 'Pushpa', 'Vani' and 'ES-18', in which alkali test indicated heterogeneity (Fig. 3), the selection for crack resistance was possible. These findings suggest the usefulness of a heterogeneity test by alkali treatment in rice varieties to decide whether the crack resistance in any particular variety can be developed by mere intravarietal selection or the property has to be transferred to that variety by hydridization using a donor parent.

Amylose content: Amylose influences the textural property of cooked rice[28,29] in general, while the GT and amylose tended to be independent of each other[27]. The varieties used in this investigation belong to a higher amylose group and therefore, all the CR and CS lines isolated possessed high amylose content ranging from 26.8-30.4 per cent (Table 3). No consistant relation on culinary properties such as volume expansion and pastiness was observed between CR and CS lines. The interaction of amylose and culinary properties with crack resistance could be critically studied if isogenic lines for CR and CS characters possessing low, intermediate and high amylose content were established in different varieties.

Pentosan content: The influence of pentosan on the physico-chemical properties of cereals was studied by several workers. Mod *et al.*[30] and Juliano *et al.*[31] have found no consistant relationship between pentosan content and amylograph viscosity of rice flour. An inverse relation of pentosans with milling and baking qualities of wheat has been reported[32]. Low pentosan was found to accelerate the degerming process in maize[33]. According to Karim and Rooney[34] the kernel hardness in sorghum is related to pentosan.

Data in Table 3 show that pentosan content of milled rice in CR lines and 'Halubbulu' varied between 3.8 and 4.5 per cent which is about twice as that of CS line (1.9 to 2.3 per cent).

Cartano and Juliano[10] have observed the association of lower alkali score with higher yield of pentosans upon extraction with dilute alkali in a white core variety BPI-6. However, they have not studied its cracking

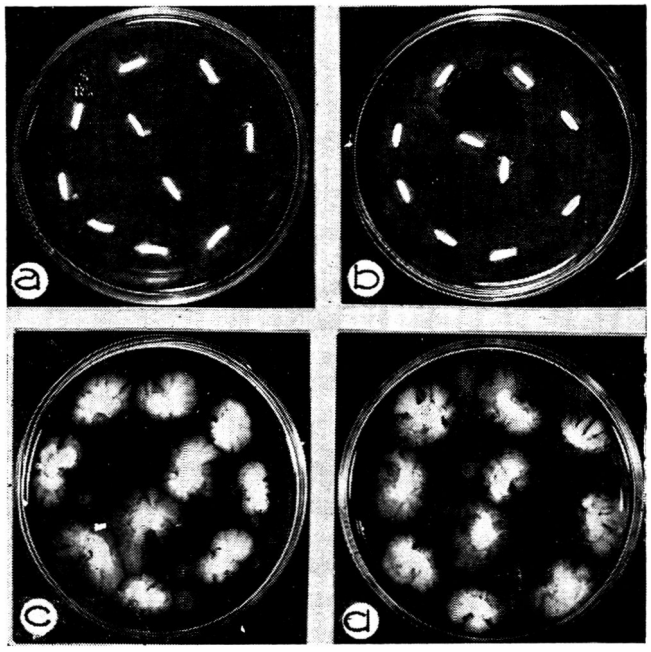

Fig. 1. Milled rice grains of crack resistant (CR) and crack susceptible (CS) lines treated with 1.4% KOH solution to show the association of crack resistance with alkali resistance.

a=FT-1 (CR); *b*=FT-12 (CR)
c=FT-2 (CS); *d*=FT-14 (CS)

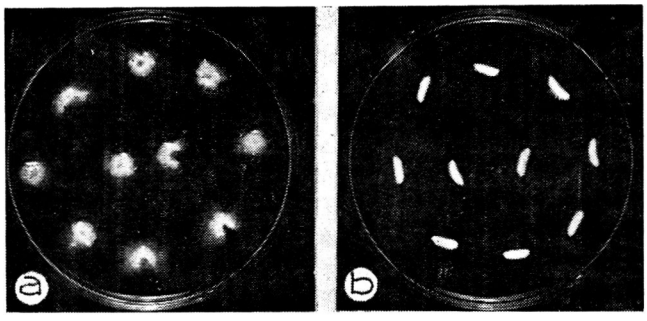

Fig. 2. Milled rice grains treated with 1.4% KOH solution to show the homogeneity of grains to alkali reaction within the variety.

a='Pusa—150'; *b*='Intan'

behaviour. It is clear that pentosan content in rice plays a very significant role in deciding the textural property of the grain and hence its cracking behaviour. It is possible that when the pentosan content increases, adhesion between two cell walls also increases and makes cleavage difficult[35]. With lower pentosan content, the adhesion decreases and cleavage becomes easy.

These results reveal that cracking in rice is a complex phenomenon controlled by several factors which can

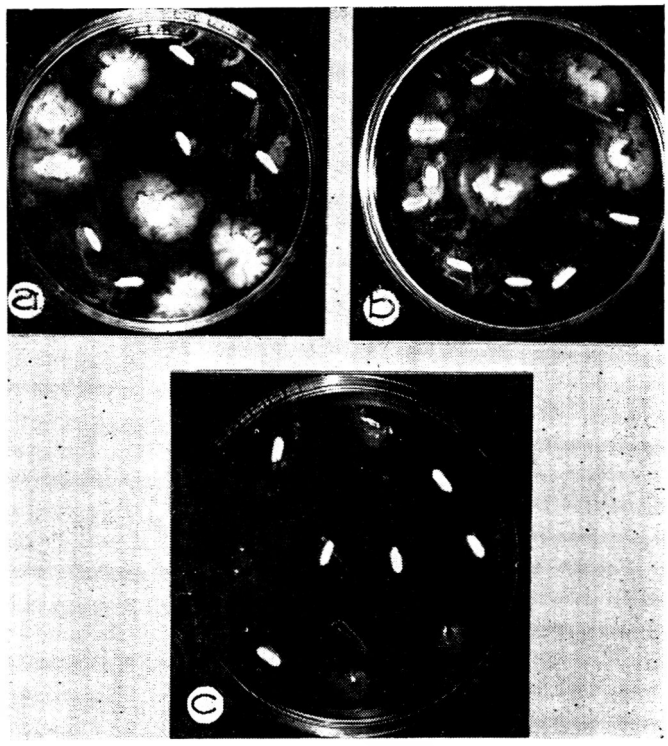

Fig. 3. Milled rice grains treated with 1.4% KOH solution to show the heterogeneity of grains to alkali reaction within the variety.

a=Pushpa; *b*=Vani; c=ES-18

be classified broadly into a 4 different groups. The first group influences the hydration of the grains and thus affects the cracking property only indirectly. The factors in the second group constitute the properties of starch and protein which may affect cracking by their differences in size, density, compactness, crystallinity, molecular size and the degree of branching of the amylopectin fractions. Much study is required to understand clearly the influence of these factors on cracking. The factors in the third group are the cell wall characteristics which may be influenced by quantitative and/or qualitative variations in cell wall constituents, such as cellulose, pentosans and pectins, which would make cleavage along the cell wall line either easy or difficult. The factors associated with the characteristic structure and orientation of cells in the endosperm and cause differences in cracking, fall in the fourth group.

Further physico-chemical and histochemical investigations are being persued to understand the complex inheritance of the cracking properties in rice.

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Effect of Calcium Carbide on Ripening and Quality of Alphonso Mangoes

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Non-uniform and delayed ripening is one of the major problems encountered in mangoes during storage and ripening under ambient conditions. Acetylene, added as calcium carbide at 2 g/kg, ensured uniform and early ripening. The period being reduced to 7 days from 14-17 days in the control. Change in skin colour was associated with softening of fruits which was also recorded with Instron Model 1140. There was sharp decline in acidity and increase in pH and total soluble solids. The rate of carotenoid synthesis in the pulp of treated fruits was higher in the initial stages. However, the total carotenoid content was lower than the control after the fruits were fully ripe. Aroma was less in the treated fruits as indicated by sensory evaluation.

In commercial practice mangoes are harvested while green and stored in bulk. As fruits vary in the degree of maturity and size, ripening is not uniform and poses a major problem. Calcium carbide (which releases acetylene) is one of the chemicals used to achieve uniformity and quick ripening in bananas[1,2] citrus fruits[3,4] and tomatoes[5,6]. The same chemical is used for mangoes also. It is reported to accelerate and ensure uniform ripening without any adverse effect on the quality of mangoes[7]. But impairment of chemical and organoleptic qualities by calcium carbide treatment is reported in 'Pairi', 'Alphonso', 'Banganapalli', 'Totapuri' and, 'Langra' varieties of mangoes[8].

In view of these conflicting observations, a systematic study on the effect of calcium carbide treatment on the ripening of 'Alphonso' mangoes was carried out and the results are incorporated in this paper.

Materials and Methods

Mature, green 'Alphonso' mangoes were harvested at early, mid and late stages (weekly intervals) from an orchard near Mysore. Fruits were sorted into water floaters (specific gravity <1) and sinkers (specific gravity >1)[9]. To prevent fungal attack, fruits were dipped in 1000 ppm Benlate solution for 5 min and air dried.

The fruits were divided into lots of 40 each and placed in ventilated wooden boxes (23 in. $\times$ 10 in. $\times$ 13.5 in.) lined with craft paper. Four packets of 5 g each of calcium carbide (at the level that is used commercially, 2g/kg) were placed in each box. The fruits were covered with straw and craft paper. The boxes without calcium carbide served as control. Five replicates were kept under each group. The boxes were kept at ambient temperature (27 ± 4°C) and at RH 55 ± 15 per cent. The boxes with calcium carbide were kept in an unventilated and controls in a ventilated room separately.

After 96 hr, the calcium carbide packets were removed from the boxes and the uncovered fruits were kept in a separate ventilated room.

Observations periodically carried out are given below:

(*i*) Visual change in surface colour was evaluated by suitably modifying the method[10] (green turning or 50 per cent yellow and edible ripe or 100 per cent orange yellow). Firmness (by finger feel) of the fruits was categorised as hard, turning soft and soft.

(*ii*) Textural studies at the edible ripe stage were conducted of both control and treated fruits using Instron Model 1140 as given below:

(*a*) *whole fruit compression:* Whole fruits were compressed with a flat circular 25.54 cm^{-2} cross section plunger. Force required corresponding to a deformation of 0.5 cm was recorded and expressed in gram per unit cross section area (g cm^{-2}).

(*b*) *Disc compression:* Compression of one cm thick discs of 2.54 cm diameter of mango-flesh was carried out using 25.54 cm^{-2} cross section cylindrical flat plunger to a final clearance of 2 mm. The first peak or the point of major slope change was recorded and the results were expressed in g cm^{-2}.

(*c*) *Magnus-Taylor puncture test*[11]: This was carried out using a flat cylindrical plunger of 1.27 cm diameter to puncture mango-flesh. The first peak was recorded and the results expressed in g cm^{-1}.

For all these tests, the cross head speed was 5 cm-min^{-1}

and the chart speed was 10 cm/min. The fruit orientation in (*a*) and the sample positions in (*b*) and (*c*) were kept constant.

(*iii*) *Chemical composition:* Chemical constituents in the pulp were analysed in triplicate from two composite samples of five randomly selected fruits each and the mean values are reported. Total acidity was determined by the AOAC method[12] and total carotenoids by the partition method[13]. Total soluble solids (expressed as °Brix) were determined by the use of a hand refractometer.

(*iv*) *Sensory analysis:* To evaluate the sensory qualities, a descriptive quality profile procedure was developed as shown in the score card and used for the ripe fruits for individual quality attributes of whole fruits and cut-pieces of each sample by a descriminative communicative panel of 15 judges. The data was compiled and analysed by two-way and three-way analysis of variance[14].

Results and Discussion

The percentage of sinkers in the early, mid and late harvests were 55, 58 and 98 per cent respectively. Hence, floaters were not considered in the late harvest group.

SENSORY EVALUATION SCORE CARD

Name:

(A) DESCRIPTIVE EVALUATION OF MANGOES— WHOLE FRUIT

Evaluate each sample through the descriptors in the scales below by crossing and writing the sample code at any position judged appropriate

Colour and appearance

Green, yellowish green	Glossy, greenish yellow	Glossy appealing yellow, reddish yellow, orange yellow	Slightly dull	Dull, shrivelling, not appealing, black spots, discoloration

Odour (Smell)

Green unripe	characteristic weak, slightly unripe	characteristic strong	characteristic less fresh, slightly overripe	Overripe, foreign/ off odour odour

Firmness (Finger feel)

Hard	Slightly hard	Firm optimal	Tending to soft	Soft

Overall quality

Excellent	Very good	Good	Fair	Poor

Indicate defectives in each sample, if any, by writing the sample code above the appropriate descriptiors given below: Discolouration, black spots, shrivelling

Signature

(B) DESCRIPTIVE EVALUATION OF MANGOES CUT PIECES

Evaluate each sample through the descriptors in the scales below by crossing and writing the sample code at any position judged appropriate

Colour

Uniform, deep yellow, orange yellow, reddish yellow	Uniform, less deep yellow	Non-uniform pale/ yellowish white, white patches, discolouration

Compactness (Visual)

Compact, clean-cut-edge	Not so compact, frayed cut-edge	Not compact, centre softening

Texture

Hard	slightly hard	firm, smooth	slightly soft	mashy

Aroma

Raw, greenisn	Typical, weak, slightly unripe	Typical, full intense	Typical, less fresn, slightly overripe	Over ripe, off/foreign

Taste

Raw, sour	Slightly sour, unbalanced	Optimally sweet, balance	Not sour, less sweet, unbalanced	Flat, bland, off/foreign

Overall quality

Excellent	Very good	Good	Fair	Poor

Indicate defectives in each sample, if any, by writing the sample code above the appropriate descriptors given below: Non-uniform colour, discolouration, white patches, centre softening, foreign/off aroma, foreign/off taste

Signature

TABLE 1. CHANGES IN THE VISIBLE COLOUR AND SOFTNESS OF CALCIUM CARBIDE TREATED ALPHONSO MANGOES

Treatment	% edible ripe coloured fruits after indicated days of harvest				% soft fruits after indicated days of harvest			
	5th	7th	14th	17th	5th	7th	14th	17th
Early harvest								
Control floaters	0.0^a	0.0^a	26.7^a	62.5	0.0^a	0.0^a	33.3^a	70.8
Carbide floaters	71.7^b	81.7^b	—	—	62.5^b	87.5^b	—	—
Control sinkers	0.0^a	3.3^c	60.0^b	—	0.0^a	9.2^c	64.2^b	—
Carbide sinkers	93.8^c	94.4^d	—	—	62.5^b	100.0^d	—	—
Mid harvest								
Control floaters	0.0^a	0.0^a	37.1^a	78.1	0.0^a	1.0^a	39.0^a	78.1
Carbide floaters	40.0^b	96.2^b	—	—	40.9^b	100.0^b	—	—
Control sinkers	0.0^a	7.7^c	81.4^b	—	0.0^a	13.6^c	86.5^b	—
Carbide sinkers	47.6^b	91.4^b	—	—	58.1^c	100.0^b	—	—
Late harvest								
Control sinkers	1.5^a	4.0^a	85.0	—	6.7^a	9.2^a	79.5	—
Carbide sinkers	96.5^b	100^b	—	—	100.0^b	100.0^b	—	—

Mean with different superscripts under each column and harvest differ significantly according to hi-square test ($P<0.05$).

(*i*) *Changes in visible colour and firmness:* Calcium carbide treatment significantly reduced the number of days required to attain edible ripe colour and softness. The percentage of fully coloured fruits was significantly higher in the treated than in the controls at their ripe stage. Control sinkers ripened earlier (14 days) than control floaters (17 days), while this difference disappeared with treatment. Treated fruits in all the batches ripened in 7 days after harvest (Table 1). The changes in softness showed a similar trend with changes in colour (Table 1). Treated fruits showed edible softness within a short duration. Contrary to this, in banana, calcium carbide treatment is reported to produce firmer fruits[1].

(*ii*) *Instrumental texture changes:* The force required to compress the whole fruit by 0.5 cm was higher for control floaters than for sinkers in both early and mid harvests. The trend was better reflected in the disc compression method. The control over treated samples recorded higher compression force. The floaters were more firm than sinkers in the control group. But this differences was narrowed by treatment. Similar trend was recorded by the Magnus-Taylor puncture test (Table 2).

(*iii*) *Chemical constituents:* There was no significant difference between the treated and the untreated fruits at the ripe stage in the levels of total soluble solids, pH and titratable acidity (Table 3). Total carotenoid content of the treated fruits, though higher at initial stages, was however lower than that of control at their edible ripe stage (Fig. 1) as reported earlier.[8].

(*iv*) *Sensory analysis:* Calcium carbide treated fruits developed good colour at the end of 4 days of treatment. Evaluation of these fruits by a small panel of 8 members indicated that the texture was firm, aroma development negligible and the taste sour. Hence, they were again evaluated on the 7th day.

TABLE 2. INSTRUMENTAL MEASURE OF TEXTURE IN ALPHONSO MANGOES TREATED WITH CALCIUM CARBIDE AT RIPE STAGE

Method	Category	Early harvest		Mid harvest	
		Control	Treated	Control	Treated
Whole fruit compression[a] ($g.cm^{-2}$)	Floaters	117	88	87	89
	Sinkers	85	88	66	78
Disc compression[b] ($g.cm^{-2}$)	Floaters	334	232	382	295
	Sinkers	294	214	316	310
Magnus-Taylor puncture test[c] ($g.cm^{-1}$)	Floaters	670	444	588	546
	Sinkers	552	464	430	520

Edible ripe stage: Control floaters-17th day, control sinkers-14th day, carbide floaters and sinkers-7th day.

a: mean of triplicates: *b* & *c*: mean of 6 replicates.

TABLE 3. CHANGES IN THE CHEMICAL CONSITUENTS IN CALCIUM CARBIDE TREATED ALPHONSO MANGOES AT THEIR EDIBLE RIPE STAGE

Treatment	Edible ripe stage (days)	Acidity (% maleic acid)	pH	Total soluble solids (°Brix)
Early harvest				
Control floaters	17	0.30	4.60	17.0
Carbide floaters	7	0.39	4.20	18.0
		(3.93)	(3.50)	(7.5)
Control sinkers	14	0.36	4.00	20.0
Carbide sinkers	7	0.32	4.20	20.0
		(4.07)	(3.45)	(9.0)
Mid harvest				
Control floaters	17	0.19	4.90	15.0
Carbide floaters	7	0.26	4.60	19.0
		(3.93)	(3.15)	(6.0)
Control sinkers	14	0.19	4.80	16.0
Carbide sinkers	7	0.30	4.60	20.0
		(3.87)	(3.10)	(7.5)
Late harvest				
Control sinkers	14	0.19	4.80	18.0
Carbide sinkers	7	0.26	4.30	18.0
		(2.95)	(3.10)	(8.0)

Values given in parenthesis are for the raw fruits in the respective floaters (both control and treated)/sinkers (both control and treated) in the corresponding groups.

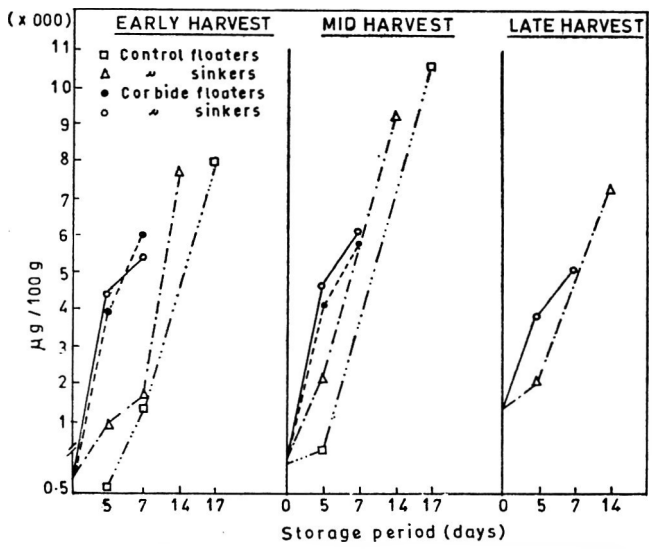

Fig. 1: Total carotenoids in the pulp of calcium carbide treated Alphonso mangoes during ripening

TABLE 4. SENSORY QUALITY OF CALCIUM CARBIDE TREATED ALPHONSO MANGOES AT THEIR EDIBLE RIPE STAGE

Quality attributes	Floaters and sinkers from early and mid harvests				Sinkers from early mid and late harvests	
	Control	Treated	Floaters	Sinkers	Control	Treated
Whole fruits						
Colour & appearance	3.16[a]	3.95[b]	3.32[c]	3.79[d]	3.53	3.77
Odour	3.71	3.79	3.49[c]	4.01[d]	3.97	3.77
Firmness (finger feel)	4.65[a]	5.45[b]	4.93	5.18	4.84[e]	5.89[f]
Overali quality*	79	93	75	97	89	84
Cut-pieces						
Colour	3.94	3.40	3.73	3.62	3.90[e]	3.31[f]
Compactness	4.24	4.36	4.31	4.28	4.09	4.29
Texture	4.85[a]	5.37[b]	5.12	5.10	5.17	5.57
Aroma	4.84[a]	3.95[b]	4.28	4.52	4.99[e]	3.98[f]
Taste	4.91	4.85	4.89	4.87	5.04	5.14
Overall quality*	81	78	68	92	90	70

Mean score: 1 to 5 to 9 unripe to optimally ripe to overripe quality.

Figures with different superscripts in the same row separately for each group differ significantly.

*% panelists grading the sample as good.

In the treated fruits, the mean score was significantly higher with respect to external colour and appearance, and they were more soft than control fruits. In the cut-pieces, the treated fruits were more soft in texture and less intense in aroma. Between floaters and sinkers from both control and treated groups, the sinkers were better in colour and appearance and odour. No difference could be seen in cut-pieces for the individual attributes. (Table 4).

Overall quality grading in the control group showed agreement between the whole fruits and cut-pieces, whereas in the treated group it was lower for the cut-fruit. Sinkers scored higher grade in overall quality for whole fruit as well as cut-fruit than the floaters (Table 4).

When only the sinkers were considered, the whole fruit was more soft in the treated. This was clearly evidenced by the textural studies (Table 2). In the cut-pieces, the flesh colour was less as reflected by the less carotenoid content (Fig. 1) and also the intensity of aroma was lower (Table 4).

Thus, calcium carbide treatment of 'Alphonso' mangoes resulted in the development of uniform and attractive yellow skin colour and quick softening of fruits. This advantage is being exploited commercially for ripening and marketing mangoes. However, there was less synthesis of carotenoids in the pulp and aroma development. This is in accordance with the report of Pattabhiraman *et al.*[15] that the intensity of aroma and flavour in the mango fruits is proportional to the carotenoid content.

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Freezing Preservation of Totapuri Mango Pulp

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The storage quality of 'Totapuri' mango pulp, as such or with addition of ascorbic acid, which was frozen as slabs in polyethylene bags (52×40×3 cm) and stored at −18°C for a period of 14 months was studied along with canned pulp for comparison. Ascorbic acid, total carotenoids, chroma and viscosity decreased in all the samples, more pronounced in the control during prolonged storage. Sensory evaluation of the nectar prepared from the frozen pulp at the end of 6, 10 and 14 months of storage indicated that the frozen product had developed an off-flavour which could be removed either by pasteurizing the pulp before freezing or heating the frozen pulp before use. Ascorbic acid added to the pulp helps to retain the colour, flavour and aroma during long storage.

Mango (*Mangifera indica* L) is the most popular and choicest fruit of the tropical countries. Pulp from mango is canned for use in ready-to-serve beverages, juices, nectars, ice-creams, squashes, jams etc. Freezing is known to retain the quality of the product for a long time during storage. Mathur *et al.*[1] and Adsule and Roy[2]

have studied the storage quality of mango pulp frozen in cans using slow freezing method. In this paper, storage quality of 'Totapuri' mango pulp quick frozen in the form of slabs in polyethylene bags and stored at –18°C for a period of 14 months is presented.

Materials and Methods

Ripe 'Totapuri' mangoes from the local market were washed, cheeks removed and pulped. The pulp was divided into three portions, one was frozen as such (control), to the second, ascorbic acid added at the rate of 50 mg per 100 g and frozen and the third was canned.

For freezing, the pulp was filled into 200 gauge polyethylene bags of the size 52×40×3 cm and sealed. Each bag contained 7 kg of pulp. The sealed bags were kept in trays and frozen in a plate freezer at –40°C. Nine slabs were made from each portion. Time required for freezing was $4\frac{1}{2}$ hr. The frozen slabs were stored at –18°C.

For canning, the pulp was mixed with citric acid to raise the acidity to 0.5 per cent, heated to 85°C, filled into plain A $2\frac{1}{2}$ cans, sealed, processed for 30 min in boiling water and cooled. These cans were stored at 4°C to prevent changes occurring at ambient temperature. The canned pulp was used as control.

After 3,6,10 and 14 months of storage, frozen and canned pulps were analysed for total soluble solids, acidity, ascorbic acid, total carotenoids, chroma and viscosity. A day prior to analysis, the frozen samples were transferred to a refrigerator for slow thawing. Brookfield viscometer at 60 rpm was used for measuring viscosity. Reflect colour was measured by using Spectronic-20 colorimeter with reflectance attachment. The samples were taken in glass cells (American Company's 5-820 cell) for the colour measurement. Chroma was calculated according to the procedure outlined earlier[3]. Ascorbic acid in the samples was determined by visual titration method with standard 2-6 dichlorophenol indophenol dye[4]. Total carotenoids in the pulps were extracted with acetone and transferred to petroleum ether (b.p. 60-80°C). Colour was measured at 450 nm in Spectronic-20 colorimeter and expressed as β-carotene[5]. All the analyses were made in triplicate and the mean values are presented.

Sensory evaluation of the frozen pulp after thawing and canned pulp was made both as pulp and after converting into nectar. The nectar having 20 per cent pulp, 15 °Brix, and 0.3 per cent acidity was subjected to sensory evaluation before and after bottling. The nectar was heated to 85°C for 5 min, filled in bottles, crown corked, processed in water at 75°C for 5 min and cooled. Colour, aroma, flavour and overall quality of the pulps were analysed by rank sum method[6], the best one was assigned rank 1, by a panel of 12-17 panelists. Bottled nectar samples were evaluated by a panel of 17-19 members for difference and preference by preference matrix analysis[7] in addition to rank sum analysis.

Results and Discussion

There was no significant change in TSS and acidity in the stored pulp from all the treatments (Table 1).

TABLE 1. CHANGES IN PHYSICO-CHEMICAL CHARACTERISTICS OF FROZEN AND CANNED TOTAPURI MANGO PULPS DURING STORAGE

Storage period (months)	TSS (°Brix)			Acidity (%)			Ascorbic acid (mg/100 g)			Total carotenoids as β-carotene (μg/100 g)			Chroma (%)			Viscosity (cp.)		
	Frozen		Canned	Frozen		Canned	Frozen		Canned	Frozen		Canned	Frozen		Canned	Frozen		Canned
	Control	A.A. added		Control	A.A. added		Control	A.A. added		Control	A.A. added		Control	A.A. added		Control	A.A. added	
0	16.5	16.5	16.0	0.45	0.45	0.50	17.0	65.0	14.0	4200	4200	4100	49.0	49.0	49.5	3950	3950	3795
3	16.5	16.5	16.0	0.46	0.46	0.51	10.0 (58.8)	53.0 (81.5)	11.5 (82.1)	3560 (84.8)	4050 (96.4)	4070 (99.3)	45.0 (91.8)	48.0 (97.9)	48.0 (97.0)	3760 (95.2)	3870 (98.0)	3740 (98.5)
6	16.2	16.5	15.8	0.45	0.46	0.52	6.1 (35.9)	45.0 (69.2)	10.5 (75.0)	3250 (77.4)	3980 (94.8)	3995 (97.4)	42. (86.7)	46.51 (94.1)	47.2 (95.3)	3452 (87.4)	3620 (91.6)	3650 (96.2)
10	16.0	16.0	15.8	0.47	0.43	0.50	1.4 (8.2)	29.5 (45.4)	9.8 (70.0)	2588 (64.0)	3570 (85.0)	3820 (93.2)	32.5 (66.3)	42.2 (86.1)	46.8 (94.5)	2650 (67.1)	3220 (81.5)	3525 (92.9)
14	16.0	16.0	16.0	0.48	0.47	0.52	0.9 (5.3)	12.8 (19.7)	8.9 (64.6)	2150 (51.2)	3240 (77.1)	3750 (91.5)	27.0 (55.1)	36.4 (74.3)	45.4 (91.7)	2260 (57.2)	2950 (74.7)	3410 (89.9)

Figures in parenthesis indicate percentage of initial content

A.A.—Ascorbic acid.

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There was reduction in ascorbic acid content in all the samples. After 10 months storage, the retention of ascorbic acid was 8.2, 45.4 and 70.0 per cent, in control frozen pulp, ascorbic acid added and frozen and canned pulps respectively. Loss of ascorbic acid in frozen control pulp was faster than in pulp fortified with ascorbic acid and frozen or canned. This may be due to higher activity of oxidative enzymes in the control pulp. Ascorbic acid in the ascorbic acid fortified pulp acted as an antioxidant. Retention of ascorbic acid was better in canned than in frozen pulps, possibly due to (*i*) heat inactivation of oxidative enzymes during processing and (*ii*) tin in the cans acting as a protectant to ascorbic acid[2].

Total carotenoid content in both frozen and canned samples decreased during storage. The losses were greater in the frozen natural sample (36 per cent) than in ascorbic acid added sample (15 per cent) and were least in canned samples (6.8 per cent). The values further decreased after 14 months of storage. Colour as per cent chroma also decreased during storage and the trend was similar to the carotenoid losses.

The viscosity of pulps before freezing and immediately after canning was 3950 and 3795 cp respectively and decreased during storage. After storage of 10 months, the values were 2650, 3220 and 3525 cp in the control frozen pulp, ascorbic acid added and frozen and canned pulps respectively. The decrease in viscosity was more in frozen pulps than in canned pulp. The decrease in viscosity in frozen pulps may be attributed to the activity of the pectin degrading enzymes.

The canned pulp had been subjected to heat treatment during processing but not the frozen pulps. At the end of 6,10 and 14 months of storage the sensory characteristics with respect to colour, aroma, flavour and overall quality of the canned pulp were significantly superior; pulp without added ascorbic acid was significantly inferior; and the pulp with added ascorbic acid was neither significantly superior nor inferior (Table 2). These results show that the canned product stored at low temperature is superior to pulp frozen without any heat treatment. The observations on the nectars prepared from these pulps subjected to sensory evaluation without any further heat treatment were similar to those observed in the case of pulps.

Nectars prepared from canned or frozen samples but

TABLE 2. RANK SUM DATA FOR SENSORY QUALITY OF PULPS AND NECTARS PREPARED FROM STORED PULPS

Storage period (months)	Judges (no.)	Canned pulp				Frozen pulp							
						Ascorbic acid added				Without ascorbic acid			
		Colour	Aroma	Flavour	Overall quality	Colour	Aroma	Flavour	Overall quality	Colour	Aroma	Flavour	Overall quality
Pulp													
6	11	13**	12**	12.5**	12.5**	25.5	19.5	22.0	22.0	28.5**	30.5**	31.5**	31.5**
		sup.	sup.	sup.	sup.	n.s.	n.s.	n.s.	n.s.	inf.	inf.	inf.	inf.
10	12	12**	13**	12**	12**	24.5	23.5	24.5	24.5	35.5**	35.5**	35.5**	35.5**
		sup.	sup.	sup.	sup.	n.s.	n.s.	n.s.	n.s.	inf.	inf.	inf.	inf.
14	16	16**	16**	16**	16**	35.5	40.5**	39.5**	40.5**	44.5**	39.5**	40.5**	39.5**
		sup.	sup.	sup.	sup.	n.s.	inf.	inf.	inf.	inf.	inf.	inf.	inf.
Nectar before pasteurization													
6	11	12**	13**	16**	13**	25.5	23.5	20.5	23.5	28.5**	29.5**	29.5**	29.5**
		sup.	sup.	sup.	sup.	n.s.	n.s.	n.s.	n.s.	inf.	inf.	inf.	inf.
10	12	12**	12**	11**	11**	23.0	23.5	23.5	23.5	32.0**	30.5**	31.5**	31.5**
		sup.	sup.	sup.	sup.	n.s.	n.s.	n.s.	n.s.	inf.	inf.	inf.	inf.
14	16	16**	16**	16**	16**	37.5	39.5**	40.0**	40.0**	42.5**	40.5**	40.0**	40.0**
		sup.	sup.	sup.	sup.	n.s.	inf.	inf.	inf.	inf.	inf.	inf.	inf.
Nectar after pasteurization													
14	20	23**	21**	23.5**	22**	44.0	41.0	37.0	38.0	53.0**	58.0**	58.0**	58.0**
		sup.	sup.	sup.	sup.	n.s.	n.s.	n.s.	n.s.	inf.	inf.	inf.	inf.

**Significant at 1% level; Sup: Superior; Inf: Inferior
n.s: not significant.

subjected to heating before sensory evaluation showed no significant difference with respect to colour, consistency and flavour at the end of 6 months of storage. The observations at the end of 10 months of storage were similar in the case of nectar prepared from canned sample and frozen pulp containing added ascorbic acid while that prepared from pulp frozen without added ascorbic acid was significantly inferior. At the end of 14 months of storage, the nectar prepared from the canned pulp was superior, from the pulp frozen without added ascorbic acid was inferior and with added ascorbic acid not significant. At the end of 6 and 10 months storage, the sensory evaluation was done by preference matrix analysis and by rank sum analysis at the end of 14 months of storage.

The results show that the mango pulp frozen without pasteurization develops off-flavour during storage, the intensity of which is less when the pulp is fortified with ascorbic acid. The off-flavour produced is, however, heat labile. Hence, when the pulp is used for products where heating is involved before consumption, this disadvantage as compared to canned product with respect to flavour is overcome. For such purposes, mango pulp frozen with added ascorbic acid is similar to canned pulp with regard to colour, consistency and flavour even after prolonged storage, while the pulp frozen without added ascorbic acid (control) is inferior.

The off-flavour which develops during frozen storage could however be overcome, if the pulp is heated before freezing. Avena and Luh[8] have recently reported the changes during frozen storage of pasteurized mango puree with and without added sugar. No such development of off-flavour were encountered during storage. Hence pasteurization before freezing could overcome this disadvantage.

The manufacturers of ready-to-serve beverages pasteurize the mango pulp and store at 4°C in wide mouthed 18 l. tins lined with polyethylene film for subsequent use. However, the storage life of such pulps is reported to be only 3-4 months. 'Totapuri' mango pulp having 0.5 per cent acidity and 'Alphonso' mango pulp having 0.6 per cent acidity would have a pH less than 4.0. Heating of such pulps to 95°C would inactivate the heat resistant enzymes and destroy the spoilage organisms. Based on the results of the present study, it may be stated that the shelf life of such pulps packed as stated above could considerably be increased by storing at −18°C. Alternately, the pasteurized pulp may be filled hot into the narrow mouthed plain 18 l containers, the lid is sealed by soldering, and the sealed container was tilted upside down to check leakage and to sterilize the lid and allowed to air cool or cooled in chlorinated water. The product would behave in the same way as the canned product when stored at 4°C or at ambient temperature. However, such cans are not suitable for reuse. This is based on the experience of one of the authors while working in a factory[9].

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Improving the Quality of Soybean (*Glycine max* (L.) Merril) for Human Consumption: Factors Influencing the Cookability of Soybean Seeds

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Cookability and associated tests conducted in this study involved determination of (i) rates of cooking, (ii) water uptake during cooking and at room temperature, and (iii) influence of seed size on cookability and water uptake. Cookability was found to be negatively associated with water uptake during cooking; soybean lines which absorbed water more rapidly tended to cook in a shorter period of time. Seed size was observed to influence cookability and water uptake. Generally small seeds tended to cook faster than larger seeds suggesting that breeding and selection for small seed sized soybean varieties may help overcome the problem of cookability encountered in soybean. In all experiments involving water uptake, the influence of temperature or seed size on water uptake was not the same on all the lines tested.

Soybean is progressively becoming a world-wide potential source of major nutrients required for normal diet. As high as 45 per cent of the dry matter is protein and the amino acid pattern approaches the optimum recommended by FAO[1]. Soybean also contains about 20 per cent oil, which is very desirable because it contains a large proportion of unsaturated fatty acids.

Soybean has not been a popular food crop in many parts of the world, despite its high nutritional value. The Protein Advisory Group Statement 22 identified cookability or difficulty-to-cook of beans as a serious, universal problem limiting the direct use of soybeans. Rice[2] and Muellar *et al*[3]. recommended cooking times of up to three hours in order to produce a food product from mature soybean seeds. Long cooking times discourage people from continuing to incorporate soybeans in their normal diets.

The objective of this study was to investigate factors that influence the cookability of soybeans in order to provide information that may be useful to breeders and food processors attempting to overcome the problem of cookability encountered in soybeans.

Materials and Methods

Soybean lines from the cross 'Big Jule'/'Bethel' were grown in replicated field plots at Hawkesbury Agricultural College of Advanced Education, Richmond, Australia. All soybean seed lots used in the tests were fully matured seeds and were stored in a controlled atmosphere seed storage room at 10°C and humidity of 30 per cent for a minimum of two weeks before being used, so as to enable the seed lots to equilibriate at the same seed moisture content. Cookability and associated tests were conducted in the experimental food technology laboratory at Hawkesbury Agricultural College of Advanced Education.

To determine line variation in cooking time and rate of water uptake, seed lots representing a number of lines and field plot sources were pressure cooked for three different times at 116°C. Seeds of nearly uniform size (about 7.6mm in diameter) of each seed lot were obtained by sieving. Clean whole seeds were weighed and placed in cans (75mm×117mm) and the cans were filled with tap water and sealed. The cans were then placed in a retort, which was steam heated at 116°C. The beans were cooked for 10, 20 and 30 min. For each of the three cooking times, timing started when the steam heated retort reached 116°C, which took about 5 min. The assumption made was that the cans and its content would also attain this temperature

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within this period given the small size of the cans and the quantity of the beans used. After cooking for the respective times, the cans were cooled in a waterbath. The cans were then opened, the water drained and the beans were weighed to determine water uptake. The cooked beans were then tested for hardness using a texture measuring instrument, the Maturometer, Australian Patent No. 143316. This instrument measures the resistance or force required to puncture beans by pins. The details of the theory and mechanism of the instrument are described by Mitchel *et al*[4].

To determine any influence of seed size on cookability and water absorption during cooking and during soaking at room temperatures, seeds of three sizes of each of six soybean lines derived from the 'Big Jule'/'Bethel' cross were used. The three seed sizes were obtained by sieving the mixed seed lots as harvested from individual field plots. The size classes contained seeds centred on 8.4 mm diameter (range 8.1-8.7 mm), 7.6 mm diameter (range 7.2-8.0 mm), and 6.5 mm diameter (range 6.5-7.1mm). Cookability and water uptake of the various seed sizes were determined as described above, excpet that in this experiment a single time of cooking of 10 min at 116°C was used. Influence of seed size on water uptake during soaking at room temperature (20-25°C) was determined by soaking weighed samples of the three size classes for 30, 60, 180, 240, 360 min.

Results and Discussion

1. *Effect of cooking time on cookability and water uptake:* A commercial product of canned beans (Sanitarium Soybeans) was used as a reference for accpetable tenderness. The Maturometer reading for the commercial product was 27.2kg. Forty one soybean lines were used to test the effect of cooking time on hardness and water uptake. Mean squares obtained from the analysis of the three periods are given in Table 1. Genetic differences among lines were shown by the significant mean square values for lines in the analysis of variance. Significant line by cooking time interaction was observed indicating line differences in the relationship of cookability and water uptake with cooking time.

Fig. 1 shows the effect of cooking time on hardness for some of the soybean lines tested. All the lines showed a decrease in hardness with cooking time, however, some lines required shorter time to reach acceptable tenderness. Fig. 2 shows the effect of cooking time on water uptake for some of the lines tested. An increase in water uptake with time was shown by some lines while others such as 'Jeth 3', 'Jeth 60' and 'Big Jule', showed a decrease in water uptake with cooking time. The decrease in water uptake exhibited by some of the lines could be due to disruption of cells during cooking with release of cell-bound water.

The correlation coefficient for the association of hardness after cooking and water uptake during cooking was $r=0.76^{**}$. The observed association is that, lines which absorb water more rapidly tend to cook in a shorter period of time.

Differences in cookability observed among lines may be due to differences in seed characteristics.

TABLE 1. MEAN SQUARES OBTAINED FROM ANALYSIS OF VARIANCE FOR COOKABILITY AND WATER UPTAKE OF PARTICULAR SOYBEAN LINES TESTED AT THREE COOKING TIMES

Source	df	Cookability	Water uptake
Blocks	1	0.68	18.40
Lines	40	714.78**	269.54**
Time	2	28081.38**	42.69**
Line×Time	80	138.76**	34.61**
Error	122	32.49	9.00

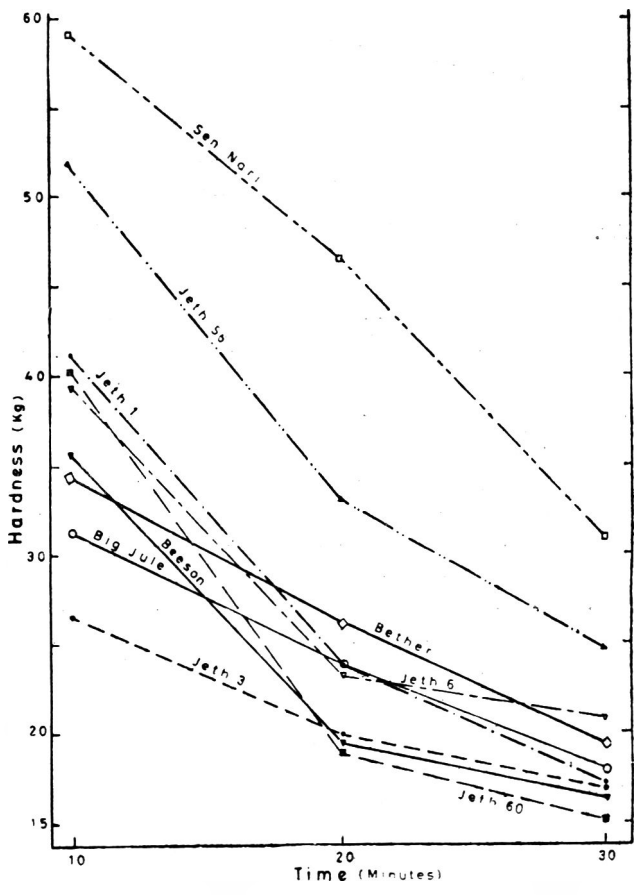

Fig. 1: Effect of cooking time on hardness of the cooked soybeans of particular lines

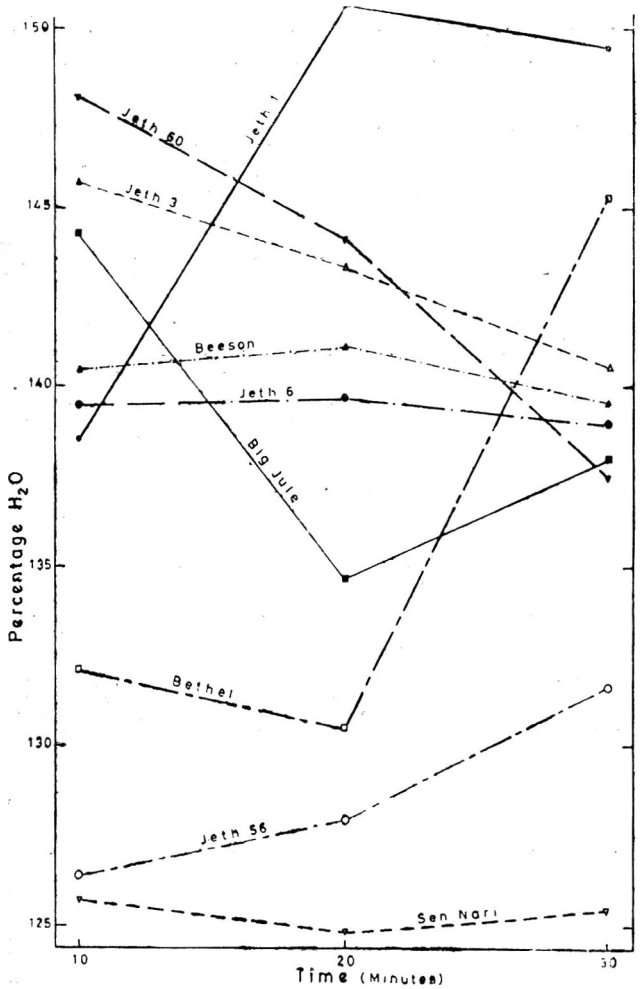

Fig. 2. Effect of cooking time on water uptake of particular lines of soybeans.

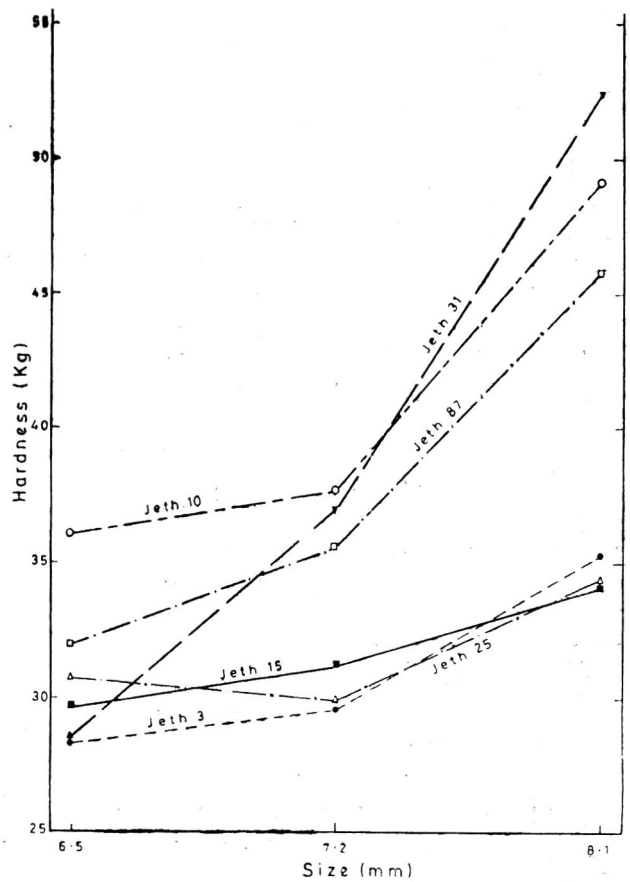

Fig. 3: Effect of seed size on hardness after cooking for 10 minutes at 116°C

Softening due to cooking occurs because of structural changes which take place during cooking. With non-oil legumes these structural changes include the breakdown of the middle lamella, leading to easy separation of cells[5]. The same authors[5] also observed that in soybeans, heating caused separation of intact cells with no evidence of the breakdown of the middle lamella. The observed differences in cookability among lines observed in this study could be due to genetic differences in cell separation during cooking and in seed coat characteristics affecting water uptake which is associated with cookability.

2. Influence of seed size on cookability and water uptake:

During cooking: The relation of seed size to hardness after 10 min of cooking is shown in Fig. 3. The six soybean lines showed an increase in hardness with increasing seed size. Within lines, small seeds tended to be softer after cooking than larger seeds. The effect of seed size on water uptake during cooking is shown in Fig. 4. Seed size appears to affect water uptake; smaller seeds absorbing more water than larger seeds.

During soaking: Soaking soybean is an essential step in preparation of soybean products for human consumption. Seed size was found to influence water uptake during soaking. Some soybean lines showed an increase in water uptake with increasing seed size while others showed a decrease in water uptake with increasing seed size (Fig. 5). Significant correlations were found between (*i*) hardness after cooking and water uptake during soaking ($r = -0.54^{**}$) and (*ii*) water uptake during cooking and water uptake during soaking at 25°C ($r = 0.49^{**}$). The amount of water absorbed during soaking increased with soaking time for all lines and seed size classes studied.

Hsu *et al*[6]. found that water uptake by soybeans during soaking was influenced by seed size. Small seeds were observed to absorb more water than larger seeds, and this was explained as being due to the fact that smaller bean kernels provide more surface area per unit mass for mass transfer. Calero *et al.*[7] found that the capacity

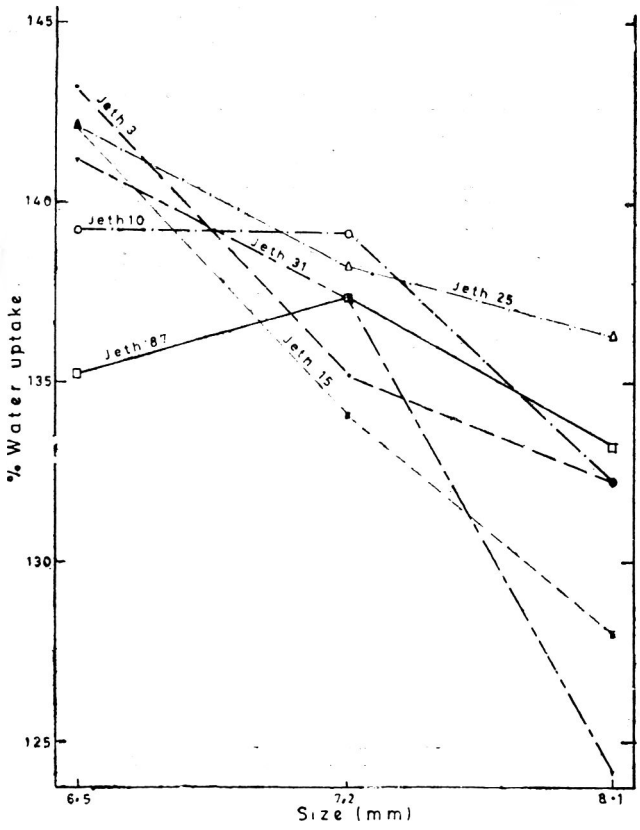

Fig. 4: Effect of seed size on water uptake by seeds of particular soybean lines during cooking for 10 minutes at 116°C

to absorb water in soybeans was dependent on the shape and size of functional pores on the seed coat, and the amount of waxy material embedded in the epidermis. Small seeds were found to have near round and functional pores, whereas large seeds were found to have distorted pores due to the size effect, thus enabling small seeds to absorb more water than large seeds. The differences in water uptake between small seeds and large seeds observed in this study could also be due to differences in shape and size of functional pores, and other structural differences.

Within homozygous lines, differences in seed size are due to environmental and/or developmental causes. The influence of seed size on cookability observed within soybean lines from the cross 'Big Jule' 'Bethel' may not be genetically important. However, the effect of seed size observed within lines shows the need to have seed samples of uniform size when carrying out tests involving selection of soybeans for cookability. All test samples need to be stored at the same moisture and temperature conditions prior to testing since cookability has been observed to be affected by these factors[8]. Lebedeff[9] found genetic differences among lines of dry beans (*P. vulgaris*) in the development of hardseededness during storage, which was also affected by storage conditions. Storage of seeds in conditions used in this study (10°C and 30 per cent RH) for two or more weeks enables samples of various origin to attain the same moisture level prior to testing. No hardseededness was observed in this study.

The results of this study also suggest that breeding and selection for small seed size may help overcome the problem of cookability encountered in soybean. However, more research is required to evaluate the genetic nature of the relationship between seed size and the cookability of soybean seeds. Mwandemele *et al.*[10] have demonstrated genetic variation in cookability in soybean and observed the character to be highly heritable. This suggests that cookability can be improved through breeding and selection, which may help reduce costs of processing and save energy.

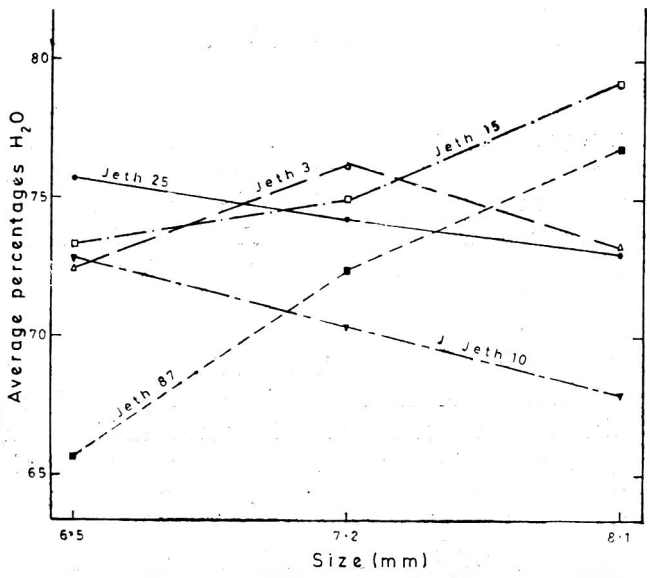

Fig. 5: Effect of seed size on water uptake by seeds of particular soybean lines during soaking at room temperature for four hours

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Adsorption of Salt Soluble Fish Proteins at Peanut Oil/Water Interface

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The adsorption charactersitics of salt extracted fish protein on peanut oil have been studied quantitatively. The effect of ionic strength, pH, denaturant and temperature on the adsorption isotherm have been compared with each other. The adsorption was found to decrease with increasing salt concentration at pH away from isoelectric point, denaturant and decreasing temperature. The adsorption of proteins is multilayer in nature and decrease in adsorption is possibly due to denaturation. From thermodynamic analysis of the results, the parameters such as $\triangle G^\circ$, $\triangle H^\circ$ and $\triangle S^\circ$ have been calculated quantitatively and the nature of adsorption discussed.

Proteins are good food emulsifiers[1]. In many proteinous foods such as fish and meat sausage, the food material is actually present in the form of oil/water or water/oil emulsion which is stabilised by the proteins. The stability of these food materials at various conditions depends on the nature of the protein film forming an adsorbed layer on the fat particles. The change in texture and toughness during frozen storage of fish have frequently been attributed to the denaturation of the adsorbed proteins during cold storage process[2,3]. These correlations are however indirect and quantitative data of fish protein adsorption on food materials seldom exist.

It is well known that in all biological systems proteins are in complicated adsorption interaction with lipids and other biological components. It is also known that proteins get denatured when adsorbed at an interface[4,5]. However, some workers[6-8] have reported that proteins may retain a certain degree of native conformation even in the adsorbed state. Fish muscle is composed of a heterogeneous mixture of proteins. Presently some information is available on the adsorption of pure and single protein at hydrocarbon oil/water interface[8-10]. But the adsorption behaviour of mixed protein systems is unknown. As mixed proteins actually exist in real biological systems, the adsorption characteristics of such systems will be more meaningful in understanding several phenomena which are known to be controlled by adsorbed proteins, like cellular aggregation[11], cellular growth[12] in cell membranes and digestion of insoluble fats[13] in food materials. Moreover, as fish is the raw material for several processed food products,

adsorption studies of fish proteins on food materials may be helpful to food processing industry.

In the present paper an attempt has been made to study the adsorption behaviour of salt extracted fish proteins at an oil/water interface. The effect of the change of pH, salt concentration, denaturant and cooling on the adsorption behavior is studied. As peanut oil is extensively used in India in the preparation of food-stuffs by the industry and also used as a cooking oil, double refined peanut oil was selected for adsorption study of fish proteins. Attempts have been made to understand the conformational aspects of the adsorbed fish protein and the amount adsorbed has been interpreted from the fundamental mechanism of the adsorption process.

Materials and Methods

Fish protein used for the experiment was taken from fresh-water fish 'Rohu' (1-1.5 kg total wt.) procured from market. Skin was removed and bone was separated from the muscle. Water distilled oevr alkaline permanganate solution was used throughout the experimental work.

All inorganic salts used were of analytical grade. Double refined peanut oil used as the oil phase was purchased from the market and used without further purification. The interfacial tension of this oil against double distilled water, viscosity and density agree well with the reported values[10]. The GLC data of this oil shows the same fatty acid compositions as reported elsewhere[10]. The oil is also highly stable as no detectable rancidity could be identified within one month storage period during which this work has been carried out.

Fish muscle was cut into small pieces after removing bone and skin. This muscle was extracted with salt solution of different molarity by rotating in a waring blender at high speed for 5 min. The slurry was then blended in an electric blender fitted with perforated socket rotated at 4000 rpm for 10 min. The slurry was then centrifuged at 5000 rpm and the centrifugate filtered through filter paper. This filtrate was diluted with salt solution having the same molal concentration as used for extraction. Thus, different protein solutions (each 40 ml volume) of different concentrations were prepared in 125 ml reagent bottles fitted with standard joints. These solutions and a definite volume of peanut oil were separately thermostated at the experimental temperature for 2 hr.

The protein solutions were transferred to a 250 ml beaker and 5.0 ml of peanut oil was added to it from a burette. The mixture was blended in an electric blender, fitted with a perforated socket at the bottom, at 4000 rpm. The time of emulsification was 5 min in all cases. Immediately after preparation, the emulsion was poured into the reagent bottles and kept under controlled temperature. During storage, the emulsions were occasionally shaken by hand.

The particle size and hence the surface area of the oil droplets in the emulsions measured after 18 hr of preparation was estimated by turbidometric method[8] in a Klett-Summerson photoelectric colorimeter '(Philadelphia, Pa.) using red filter. Surface area was directly read out from a turbidity vs. surface area standard plot. To draw the standard plot, five emulsions having same composition but different particle size were taken. Definite amount of emulsion was taken in test tubes and diluted five times with the salt solution in which protein was extracted. The turbidity of the emulsions were measured by Klett-Summerson colorimeter. To measure surface area, 1 ml of each emulsion was diluted 25 to 50 times with the continuous phase. Two drops of this diluted emulsion were placed on a clean glass slide, covered with a cover slip (15 × 15 mm), and placed under a microscope (Leitz, Germany) fitted with micrometer scale. Magnification was kept in the range of 40 × 16. The diameters of droplets of different sizes were measured with the help of micrometer scale from 4/5 different representative field of the slide. 250/300 droplets were measured for each emulsion. The mean volume surface diameter (d_{vs}) was calculated from the following relations:

$$d_{vs} = \frac{N_1 d_1^3 + N_2 d_2^3 + N_3 d_3^3 + \ldots}{N_1 d_1^2 + N_2 d_2^2 + N_3 d_3^2 + \ldots} = \frac{\sum_i N_i d_i^3}{\sum_i N_i d_i^2} \quad \ldots (1)$$

where N_i is the number of droplet, having a diameter d_i. d_{vs} is related to the average surface area S by the equation

$$S = \frac{6}{d_{vs}} \quad \ldots (2)$$

where S is in units of square meters per milliliter of oil, if d_{vs} is expressed in micrometers.

Protein concentration was determined by the method of Lowry *et al*[14]. After turbidity measurement, the emulsions were allowed to cream by keeping them undisturbed for nearly 5 hr. The turbid protein solution below the cream was taken out by means of a syringe. During protein concentration determination, turbidity corrections of these solutions against blank was made. Before addition of the colouring reagents, solutions were diluted in the range of 10 to 150 μg/ml. Readings were taken in Klett-Summerson photoelectric colorimeter using red filter. Protein concentration was determined using calibration curve made with standard solutions of BSA (bovine serum albumin).

Knowing the percentage concentration of the protein solution before and after adsorption and the mean

volume surface diameter of the emulsion droplets, T_p, the amount of protein adsorbed, in milligrams per square meter of the interface, was calculated from the relation

$$T_p = \frac{5V_c\,(C_i - C)\,d_{vs}}{3V_o} \quad \ldots\ (3)$$

where V_c and V_o are the volumes of the continuous phase and the oil phase, respectively and C_i and C are the initial and final percentage concentrations of the protein solutions, respectively.

Results

The usual way to present the adsorption data is in the form of adsorption isotherm, in which amount adsorbed is plotted against equilibrium concentration. In the present case also, the data are presented in the form of adsorption isotherms and the effects of some parameters such as pH, ionic strength, denaturant and temperature on the isotherms have been shown.

The results of the effect of pH at a constant temperature of 28°C have been presented in Fig 1. Adsorption experiments were carried out at pH 5.7, 4.3 and 8.1. Salt extract of 0.50 M strength was used as protein solution so that the ionic strength remains constant at 0.50, pH was adjusted by suitable addition of HCl or NaOH. The results show that the amount of adsorption varies with pH. Adsorption is highest at pH 5.7 and least at pH 4.3 and that at pH 8.2 being intermediate between the two. Moreover, the initial slopes of the adsorption isotherm is in the same order of adsorption, i.e. pH 5.7>pH 8.2>pH 4.3. The other interesting point that can be noted from Fig 1 is regarding the shape of the isotherm. All the isotherms pass through a maxima before reaching the constant saturation value and the maxima occurs in the equilibrium concentration range of 0.020 to 0.035 per cent. The saturation values at pH 5.7, 8.2 and 4.3 are 37, 16 and 10 mg/m² respectively. Another observation is that the initial rise of the amount

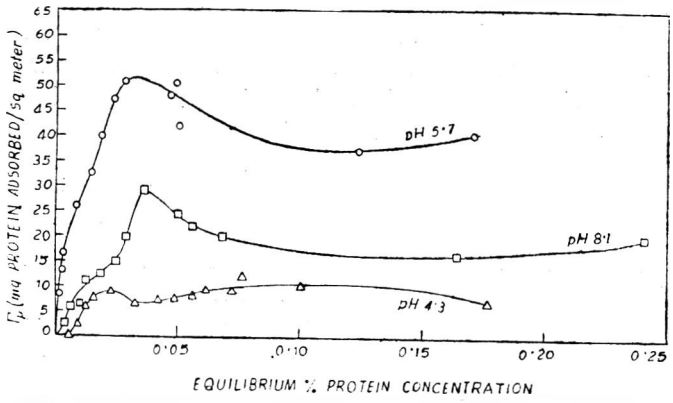

Fig. 1. Adsorption isotherms of salt soluble fish protein on peanut oil at different pH.

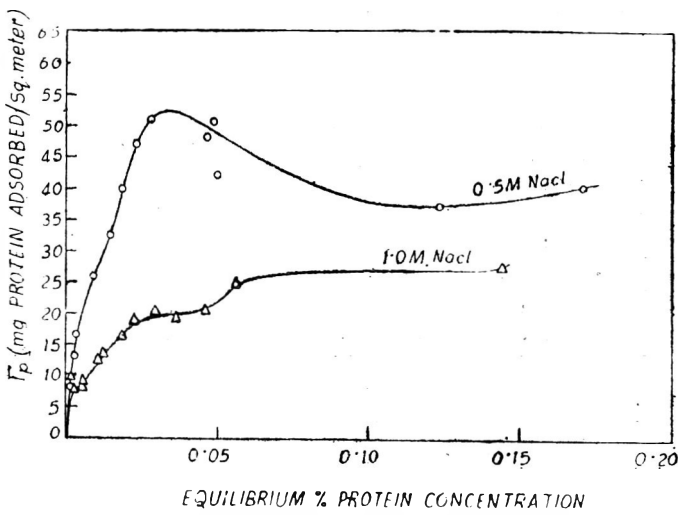

Fig. 2. Adsorption isotherms of salt soluble fish protein on peanut oil at different ionic strength.

of adsorption with rise in equilibrium concentration is not linear in nature.

In Fig 2 two isotherms at different ionic strengths maintained by 0.5 M NaCl and 1.0 M NaCl have been compared. It is found that amount of protein adsorbed decreases with increasing ionic strength and saturation value of adsorption (T_p^m) for 1 M NaCl is attained at 27 mg/m² as compared to 37 mg/m² for 0.5 M NaCl. Also the maxima which occurs at 0.5 M NaCl is not found at 1.0 M NaCl.

The effect of urea on the isotherm has been depicted in Fig 3 in which two isotherms, one at 0.5 M NaCl and the other at 2.0 M urea have been presented. It is seen that the isotherm in presence of urea shows some distinct steps. Initially at very low equilibrium protein contentration (region AB) adsorption is very small. Then adsorption increases (region BC) and ultimately reaches a state of apparent saturation (region CD) and this corresponds to only 5 mg/m². After this region, adsorption increases slowly and tends toward saturation

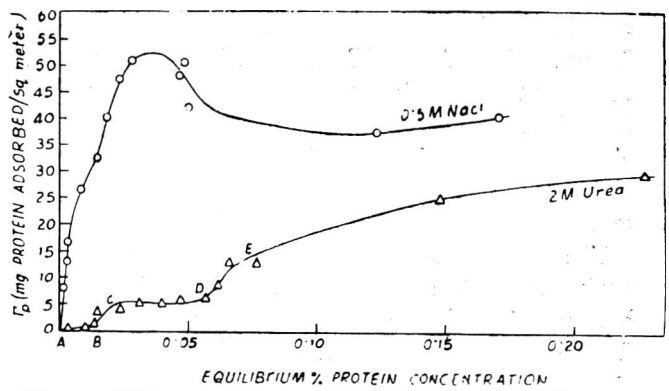

Fig. 3. Adsorption isotherms of salt soluble fish protein on peanut oil in presence of urea.

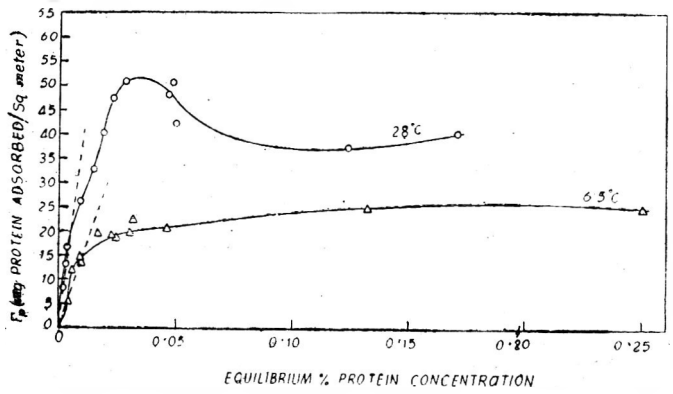

Fig. 4. Adsorption isotherms of salt soluble fish protein on peanut oil at different temperatures.

(region DEF) through wide range of equilibrium cocentrations (0.07 to 0.25 per cent). The isotherm in the region ABCD is S-shaped. The maxima also disappears at 2.0 M urea. At the highest equbilirium concentration studied for 2.0 M urea, the maximum adsorption corresponds to 30 mg/m^2.

In Fig 4 two isotherms at 28 and 6.5°C, other physical conditions remaining same, have been compared. It is found that at 6.5°C adsorption becomes less compared to that at 28°C and saturation adsorption at 6.5°C corresponds to 25 mg/m^2. Also the initial slope decreases with fall in temperature.

Discussion

That the salt extract of fish muscle contains a mixture of proteins has been reported by Dyer and Dingle[14a]. The composition and the physical characteristics have been studied by them by electrophoretic, ultracentrifugal and sedimentation techniques. The primary effect of changing pH of a protein solution is the alteration in the net charge of the protein molecule. The average isoelectric pH (IEP) of the mixed proteins in the 0.5 M NaCl extract of fish is about 5.2 in the adsorbed state as measured by the microelectrophoresis technique (Basu *et al.*, unpublished data). So at pH 4.3 there will be a net positive charge on the protein molecules and at pH 8.2 there will be a net negative charge. The pH 5.7 being very close to isoelectric pH will impart only slight excess negative charge on the protein molecules. The decrease in adsorption of fish protein on the peanut oil/water interface at pH away from IEP is due to the net charge on the protein segments. Due to the electrical repulsion among the charged segments, the protein molecules may undergo extensive surface denaturation leading to the low adsorption. However, at pH 5.7, the electrostatic repulsion is small, so that the protein molecule may retain its compact structure. More molecules can now be accommodated in unit area of the surface leading to greater extent of adsorption. It may be mentioned in this connection that bovine serum albumin (BSA) adsorbs in similar fashion on peanut oil[10].

One important difference between the adsorption of fish protein extract and BSA is that the adsorbed amount in case of BSA lies between 0 and 4 mg/m^2, whereas that for fish proteins leads to a value as high as 40 mg/m^2. Such high values of adsorption suggest multilayer adsorption of fish proteins. Such multilayer adsorptipn, specially with macromolecules, is very common[9,15]. The first layer in such adsorption is possibly surface denatured and additional protein molecules are adsorbed on this denatured film in a manner similar to the formation of duplex film[16]. However, alternative mechanisms are also suggested for the formation of such films[17,18]. Such multilayer formation may lead to increased stability of the fish products.

The initial slopes of the adsorption isotherm indicates the affinity[19] of the protein molecules towards the peanut oil/water interface. This affinity is primarily dependent on the kinetics of adsorption which is again a diffusion controlled process[20]. A protein molecule with compact structure should diffuse faster than an unfolded molecule[20]. This leads to greater rate of adsorption at pH 5.7 which is close to IEP than at pH 8.2 or 4.8. This may also explain magnitude of adsoprtion at the three pH studied.

The maxima observed in Fig 1 is frequently observed in adsorption experiments both with micro-and macromolecules[21]. With proteins such maxima has been obtained by Bull[22] and Mitra *et al.*[23] at lower equilibrium concentrations. These authors attributed the formation of such maxima at lower values of equilibrium concentrations to conformational alterations of the adsorbed proteins.

Addition of a neutral salt increases the ionic strength of the medium. which reduces the electrostatic repulsions between the similarly charged segments of the protein molecule, both in the bulk as well as in the adsorbed state. This may favour the compact structure of the protein molecule resulting in an increase of the amount of adsorption. However, near the isoelectric pH such effect will be small. Moreover, at 1.0 M NaCl concentration, the protein molecules may undergo extensive dehydration[24] leading to denaturation of the adsorbed protein. This may lead to decrease in the amount of adsorption (Fig 2). Similar decrease in the amount of protein adsorption with increase in ionic strength was also reported by Suzawa *et al.*[25]. The unfolding at 1.0 M NaCl is further supported from the initial slopes of the curves at Fig 2. Decrease in the affinity at 1.0 M NaCl concentration is definitely a result of slower rate of diffusion which again should be due to unfolding of the protein.

Urea is known to be a common denaturant of proteins[26]. The isotherm shows a region of apparent saturation (region CD) above which adsorption fruther increases. This behaviour suggests interfacial coagulation which is known to occur readily in proteins at interfaces[27,28]. The initial saturation probably corresponds to a monolayer adsorption and further adsorption increases the number of layers. Similar results were also found for adsorption of gelatin[10] which is known to be a denatured protein. The very low affinity (region AB) may reflect extensive unfolding of the protein molecule even in the bulk.

The decrease in the amount of adsorption with decrease in temperature is contrary to general observation where an increase in the adsorption is usually obtained[10,19]. The results for the fish protein (Fig 4) may imply a kinetically controlled process which can offer an explanation of the apparent anamolous results. The first step in adsorption is diffusion of protein molecules from bulk to the interface[5,20]. The second step is unfolding and reconformation of adsorbed protein[5,20]. At lower temperature, the rate of diffusion may be slow compared to the rate of unfolding. As a result, the protein molecule which reaches the interface gets unfolded completely before a second molecule reaches the interface. That is, the adsorbed molecules are mostly denatured. At moderate temperature the rate of diffusion may be faster than the rate of unfolding and thus the adsorbed film may be partly native proteins. This may increase the amount of adsorption. Of course, some other complex factors may beresponsible for the result. The results clearly imply that hydrophobic interaction plays the important role in the adsorption process. The positive entropy value given in Table 1 also supports this.

Thermodynamic treatment of adsorption data is important but is often very complex. Because of the complex nature of the isotherms and irreversibility of the adsorption process[22], application of equations based on classical thermodynamics is questionable and less justified. However, such treatment may be extremly helpful for a comparative purpose. The usual procedure to calculate thermodynamic parameters is to consider the initial slopes of the adsorption isotherm[19]. The limitation of such considerations for the adsorption at liquid-liquid interfaces has been discussed previously[10].

Free energy change ($\triangle G$) for the adsorption of fish proteins to peanut oil has been calculated on the basis of Bull's equation[22] which, in simple form reads,

$$\triangle G = +RT\Gamma_p \ln Cp - RT \int_0^{Cp} \Gamma_p \cdot \frac{dCp}{Cp} \quad \ldots\ (4)$$

The limitations of the applicability of this equation to adsorption data has been discussed elsewhere[10]. The standard free energy change ($\triangle G^\sigma$) of adsorption per mole of protein has been calculated from the slope of the linear plot of $\triangle G$ vs Γp (Fig 5). The standard enthalpy change $\triangle H^\sigma$ for the transfer of one mole of protein to the peanut oil/water interface may be calculated from the relation

$$\frac{\triangle G_2^0}{T_2} - \frac{\triangle G_1^0}{T_1} = \triangle H^\sigma_{av} \left(\frac{1}{T_2} - \frac{1}{T_1}\right) \quad \ldots\ (5)$$

Here $\triangle G_1^0$ and $\triangle G_2^0$ are the standard free energy changes at T_1 and T_2 temperatures respectively. $\triangle H^\sigma_{av}$ stands for the average enthalpy change at an average temperature of $\frac{1}{2}(T_1+T_2)$ corresponding to an average free energy change $\triangle G^\circ_{av} = \frac{1}{2}(\triangle G^\sigma_1 + \triangle G^\sigma_2)$. The average entropy change was calculated from the equation

$$\triangle G^\circ_{av} = \triangle H^\circ_{av} - T\ \triangle S^\circ_{av} \quad \ldots\ (6)$$

The values of $\triangle G^\sigma$ calculated from various adsoprtion isotherms along with maximum adsorption (Γ_p^m) are reported in Table 1. The average free energy, enthalpy

TABLE 1. THERMODYNAMIC AND OTHER RELATED PARAMETERS FOR ADSORPTION OF FISH PROTEINS TO PEANUT OIL/WATER INTERFACE

Salt	pH	Temp (°C)	Salt concn (M)	Γ_p^m mg/sq.m.	$\triangle G^\circ$ KJ/mole
NaCl	5.7	6.5	0.5	25.13	-10.67 T_{av}=290.25°K
NaCl	5.7	28	0.5	50.71	-10.92 $\triangle H^\circ_{av}$ = +8.0 KJ/mole
NaCl	8.2	28	0.5	29.06	-9.49 $\triangle G^\circ_{av}$ = -10.8 KJ/mole
NaCl	4.3	28	0.5	12.33	-8.40
NaCl	5.7	28	1.0	27.44	-14.41 $\triangle S^\circ_{av}$ = +0.064 KJ/mole/K
Urea	6.9	28	*2.0	29.50	-6.80

*No other salt was used for extraction.

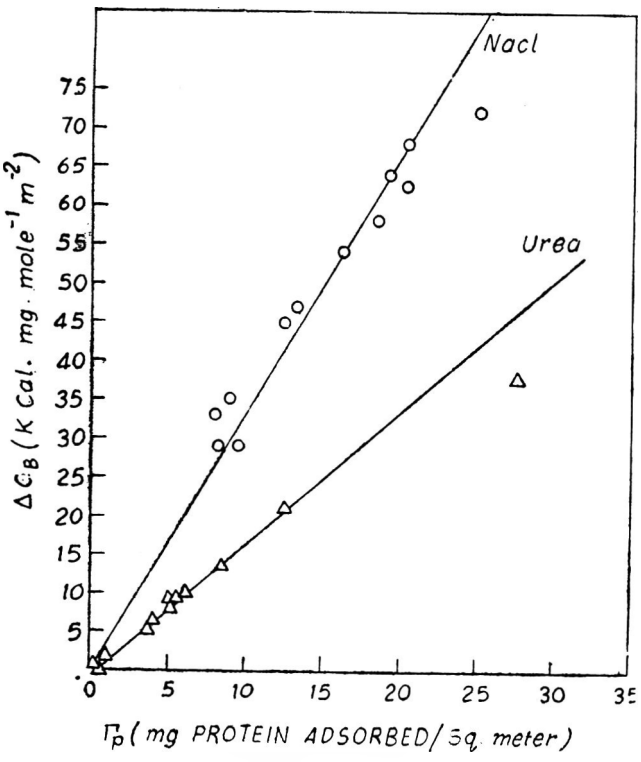

Fig. 5. Plot of $\triangle G$ vs. Γ_p.

and entropy values are also reported in Table 1. It is noted from Table 1 that $\triangle G^\circ$ does not reflect the trend in saturation adsorption. Also, it is found that $\triangle H^\circ_{av}$ and $\triangle S^\circ_{av}$ are both positive in agreement with the previous results[10,19].

Conclusion: Adsorption of fish protein is maximum near the isoelectric point. At pH away from IEP., protein gets surface denatured. Increase in the salt concentration increases the degree of denaturation in the adsorbed film leading to less amount of adsorption. Denaturant decreases adsorption. Cooling below room temperature possibly enhances denaturation. This indicates that the interactions of the fish proteins with peanut oil is hydrophobic in nature. This also explains the development of toughness during cold storage of fish.

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Studies on *Anardana* (Dried Pomegranate Seeds)

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Forty samples of *Anardana* (sun-dried seeds of ripe sour pomegranate) comprising authentic as well as commercial/ market samples, were analysed for their important physico-chemical characteristics. The range of values was moisture 5.4-14.7%, acidity 7.8-15.4%, total ash 2.04-4.04%, acid insoluble ash 0.73-1.98%, crude fibre 22-30% and protein (N×6.25) 4.74-6.25%. Variation in colour was also noticed. Quality standards have been proposed for the product. Sorption studies indicated that an initial moisture content of 8.7% exerts an ERH of 62%. Storage studies over a period of 9 months revealed that friction-top tins were the best followed by glass containers. In 200 gauge polythene bags, there was loss of moisture during summer months which was regained during rainy season. Darkening in storage was the least in friction-top tins.

Dried, sour, whole pomegranate (*Punica granatum* L) seeds popularly known as *Anardana*, is used as an acidulant in Indian curries, chutneys, etc. in place of tamarind and *amchur* (dried raw mango) in North India. It is also used in Ayurvedic system of medicine[1]. Fruits are hand picked, cracked and seeds with flesh are separated from rind and septa which are then sun-dried. It is packed in gunny bags for the market. It is reported that the total value of *Anardana* produced in the states of Jammu and Kashmir and Himachal Pradesh is Rs. 60 lakhs per annum[1]. *Anardana* has also some export market in East Asian countries. There is little published information on the manufacture, composition, packaging, storage and quality standards for *Anardana*[2]. Studies were undertaken on the above mentioned aspects, the results of which are presented in this paper.

Materials and Methods

Raw material: Samples of *Anardana* were collected from Divisional Forest Officers, General Managers (District Industries Centres) of Jammu and Kashmir and Himachal Pradesh. Market samples were also procured from Ludhiana, Jammu, Amritsar and Simla. The collected samples were filled into clean airtight glass jars and stored at 4°C.

Foreign matter in *Anardana* was determined by separating the septa, rind, stone, mud, etc from 100 g of the sample and weighing. Colour was noted by a panel of judges by visual observation. Aliquots of powdered samples were utilized for the determination of moisture, acidity, total ash, acid insoluble ash, crude fibre, reducing, non reducing and total sugars, ether extract and crude protein following standard A.O.A.C. methods[4]. For the objective evaluation of the colour of *Anardana*, optical density at 435nm of 100 ml methanolic extract obtained from 10 g of sample using methyl alcohol containing 1 per cent HCl in a Spectrocol photoelectric colorimeter, model CL-23.

Equilibrium relative humidity (ERH) studies were conducted by Wink's Weight Equilibrium method[3].

Storage: Anardana samples were stored in glass jar with screw caps, friction top tin cans, and heat sealed low density polythene bags (200 gauge) (LDPE) for 9 months at room temperature (13 to 38°C) and 30 to 90 per cent RH. Cnanges in visual colour, moisture, acidity and optical density of methanolic extract were determi-

ned at intervals of 0,3,6 and 9 months storage by A.O.A.C. procedures[4].

Frequency distribution histograms and formulation of quality standards for Anardana: Based on the results of analysis of 40 samples of *Anardana* for a few important quality attributes like absence of foreign matter, moisture, acidity, total ash, acid insoluble ash, and crude fibre, frequency distribution histogram of each quality attribute was constructed which helps in subsequent formulation of draft quality standards for each quality characteristic.

Results and Discussion

Chemical composition: All the 40 samples contained foreign matter like septa, rind, mud and small stone pieces. It ranged from 1.5 to 6.5 per cent. Himachal Pradesh (HP) samples had slightly less foreign matter (1.5-2.5 per cent). The moisture ranged from 5.4 to 14.7 per cent, acidity (as citric acid) from 7.8 to 15.4 per cent, total ash from 2.04 to 4.04 per cent, acid insoluble ash from 0.75 to 1.98 per cent, reducing sugars from 9.26 to 16.8 per cent (total sugars were almost same or slightly higher than reducing sugars), crude fibre from

TABLE 1. COMPOSITION OF *ANARDANA* PROCURED FROM JAMMU AND KASHMIR, HIMACHAL PRADESH AND PUNJAB

Source	Visual colour	Foreign matter* (%)	Moisture (%)	Acidity (as % citric)	Sugar			Ash		Crude fibre (%)	O.D. at 435 nm
					Reducing (%)	Non-reducing (%)	Total (%)	Total (%)	Acid insoluble (%)		
Jammu & Kashmir State											
DFO Jammu	LB & R	3.5–	8.3–	9.6–	9.3–	0.1–	9.3–	2.52–	0.95–	22.6–	0.83–
		4.5	10.4	12.9	16.2	0.8	16.6	3.51	1.05	30.0	0.98
Jammu market	,,	3.5–	6.4–	11.9–	11.8–	0.7–	14.2–	2.96	1.10–	25.0–	0.67–
		4.0	9.4	12.8	13.4	4.6	16.9	3.23	1.98	25.5	0.70
DFO Udhampur	BR.LB,DR	4.0–	7.4	11.1	13.2–	0.3–	13.5–	2.65–	0.87–	25.6	0.83–
		6.5	12.2	12.1	15.8	0.7	16.5	3.25	1.12	29.5	0.96
Udhampur market	,,	4.5–	7.4–	12.1	13.2–	0.6–	13.8–	2.82–	1.25-	23.4–	0.86–
		5.0	7.8		13.9	0.9	14.9	2.85	1.82	25.2	0.89
DFO Batote	LB & R	2.5–	5.3–	11.7–	11.2–	4.1–	16.5–	2.45–	0.91–	26.0–	0.88–
		2.6	5.5	12.7	12.0	5.2	17.0	2.70	1.12	27.0	0.89
DFO Riasi	R	2.8–	8.7–	10.2–	14.3–	1.1–	15.5–	2.40–	1.05–	26.7–	1.00–
		3.9	10.6	15.4	15.4	1.6	16.9	3.35	1.10	29.5	1.15
Himachal Pradesh State											
DFO Padar	LB & R	2.1	10.5	10.3	14.0	0.4	14.4	2.42	0.80	25.6	0.65
DFO Suiahan	,,	2.0	10.5	11.0	16.8	0.4	17.2	2.54	0.98	25.0	0.66
DFO Dalaha	,,	2.5	11.0	12.0	16.0	0.2	16.2	2.32	0.95	22.0	0.72
DFO Diang	,,	2.5	8.8	11.9	14.0	0.2	14.3	2.04	0.95	26.0	0.76
DFO Nagarota	,,	1.5	9.5	11.3	13.0	0.4	13.5	2.04	1.02	25.0	0.70
DFO Simla	,,	2.0	9.8	12.0	12.3	0.5	12.8	2.35	1.12	22.5	0.75
Simla market	,,	2.0	6.7	12.2	12.5	3.3	16.2	3.46	1.16	23.5	0.80
Punjab State											
Ludhiana Market	DR & LB	2.5–	5.9–	7.8–	11.0–	0.2–	14.4–	3.02–	0.73–	26.5–	1.20–
		5.0	12.0	12.2	15.6	4.5	17.0	4.04	1.58	29.0	1.80
Amritsar market	LB & DB	3.0–	6.8	8.9–	11.0	2.7–	14.6–	2.87–	0.92–	26.5–	1.10–
		4.5	14.7	11.8	13.1	5.2	17.5	3.73	1.84	28.2	1.65

Results are expressed on as is basis.

*Includes rind, septa, stone and mud pieces.

LB: Light brown; R: Red, BR: Bright red; DR: Dark red; DB: Dark brown.

22 to 30 per cent, ether extract from 0.25 to 0.63 per cent, (estimated in 21 samples) and crude protein from 4.74 to 6.25 per cent (Table 1).

Equilibrium relative humidity studies: With an initial moisture content of 8.7 per cent, *Anardana* exerted an ERH of 62 per cent and there was practically no change in colour (darkening) and texture (softening) (Table 2).

TABLE 2. R.H. AND EQUILIBRIUM MOISTURE RELATION FOR DRIED POMEGRANATE SEEDS (*ANARDANA*)

RH(%)	E.M.C.%	Colour	Texture	Remarks
20	3.6	Light brown	Crisp, free flowing	No darkening
30	4.2	,,	,,	,,
40	4.9	,,	,,	,,
50	6.7	,,	,,	,,
60	7.7	,,	,,	,,
70	11.8	Slight dark brown	Slight caking	Darkened
80	18.8	Dark brown moist sample	Heavy caking	,,
90	57.7	,,	,,	Darkened, mould appeared

Initial moisture 8.7%

TABLE 3. CHANGES IN *ANARDANA* DURING STORAGE PACKED IN GLASS JAR, POLYTHENE BAG (200 GAUGE) AND FRICTION-TOP TIN

Storage period (months)	Moisture (%)	Acidity (as % citric acid) (MFB)	O.D. at 435-450 nm
	Glass jar		
0	10.9	15.8	1.20
3	9.3	14.0	1.45
6	9.9	10.0	1.70
9	9.8	9.9	1.75
	Polythene bag		
0	10.9	16.8	1.10
3	5.8	13.6	1.15
6	10.4	10.2	1.90
9	10.0	9.9	1.95
	Tin can		
0	10.9	15.8	1.10
3	9.3	14.0	1.55
6	9.6	10.5	1.85
9	9.6	9.9	1.85

Visual colour of samples in all the containers was: at initial, light to dark red; 3 months, light brown to dark brown; 6 months-light brown to dark brown; and at 9 months-dark brown to black.

Further, practically no change in colour and texture was noticeable upto 60 per cent RH, while at 70 per cent RH, slight caking took place and colour also darkened. Mould growth appeared only in one sample at 57.7 per cent moisture level at 90 per cent RH but in all other samples, it did not appear even at 90 per cent RH.

During 9 months' storage of *Anardana* in three types of containers, the colour changed from red to brown. Samples showed slight change in moisture (1 to 1.2 per cent) in glass jar and tin containers, but in LDPE bags, moisture decreased during summer months from 10.9 to 5.7 per cent but increased during rainy and winter months (5.7 to 10.4 per cent) which can be attributed

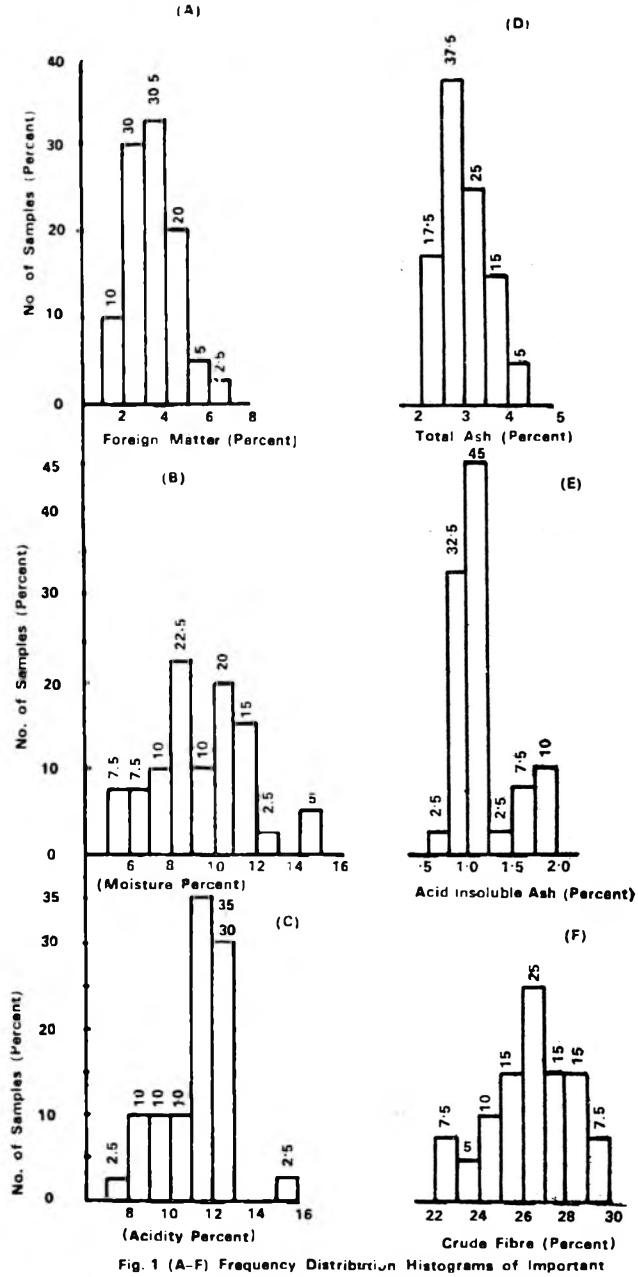

Fig. 1 (A-F) Frequency Distribution Histograms of Important Physico Chemical Quality Parameters of Anardana.

TABLE 4. REGIONAL VARIATIONS IN THE CHEMICAL COMPOSITION OF *ANARDANA*

Parameters	J & K		Himachal Pradesh		Punjab	
	Range (19)	Ave-rage	Range (7)	Ave-rage	Range (14)	Ave-rage
Foreign matter (%)	2.5- 6.5	4.2	1.5- 2.5	2.1	2.5- 5.0	3.5
Moisture (%)	5.2-12.2	8.7	6.7-11.0	9.6	5.9-14.7	10.5
Acidity (%)	9.6-15.4	11.9	10.2-12.2	11.5	7.8-12.2	10.2
Total ash (%)	2.4- 3.5	2.9	2.0- 3.5	2.6	2.8- 4.0	3.4
Acid insoluble ash (%)	0.9- 2.0	1.1	0.8- 1.2	1.0	0.7- 1.8	1.3
Crude fibre (%)	22.6-30.0	26.6	22.0-26.0	24.2	26.5-29.0	27.6

All the results are expressed on as is basis.

Figures in the parentheses are number of samples analysed.

the permeability of water vapour through the polythene bags. Acidity (as citric acid) decreased with increase of storage period. Initial acidity was 15.8 per cent on moisture free basis (MFB) which decreased to 9.9 per cent MFB after 9 month's storage. Optical density (O.D.) of methanolic extract also increased with storage period which confirmed the visual observations. Initial O.D. was 1.1-1.2 and after storage, it increased to 1.75-1.95 (final) at 435-450 nm (Table 3). No insect or mould infestation was noticed. Because of the minimum physico-chemical changes, storage of *Anardana* in friction top tins is recommended but cost considerations favour LDPE bags.

Proposed quality standards: Based on the study on frequency distribution histograms (Fig 1 A-F), the following quality specifications are proposed.

Foreign matter: max 5 per cent (W/W); moisture, max 12 per cent; acidity not less than 9 per cent (W/W); total ash, max 4 per cent; acid insoluble ash, max 1.25 per cent and crude fibre max 29 per cent.

Acknowledgement

Authors wish to thank Mr. C. P. Natarajan, former Director and Dr. D. P. Sen, former Chairman, Experiment Stations, CFTRI, Mysore for their keen interest in this investigation.

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Variability in the Physico-chemical Characteristics of Spiced Papads of Punjab

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Proximate physico-chemical composition of commercial spiced papads of Punjab was determined. Results indicate wide variations in moisture content from 10.7 to 18.2%, total ash from 8.4 to 11.65 %, acid insoluble ash from 0.18 to 0.46%, ether extract from 2.1 to 3.65%, pH from 7.55 to 10.05 and alkalinity of ash from 1.38-3.07%. Frequency distribution histograms were constructed for physico-chemical characteristics of papads and results were critically examined and compared with the existing quality standards. Analysis of commercial papads also indicate wide variation in average weight (15-24 g), mean diameter (15.8 to 18.6 cm), and mean thickness (0.72 to 1.27 mm) which were in accordance with the existing quality standards.

Among the traditional savoury foods, 'Papad', 'Appalam', 'Papadam' constitute an important group which are popular in the Indian dietary. They are mostly manufactured on cottage scale or home scale. During

1979–80, papads worth about Rs. 221.8 lakhs were exported[1] from India.

Physico-chemical analysis of commercial samples of papads in India, is reported in the literature[2]. The present paper covers the results of physico-chemical composition of 35 samples of salted and spiced black gram papads. Studies were undertaken on the variations in physico-chemical composition of spiced papads from different producing centres.

Materials and Methods

Thirty five samples of salted and spiced (black gram flour) papads were procured from the market in three important towns of Punjab, namely, Amritsar, Jullundur and Ludhiana. All the samples were kept in airtight closed bottles in a refrigerator for analysis. These samples were then powdered (30 mesh) and analysed for total ash, acid insoluble ash, ether extract, crude fibre, alkalinity of ash, weight, diameter, thickness and pH, by ISI methods[3]. Protein and common salt were determined by standard AOAC methods[4].

Frequency distribution histograms were drawn for each physico-chemical characteristic of papads.

Results and Discussion

Physical characteristics of papad: Mean diameter of papads ranged from 15.8 to 18.6 cm., mean weight ranged from 15.7 to 24.0 g and mean thickness ranged from 0.72 to 1.28 mm which conform to ISI specifications (Table 1).

Proximate chemical composition of papads: Moisture content of papads varied from 10.7 to 18.2 per cent, total ash from 8.40-11.65 per cent, acid insoluble ash from 0.16-0.46 per cent, ether extract from 2.10-3.75 per cent, pH from 7.55 to 10.05 and alkalinity of ash from 1.38-3.07 per cent. Papad contains on an average 5.08 per cent salt as sodium chloride. Results were summarised in Table 1.

Frequency distribution histogram: Frequency distribution histogram on physico-chemical attributes viz, moisture, total ash, acid insoluble ash, salt, fat, alkalinity of ash, pH, crude fibre, mean weight, mean diameter and mean thickness of 35 samples of spiced papads were constructed and shown in Fig 1. The range and mean values for each parameter is represented in Table 1 and compared with the ISI specification[3]. Moisture content, acid insoluble ash, fat and pH of the aqueous extract of papads do not conform to the ISI specification while the remaining parameters conform to the ISI specification.

TABLE 1. VARIATION IN SOME PHYSICO-CHEMICAL CHARACTERISTICS OF PUNJAB SPICED PAPADS

Characteristics	Range	Mean	ISI[3]
Weight (g)*	24.00–15.67	20.48	15-23
Diameter (cm)*	18.64–15.84	17.14	5-23
Thickness (mm)*	1.26– 0.72	1.00	0.5-1.2
Moisture (%)	18.20–10.70	13.23	12-15
Total ash (%)	11.65– 8.40	9.63	max. 12.0
Acid insoluble ash (%)	0.46– 0.16	0.30	max. 0.2
Fat (%)	3.75– 2.10	2.92	max. 3.0
Salt (as NaCl) (%)	6.66– 3.06	5.08	max. 6.0
Alkalinity of ash (as Na_2CO_3) (%)	3.07– 1.38	1.74	max. 2.2
pH aq. ext.	10.05– 7.55	8.96	8.0
Crude fibre (%)	0.54– 0.34	0.46	max. 1.0

*Samples analysed were only 30, whereas for others 35 samples were analysed.

Moisture:	85.7 per cent of samples fall between the moisture content of 10-15 per cent.
Total ash:	All the samples (100 per cent) have ash content of 8.0-12.0 per cent.
Acid insoluble ash:	82.9 per cent of samples contain 0.16–0.35 per cent.
Common salt (as NaCl):	94.3 per cent of the samples have 3.0 to 6.0 per cent common salt.
Fat:	88.6 per cent fall between 2.0 and 3.5 per cent.
Alkalinity of ash:	72.9 per cent fall between 1.25 and 2.0 per cent.
pH:	77.2 per cent fall between 7.50 and 9.50.
Crude fibre:	All the samples have crude fibre content within the limit prescribed by ISI.
Mean weight:	96.7 per cent of the samples fall within the prescribed ISI limit.
Mean diameter:	All the samples have the mean diameter as prescribed by ISI.
Mean thickness:	93.3 per cent of the samples have the mean thickness prescribed by ISI.

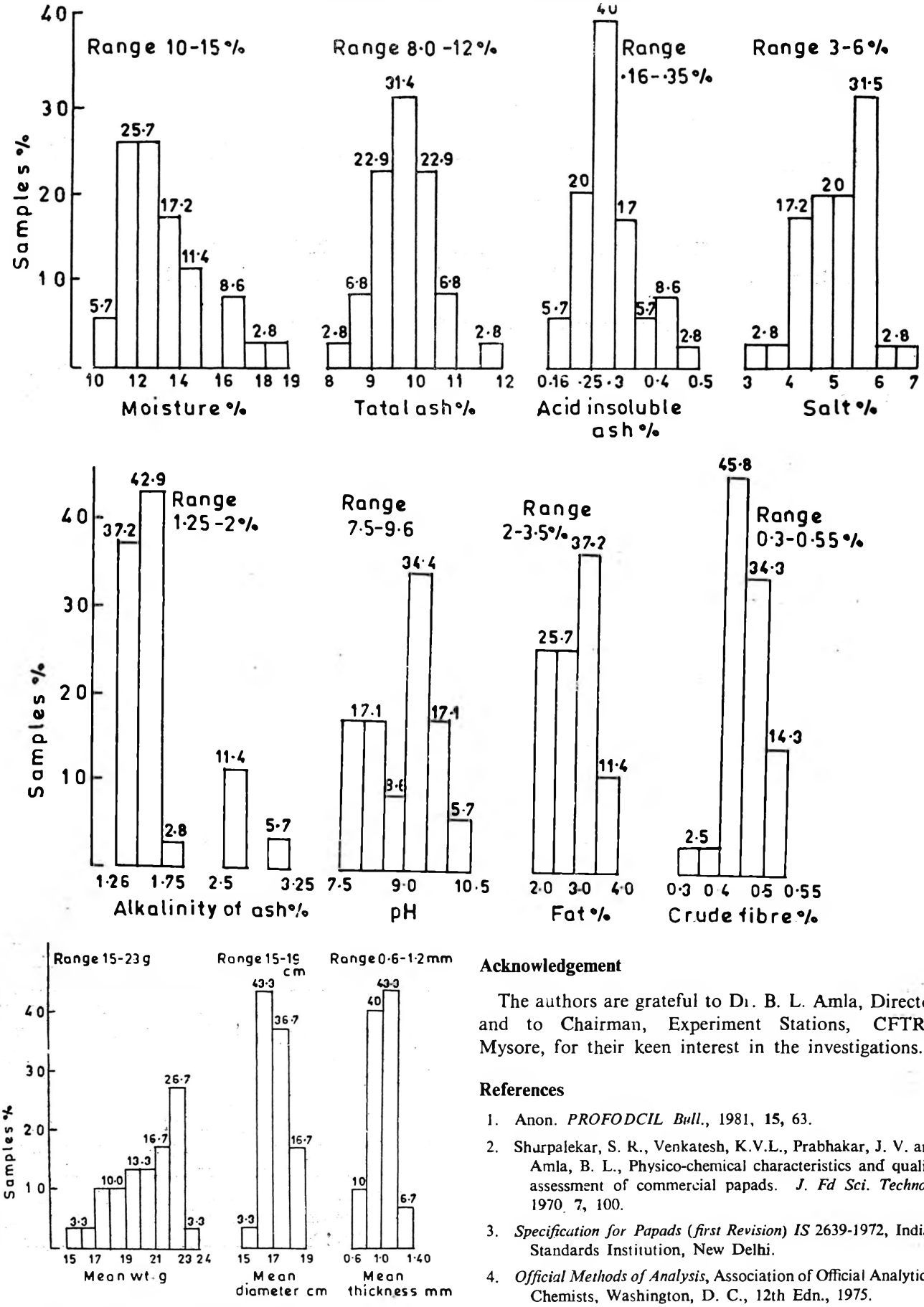

Fig. 1. Frequency distribution histograms of papads

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Silicophosphate as New Insecticide. I. Evaluation of Silicophosphates for the Control of Stored Grain Pests in Milled Rice

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Coordination compounds of silicophosphates such as tricalcium trisilicophosphate CSP-332, bulk density 0.28 g/ml; tricalcium trisilicophosphate, CSP-332A, bulk density 0.16 g/ml; hydrogen form of tricalcium trisilicophosphate, CSP-332H, bulk density 0.14 g/ml; monocalcium silicophosphate, CSP-112; and monocalcium silicophosphate (hydrogen form), CSP-112H were tested to elucidate their effect on the development of *Tribolium castaneum*, *Rhizopertha dominica*, *Oryzaephilus surinamensis*, *Sitophilus oryzae*, and *Trogoderma granarium* in milled rice. The general expectation of these silicophosphate compounds, proved to be toxic to the test insects and toxicity manifested itself in the form of extended developmental period, retarded growth and lowered rate of progeny (F_1) emergence. Out of these silicophosphate compounds, tricalcium trisilicophosphate, CSP-332H (hydrogen form), resulted as highly effective to control all five species of test-insects at 1.0 and 1.5% concentrations in milled rice. Rice treated with 0.5% concentration of CSP-332H was also found to be least suitable for the development of *T. castaneum* and *R. dominica*. No growth indices of *T. castaneum*, *T. granarium* and *O. surinamensis* were observed in rice treated with 1.0 and 1.5% concentration of tricalcium trisilicophosphate CSP-332A. Same concentrations of monocalcium silicophosphate CSP-112H (hydrogen form) were effective to control *O. surinamensis*. Moreover, no growth of *T. castaneum* and *T. granarium* were recorded in rice treated with 1.5% concentration of this compound. Rice treated with monocalcium silicophosphate CSP-112 was found to be susceptible to the infestation of all species of test insects.

In order to prevent insect infestation in food grains, non-toxic grain protectants could be used which are highly effective against most stored grain pests. They do not leave hazardous chemical residues, nor influence the colour and flavour of treated grain nor impair germination. Krishnamurthy *et al*[1]. reported that tricalcium phosphate and naturally occurring silicate compounds exerted a beneficial action on growth when fed to rats in their diets.

Little attention is given to evaluate non-toxic inorganic compounds as insecticides to control stored grain pests. From the findings reported by Majumder *et al.*[2], it is concluded that all the stored grain pests are susceptible to calcium phosphate in milled products and whole grain. Wohlgemuth[3] and Singh[4] observed that the glazing of rice coated with hydrous silicate of magnesium (mineral talc) has a limited effect against the infestation of some species of stored grain pests.

No attempt has been made to study the effect of silicophosphate compounds on the development of stored grain pests. Hence, in the present study, efforts have been made to determine the effect of silicophosphates on the development of five common species of stored grain pests in milled rice.

Materials and Methods

The silicophosphates utilized in this investigation have been synthesized by the procedure developed in this laboratory[5,6]. Calcium silicophosphates of two different compositions, $Ca_3(SiO_2)_3(PO_4)_2$ and $CaH_4(SiO_2)(PO_4)_2$ were obtained as white amorphous materials. Also, silicophosphate in the hydrogen form having the chemical formulae $H_6(SiO_2)_3(PO_4)_2$ and $H_3(SiO_2)_{0.5}PO_4$ were prepared as ion-exchange derivatives of calcium silicophosphates. Calcium silicophosphates having different bulk densities in the range of 0.14 to 0.28 g/ml were also obtained by varying the experimental conditions for their formation.

Five species of insects, namely *Sitophilus oryzae* Linn, *Tribolium castaneum* Herbst, *Oryzaephilus surinamensis* Linn, *Rhizopertha dominica* Fab. and *Trogoderma granarium* Everts were subjected to this study.

Milled rice was disinfested by subjecting to a temperature of 55°C for 4 hr and afterwards treated separately

with 0.3, 0.5, 1.0 and 1.5 per cent of tricalcium trisilicophosphate, CSP-332*, bulk density 0.28 g/ml, tricalcium trisilicophosphate, CSP-332A*, bulk density 0.16 g/ml, hydrogen form of tricalcium trisilicophosphate, CSP-332H*, bulk density 0.14 g/ml, monocalcium silicophosphate, CSP-112* and hydrogen form of monocalcium silicophosphate, CSP-112H*. The treated rice was then conditioned by keeping for three weeks in petri dishes in desiccators maintaining 75 per cent RH by means of NaCl saturated solution.

The experiment was designed to determine the degree of insect mortality in treated milled rice in each of four replicates by releasing separately 100 adults of one week old experimental insects. In case of *T. granarium*, adults were 24 hr old. Each replicate consisted of 250 g of rice and the insects were exposed for seven days. At the end of the seventh day, per cent adult mortality were noted whereas in case of *T. granarium*, mortality was counted at the end of fourth day. Wherever the control registered mortality, the percentage mortality in the treated rice was corrected for true mortality[7].

Fifty adults of each experimental insects, 0-7 days were placed separately in glass jars containing 250 g of disinfested rice and left for one week. After the adults were discarded, the rice was allowed to remain until the full emergence of next generation. This was carried out to acclamatize the insect to the test milled rice.

The reproductivity trial was carried out with the adults of test insects emerged in the rice of above trials. Fifty adults were released on experimental 250 g rice treated with different levels of silicophosphate compounds for one week. After the adults have been removed from the rice, it was left undisturbed until the first adult of F_1 generation was seen to have emerged. These adults were removed subsequently at frequent intervals (usually each day), until all the F_1 generation was seen to have emerged.

The suitability of rice grain coated with silicophosphates was assessed for the development of insect on the basis of adults emerged (F_1) and length of developmental period (the days needed for emergence of 50 per cent of F_1 generation), i.e., on the basis of suitability index[8]. All tests were carried out at $28 \pm 2°C$ and 75 per cent RH.

Results and Discussion

The biological effectiveness of silicophosphates against *T. castaneum*, *R. dominica*, *O. surinamensis*, *S. oryzae* and *T. granarium* were judged on the basis of mortality, emergency of progeny and index of suitability.

Insect mortality: The results of the insect mortality (Fig. 1) indicate that the mortality of adults of *T.*

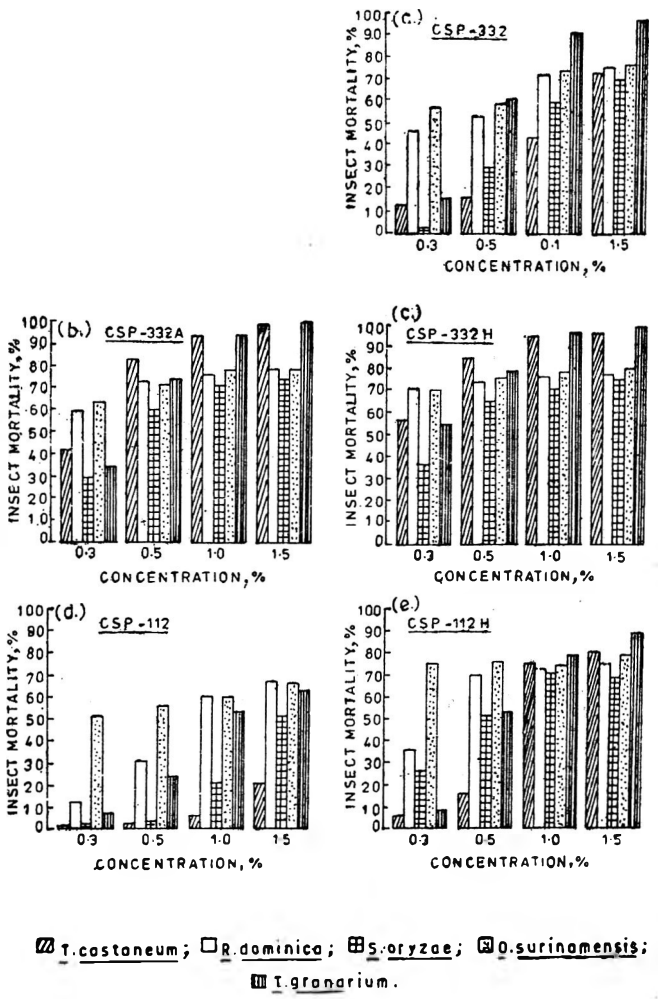

Fig. 1: Mortality of different insects released in the grain treated with different concentrations of CSP-332, CSP-332A, CSP-332H, CSP-112 and CSP-112H

castaneum in milled rice treated with CSP-332A at 0.1, 1.0 and 1.5 per cent levels were 82.5, 93.5 and 99.5 per cent respectively. Whereas 73.5 per cent mortality of *T. castaneum* was recorded in rice treated with CSP-332 at 1.5 per cent level, and at the levels of 0.3, 0.5 and 1.0 per cent, the mortality was poor. The levels of 1.0 and 1.5 per cent of CSP-112H registered 75.0 and 80.7 per cent mortality of *T. castaneum*. In the case of this insect, the 0.3, 0.5, 1.0 and 1.5 per cent levels of CSP-112 registered mortalities of only 0.5, 1.5, 5.5 and 21.5 per cent, respectively. It was observed that CSP-332H, produced comparatively high mortality of *T. castaneum* and *T. granarium* at 0.3, 0.5, 1.0 and 1.5 per cent levels. In case of *T. granarium*, a 100 per cent mortality was recorded in the rice treated at 1.5 per cent level of CSP-332H and CSP-332A. Whereas at level of 1.0 per cent of these

*These abbreviations are used throughout.

TABLE 1. MEAN NUMBER OF PROGENY (F_1) EMERGED AND DEVELOPMENTAL PERIOD IN MILLED RICE TREATED WITH DIFFERENT LEVELS OF SILICOPHOSPHATES

Insects	Progeny emerged (nos) and developmental period (days) at the indicated levels of treatment				
	0.3	0.5	1.0	1.5	Control
Tricalcium trisilicophosphate CSP-332					
Tribolium castaneum	15.25^{a} (32.25)	10.00^{b} (33.00)	5.00^{c} (36.25)	2.75^{d} (35.25)	47.00^{g} (29.00)
Rhizopertha dominica	23.75^{a} (34.75)	13.00^{b} (36.00)	10.50^{bc} (37.50)	4.50^{d} (41.25)	34.00^{e} (30.00)
Sitophilus oryzae	85.50^{a} (31.25)	45.50^{b} (32.50)	17.00^{c} (34.25)	12.50^{d} (33.00)	181.30^{b} (28.50)
Oryzaephilus surinamensis	8.50^{a} (29.00)	7.50^{ab} (31.75)	5.50^{ab} (32.50)	0.75^{c} (35.00)	21.00^{f} (127.50)
Trogoderma granarium	16.50^{a} (39.25)	14.50^{a} (42.25)	5.25^{b} (46.00)	3.00^{c} (47.50)	35.00^{g} (34.00)
Tricalcium trisilicophosphate CSP-332A					
Tribolium castaneum	10.50^{ab} (33.50)	5.75^{c} (36.50)	0.25^{f} (38.00)	0.25^{f} (40.00)	47.00^{g} (29.00)
Rhizopertha dominica	15.75^{b} (35.75)	9.50^{bc} (37.25)	7.00^{c} (39.00)	2.00^{f} (42.33)	34.00^{e} (30.00)
Sitophilus oryzae	26.25^{a} (32.00)	21.00^{c} (33.50)	12.50^{d} (36.00)	9.00^{d} (37.75)	181.50^{h} (28.50)
Oryzaephilus surinamensis	7.50^{ab} (29.50)	4.50^{b} (34.00)	0.25^{c} (36.00)	0.00 (-)	21.00^{f} (27.50)
Trogoderma granarium	3.25^{b} (41.50)	6.50^{b} (44.50)	2.25^{c} (47.50)	1.25^{d} (42.00)	35.00^{g} (34.00)
Tricalcium trisilicophosphate CSP-332H					
Tribolium castaneum	7.00^{c} (38.00)	4.25^{c} (38.00)	0.00 (-)	0.00 (-)	47.00^{g} (29.00)
Rhizopertha dominica	5.75^{a} (35.50)	3.50^{d} (39.00)	1.25^{f} (40.66)	0.00 (-)	34.00^{e} (30.00)
Sitophilus oryzae	14.25^{c} (35.00)	10.50^{d} (35.75)	6.50^{f} (36.50)	0.50^{g} (37.00)	181.50^{h} (28.50)
Oryzaephilus surinamensis	5.50^{ab} (29.23)	3.00^{d} (34.00)	0.00 (-)	0.00 (-)	21.00^{f} (27.50)
Trogoderma granarium	8.50^{b} (42.25)	4.50^{c} (45.23)	1.00^{d} (48.35)	0.25^{c} (47.00)	35.00^{f} (34.00)
Monocalcium silicophosphate CSP-112					
Tribolium castaneum	22.50^{a} (28.00)	20.50^{a} (28.50)	14.00^{b} (31.00)	9.50^{bc} (31.75)	47.00^{g} (29.00)
Rhizopertha dominica	27.50^{cd} (31.75)	22.50^{a} (31.75)	20.50^{a} (32.00)	8.00^{b} (35.50)	34.00^{e} (30.00)
Sitophilus oryzae	178.25^{h} (28.25)	141.50^{i} (28.25)	107.75^{j} (27.25)	51.25^{b} (32.75)	181.50^{h} (27.50)
Oryzaephilus surinamensis	13.50^{f} (26.75)	10.50^{a} (27.75)	10.75^{a} (30.00)	8.50^{a} (29.75)	21.00^{f} (27.50)
Trogoderma granarium	28.00^{f} (35.50)	26.00^{f} (41.25)	25.50^{f} (42.75)	19.00^{a} (43.50)	35.00^{g} (34.00)
Hydrogen form of monocalcium silicophosphate CSP-112H					
Tribolium castaneum	15.00^{a} (36.00)	12.50^{ab} (36.75)	7.75^{c} (37.50)	0.50^{f} (40.00)	47.00^{g} (29.00)
Rhizopertha dominica	9.50^{bc} (35.75)	7.00^{ad} (36.50)	5.00^{d} (36.00)	1.75^{f} (41.00)	34.00^{e} (30.00)
Sitophilus oryzae	17.75^{c} (31.25)	13.75^{cd} (31.00)	10.50^{d} (33.00)	2.50^{g} (33.00)	181.50^{h} (28.50)
Oryzaephilus surinamensis	7.50^{ab} (30.25)	5.75^{b} (31.00)	1.75^{c} (35.15)	0.25^{c} (38.00)	21.00^{f} (27.50)
Trogoderma granarium	20.50^{a} (42.00)	14.50^{a} (42.75)	7.00^{b} (75.75)	1.25^{d} (54.50)	35.00^{g} (34.00)

The figures in parenthesis denote the average developmental period in comparison of mean reproductivity on rice treated with different levels of silicophosphates are match of single species. The means followed by the same letter do not differ significantly at 0.05 probability level.

Each figure is the mean of four replicates.

silicophosphates, 97.0 and 94.5 per cent mortalities, respectively, were recorded.

Progeny: The results obtained on the progeny emerged in rice treated at different levels of silicophosphates are summarized in Table 1. The rice treated with CSP-112 at 0.3, 0.5 and 1.0 per cent levels registered significantly higher progeny emergence of all experimental insects, and significantly low progeny emergence was recorded in the rice at 1.5 per cent level. Whereas considerably poor progeny was produced in the rice treated at 0.5 and 1.0 per cent levels of CSP-112H and the rice treated with 1.5 per cent CSP-112H was found to be least suitable for the reproduction of *O. surinamensis* and *T. castaneum*. Similar results were obtained for rice treated with 1.0 and 1.5 per cent levels of CSP-332A. No progeny emergence of *T. castaneum*, *O. surinamensis* and *R. dominica* was recorded in the rice treated with 1.5 per cent CSP-332H. However, rice

treated with 1.0 percent level of CSP-332H was recorded to be immune to the infestation of *O. surinamensis*. Significantly poor reproductive rate of *T. granarium* and *S. oryzae* was recorded in rice treated with CSP-332H at 1.5 per cent levels. Whereas moderate progeny of *T. castaneum* and *T. granarium* emerged in the rice treated with CSP-332A at 1.0 and 1.5 per cent levels.

Developmental period: Offspring emerged in the experiment carried out for determining the development period from rice treated with different levels of silicophosphate compounds which were exposed to oviposition for 7 days. The developmental period of each species of experimental insects is best measured from the middle of oviposition period to the middle of period in which the adult emerged. The developmental period (days) (Table 1) of *T. castaneum* varied from 32.25 to 35.25; 32.5 to 36.25 and 32.25 to 33.00 days on the rice treated with CSP-332 at 0.3 to 1.5; 0.3 to 1.0 and 0.3 to 0.5 per cent level. The developmental period of all experimental insects on rice treated with CSP-112 was comparatively short. The developmental period of *R. dominica* varied from 34.75 to 41.25; 34.75 to 36.00; 36.00 to 41.25 and 37.5 to 41.25 days on rice treated with CSP-332 at 0.3 to 1.5; 0.3 to 0.5; 0.5 to 1.5 and 1.0 to 1.5 per cent levels, respectively. Similar trend of developmental period was recorded in case of *S. oryzae*, *O. surinamensis* and *T. granarium*. The developmental period of *T. granarium* on rice treated with CSP-332H was comparatively longer. It varied from 48.33 to 57.00 days on grain treated with 1.0 to 1.5 per cent levels of CSP-332H as compared to 46.00 to 47.00 days on rice treated with same levels of CSP-332A, and 45.75 to 54.50 days on rice treated with identical levels of CSP-112H.

The length of developmental period was found to be inversely proportional to the reproductive rate in rice treated with different levels of these silicophosphates.

Index of suitability: The results pertaining to the suitability of rice treated with silicophosphates to the growth of five species of experimental insects are summarized in Tables 2 and 3. The data indicate that rice treated with CSP-332A at 1.0 and 1.5 per cent levels were immune to the attack of *T. castaneum* and *O. surinamensis*. No growth indices of *T. castaneum*, *R. dominica*, *O. surinamensis* and *T. granarium* were recorded in the rice treated with CSP-332H at 1.0 per cent level and no growth index of *S. oryzae* was registered in the rice treated with CSP-332H at 1.5 per cent level. The rice treated with CSP-112 was comparatively susceptible to the infestation of all species of experimental insects. However, slow growth of *T. castaneum*, *R. dominica* and *T. granarium* were recorded on the rice treated with CSP-112H, at 1.0 per cent level and no growth of *O. surinamensis* and

TABLE 2. COMPARATIVE SUSCEPTIBILITY OF GRAIN TREATED WITH DIFFERENT LEVELS OF SILICOPHOSPHATES

Silicophosphate	Susceptibility index* at indicated levels			
	0.3	0.5	1.0	1.5
	Tribolium castaneum			
CSP-332	8.44^{ai}	6.97^{ab}	4.43^{b}	2.86^{c}
CSP-332A	6.62^{a}	4.79^{a}	0.00	0.00
CSP-332H	5.12^{a}	3.81^{b}	0.00	0.00
CSP-112	11.11^{a}	10.59^{ab}	8.51^{abc}	7.09^{abc}
CSP-112H	7.52^{a}	6.87^{ab}	5.46^{ab}	0.00
Untreated	13.27^{a}	13.27^{a}	13.27^{a}	13.27^{a}
	Rhizopertha dominica			
CSP-332	9.11^{a}	7.12^{a}	6.27^{ab}	3.64^{c}
CSP-332A	7.71^{a}	6.04^{a}	4.98^{ab}	1.63^{c}
CSP-332H	4.92^{a}	3.21^{a}	0.00	0.00
CSP-112	10.40^{a}	9.80^{a}	9.43^{a}	5.85^{b}
CSP-112H	6.20^{a}	5.33^{a}	4.47^{ab}	1.36^{c}
Untreated	11.72^{a}	11.72^{a}	11.72^{a}	11.72^{a}
	Sitophilus oryzae			
CSP-332	14.23^{a}	11.74^{a}	8.26^{ab}	7.21^{abc}
CSP-332A	10.21^{a}	9.00^{a}	7.01^{ab}	5.82^{abc}
CSP-332H	7.59^{a}	6.57^{a}	5.12^{ab}	0.00
CSP-112	18.34^{a}	17.53^{a}	17.17^{a}	12.02^{b}
CSP-112H	9.20^{a}	8.19^{a}	7.19^{ab}	2.77^{c}
Untreated	18.25^{a}	18.25^{a}	18.25^{a}	18.25^{a}
	Oryzaephilus surinamensis			
CSP-332	7.37^{a}	6.34^{a}	5.24^{ab}	0.00
CSP-332A	6.83^{a}	4.42^{b}	0.00	0.00
CSP-332H	5.82^{a}	3.18^{b}	0.00	0.00
CSP-112	9.72^{a}	8.47^{a}	7.99^{a}	7.19^{ab}
CSP-112H	6.66^{a}	5.64^{a}	0.00	0.00
Untreated	11.07^{a}	11.07^{a}	11.07^{a}	11.07^{a}
	Trogoderma granarium			
CSP-332	7.14^{a}	6.32^{a}	3.60^{b}	2.30^{bc}
CSP-332A	5.08^{a}	4.20^{a}	1.70^{b}	0.00
CSP-332H	5.06^{a}	3.32^{b}	0.00	0.00
CSP-112	9.32^{a}	7.89^{a}	7.48^{b}	6.84^{ab}
CSP-112H	7.19^{a}	6.25^{a}	4.25^{ab}	0.00
Untreated	10.45^{a}	10.45^{a}	10.45^{a}	10.45^{a}

The comparison of index in the different treated rice are made for single species and chemicals. The means followed by the same letter do not differ significantly at the 0.05 probability level.

Abbreviations as in Table 1.

Each figure is the mean of four replicates.

*Susceptibility index=$\log e \frac{(F_1)}{d} \times 100$

TABLE 3. GROWTH TEST INSECTS IN RICE TREATED WITH DIFFERENT CONCENTRATIONS OF SILICOPHOSPHATE

Silicophosphate	0.3	0.5	1.0	1.5
		T. castaneum		
CSP-332	—	—	—	++
CSP-332A	±	±	+++	+++
CSP-332H	±	++	+++	+++
CSP-112	—	—	—	—
CSP-112H	—	±	±	+++
Untreated	—	—	—	—
		R. dominica		
CSP-332	—	—	±	±
CSP-332A	—	±	±	++
CSP-332H	±	++	+++	+++
CSP-112	--	—	—	—
CSP-112H	±	±	±	++
Untreated	—	—	—	—
		S. oryzae		
CSP-332	—	—	—	—
CSP-332A	—	—	—	±
CSP-332H	—	—	±	+++
CSP-112	—	—	—	--
CSP-112H	—	—	—	++
Untreated	—	—	—	—
		O. surinamensis		
CSP-332	-	±	±	+++
CSP-332A	±	±	+++	+++
CSP-332H	±	±	+++	+++
CSP-112	—	—	—	—
CSP-112H	±	±	+++	+++
Untreated	—	—	—	—
		T. granarium		
CSP-332	—	±	±	++
CSP-332A	±	±	++	+++
CSP-332H	±	±	+++	+++
CSP-112	—	—	—	—
CSP-112H	—	±	±	+++
Untreated	—	—	—	—

Abbreviations as in Table 1
+++ = Immunity; ++ = least suitability; ± = corresponding slow growth and — =more suitability of growth.

T. castaneum were recorded in rice treated with 1.5 per cent level of CSP-112H. The rice treated with CSP-332H was least suitable for the growth of *T. castaneum* and *R. dominica* at 0.5 per cent level. Whereas rice treated with 1.5 per cent level of CSP-332 was also least suitable for the growth of *T. castaneum* and *T. granarium*.

From the foregoing results, it is evident that rice treated with CSP-332 and CSP-112 was more suitable for the growth of *S. oryzae* than the rice treated with CSP-332A, CSP-332H and CSP-112H. Moreover, CSP-112 was not found effective to control even a single species of experimental insects. Poor reproduction of *T. castaneum*, *R. dominica* and *T. granarium* were recorded on rice treated with CSP-332. Rice treated with CSP-332A was found least suitable for the development of *R. dominica*. Rice treated with CSP-332A was immune to the infestation of *T. granarium* at 1.5 per cent level. Rice treated with CSP-112H was found to be effective to control *T. castaneum*, *O. surinamensis* and *T. granarium* at higher levels only. Rice treated with CSP-332H was immune to the infestation of all species of experimental insects at 1.0 and 1.5 per cent level and rice treated at 0.5 per cent level was also found to be least suitable for the growth of *T. castaneum* and *R. dominica*. Majumder and Banu[2] have observed that all the stored grain pests are susceptible to tricalcium phosphate in milled products and whole grain. Recently Wohlgemuth[3] and Singh[4] reported that glazing of rice coated with hydrous silicate of magnesium has a limited effect against the infestation of some species of stored product pests. However, rice treated with silicophosphate in hydrogen form, CSP-332H was observed to be immune to the infestation of 5 species of experimental insects at 1.0 and 1.5 per cent level. The biological effect of silicophosphates was more enhanced than other sorptive materials though all these compounds contained silica as the common radical. Among the silicophosphates studied, the hydrogen form of silicophosphate was found to be highly effective to control test insects in milled rice as compared to silicophosphates in calcium form. However, the calcium form of silicophosphate CSP-332A, having the lowest bulk density of 0.14 g/ml was also considerably effective in controlling *T. granarium*, *T. castaneum* and *O. surinamensis*. This may be due to the reduction in moisture content in rice to certain degrees at difiifferent levels. For similar purpose, a compound called 'silicron' containing fine particle silica is used to protect stored products against moisture[9].

Silicophosphates have been found to have ion-exchange property by virtue of three negatively charged phosphate ionic bonds as fixed matrix at which cation exchange takes place resulting in *in vitro* hydrogenation and dehydrogenation in insects thereby killing them.

The *in vitro* hydrogenation and dehydrogenation of enzyme molecules were caused by CSP-332H and CSP-332A, respectively. However, the *in vitro* hydrogenation of enzyme molecules by CSP-332H was more severe than their dehydrogenation by CSP-332A as enzyme molecules seem to have more affinity for H ions (hydroge-

nation) than that for Ca ions (dehydrogenation). Secondly, the difference in the degree of *in vitro* hydrogenation with CSP-332H and CSP-112H can be attributed to the more number of available H ions in CSP-332H than in CSP-112H that can inhibit the enzyme-activity.

Conclusion: The results of this study lead us to the conclusion that 1.0 and 1.5 per cent levels of CSP-332A and CSP-332H, could be used as they portect the rice against *O. surinamensis* and *T. castaneum*, whereas 1.0 and 1.5 per cent levels of CSP-332H could be used to control *R. dominica* and *T. granarium*. However, where *S. oryzae* infestation occurs, higher level (1.5 per cent) of this coordination compound may have to be used. CSP-332 at 1.5 per cent level; CSP-332A and CSP-332H at 1.0 and 1.5 per cent levels and CSP-112H at 1.0 per cent level can be used against *O. surinamensis* and *T. granarium*. Moreover, 0.5 per cent level of CSP-332H could be used to control *T. castaneum* and *R. dominica* at the initial stage of infestation. As such coordination compounds of CSP-332H, CSP-332A and CSP-112H could be used as a rice protectant at 1.0 and 1.5 per cent levels for its safe storage.

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Nutritional Evaluation of *Eucalyptus kirtoniana* Seed Meal and *Acacia auriculaeformis* Seed Protein in Rats

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Defatted *Eucalyptus kirtoniana* seed cake was analysed for nutrient composition and amino acid profile before and after detoxification. *Acacia auriculaeformis* seed (ASM) protein isolate was also analysed for its chemical composition including amino acid profile. Nutritional evaluations of eucalyptus seed meal of either crude (CEM) or detoxified (PEM) and ASM-protein isolate were carried out with growing male albino rats. Comparison was made with casein. The growth promoting effects and certain biological indices were evaluated using the protein efficiency ratio (PER), biological value (BV) and net protein utilisation (NPU). The BV for the PEM were considerably better than that of CEM. The addition of methionine and cystine to PEM and ASM-protein isolate diets improved growth, PER. BV and NPU but other amino acids had no such effect so far. CEM diet affected the blood and liver biochemical parameters adversely while PEM and ASM-protein isolate diets did not reveal any abnormality in evaluations of blood and liver biochemical parameters.

Better and fuller utilization of waste seeds from forest plants is essential for meeting a variety of shortages facing the country today. But the nutritional potentialities of such forest resources have not been adequately

studied, a factor that limits their utilization as animal food ingredients, though they are rich in protein. However, based on the surveys conducted by the Indian Central Oilseeds Committee and the Hindustan Lever Ltd., it has been shown that at present hardly 7 per cent of the total potential is being tapped[1], which shows a wastage of large quantities of those seeds from forest plants. The main problem in the nutritional exploitation of these unconventional forest resources is the presence of antinutritional factors. Thus, such unconventional food sources need to be detoxified and then nutritionally evaluated prior to their use in animal feedstuffs. Only a few studies on the processing and utilization of such forest resources have been recently reported[2-4]. In our earlier studies[5-8], it has been shown that eucalyptus and teak seed proteins may be usefully exploited. Such nutritional studies are limited and scanty. In our laboratory, we have started a systematic study on the nutritional evaluation of different unconventional seed meals and seed proteins in raw or processed condition. As a part of this investigation, nutritional evaluations of *Eucalyptus kirtoniana* (eucalyptus) seed meal and *Acacia auriculaeformis* (*akashmoni*) seed protein isolate were carried out with albino rats.

Materials and Methods

Eucalyptus kirtoniana (Myrtaceae) and *Acacia auriculaeformis* (Leguminosae) seeds were collected from the local forest of Burdwan, powdered and completely defatted in a solvent extraction plant.

Defatted eucalyptus seed cake (CEM) was analysed for moisture, crude protein (CP), carbohydrates, crude fibre and total ash content by AOAC method[9]. Tannin content was estimated following the gravimetric method based on the absorption of tannins by hide powder as described by Attal *et al*[10]. Saponin content was estimated according to the method of Van atta *et al*[11]. Total nitrogen was estimated by micro kjeldahl method. Amino acid analysis was done by column chromatography as described earlier[5]. Eucalyptus seed meal was detoxified by removal of tannins using the method in which seed meal was stirred with 15 per cent acetone in water for 5 hr and discarding the filtrate. The detoxified meal (PEM) was also analysed for its nutrient composition and amino acid profile

The protein was isolated from the defatted *akashmoni* seed cake following the method of Felker and Bandurski[12] and analysed for its chemical composition including amino acid profile.

Animals and diets: Growing male albino rats (inbred in our laboratory) weighing 45-50 g, of 25-28 days were used in this study. The animals were individually caged and maintained under controlled temperature (21 ± 1 °C) and humidity (55 per cent) conditions. The rats were assigned six per group, equalized for body weight. One group was fed the protein-free diet, another group was given diet based on casein, the other group was assigned to diet containing *akashmoni* seed protein isolate and the remaining two groups fed the diets containing either crude or detoxified eucalyptus seed meal. The composition of the experimental diets are given in Table 1. All the experimental diets were adequate with respect to minerals and vitamins and provided about 16 g N/kg (except the protein-free diet). The experimental animals received their respective diets and water *ad libitum* for 28 days.

TABLE 1. COMPOSITION (G/KG) OF THE EXPERIMENTAL DIETS

Ingredients	Protein-free diet	Casein diet	CEM diet	PEM diet	ASM-protein isolate diet
Starch	650	535	350	350	535
Sucrose	200	200	200	200	200
Cellulose powder	50	50	50	50	50
Groundnut oil	50	50	50	50	50
Mineral salts	40	40	40	40	40
Vitamin mixture	10	10	10	10	10
Casein	—	115	—	—	—
CEM	—	—	300	—	—
PEM	—	—	—	300	—
ASM-protein isolate	—	—	—	—	115
Total	1000	1000	1000	1000	1000
Total N (by analysis)	0.62	16.7	16.1	16.6	16.3

CEM: Crude eucalyptus meal; PEM: Detoxified eucalyptus meal; ASM: *Acacia auriculaeformis*.

Nutritional indices: PER was calculated as bodyweight gain/unit CP intake (CP was based on actual N analysis of the test diet). TD and BV were calculated from information on carcass composition with protein-free and test diet. Metabolic and endogenous N were determined from the group of rats fed protein-free diet and mean values were used in the determination of TD and BV. NPU was calculated as BV × TD. Nutritional indices were measured following the methods as described by Mitchell[13].

Biochemical estimations: At the end of 28 days, animals were sacrificed and blood and livers were collected for biochemical estimations. Blood Hb was measured using haemoglobinometer (Coulter Electronics, Hialeah, Florida, USA). Blood sugar was estimated

following the method of Somogyi[14]. Estimation of total protein was done by folin-phenol method[15]. Total lipid materials of serum and liver were extracted and washed according to the method of Folch *et al.*[16] and estimated by evaporating the measured amount of extract and weighing. Cholesterol and phospholipids were determined by the methods of Sperry and Webb[17] and Fiske and Subbarow[18] respectively.

Results and Discussion

The data on the nutrient composition and amino acid profile are presented in Table 2. The results revealed that the nutrient composition of the defatted eucalyptus seed cake before and after detoxification was essentially the same. Antinutritional factors, tannins and saponins were almost removed and about 15 per cent solids were lost during detoxification process. The amino acid composition of the eucalyptus seed meal was almost similar before and after detoxification and contained 17 amino acids including 10 essential amino acids. ASM-protein isolate also contained 17 amino acids of which 10 amino acids were essential amino acids.

In comparision with the FAO/WHO[19] amino acid pattern, CEM and PEM contained insufficient methionine and cystine and the remaining essential amino acids were also present at levels that were somewhat lower than those recommended by FAO/WHO[19] (Table 3). The essential amino acid pattern of ASM-protein isolate compared well with that of FAO/WHO[19] pattern except for lower levels of methionine and cystine (Table 3).

When the values of essential amino acids (Table 4) were expressed as essential amino acid content: total

TABLE 2. COMPOSITION OF CEM, PEM AND ASM-PROTEIN ISOLATE

Composition	CEM	PEM	ASM-protein isolate
Total carbohydrates (%)	51.2	52.8	—
Total nitrogen (%)	2.3	2.5	14.2
Total moisture (%)	10.0	7.0	8.0
Total ash (%)	6.1	6.0	1.2
Crude fibre (%)	4.4	4.2	—
Crude fat (%)	2.1	0.9	—
Tannins (%)	6.4	0.7	—
Saponins (%)	1.2	0.4	0.5
Amino acid profile (g/16 gN)			
Glycine	4.6	3.9	4.7
Alanine	3.4	3.4	4.9
Threonine	3.2	3.1	3.3
Serine	5.0	5.0	6.4
Valine	4.3	4.1	4.0
Leucine	6.2	6.2	10.6
Isoleucine	3.1	3.1	4.8
Proline	2.7	2.7	6.0
Phenylalanine	2.6	3.0	5.4
Methionine	0.9	0.9	0.9
Cystine	0.7	0.7	0.5
Tyrosine	3.8	4.2	5.8
Histidine	2.8	2.6	1.0
Arginine	8.6	7.6	8.0
Lysine	6.1	6.1	8.9
Aspartic acid	7.8	7.8	11.2
Glutamic acid	22.4	21.3	18.4

Average of three replicates. Legend for abbreviations as under Table 1.

TABLE 3. RECOMMENDED ESSENTIAL AMINO ACID (G/16 GN) PATTERN OF FAO/WHO[19] AND ESSENTIAL AMINO ACID PATTERN OF CEM, PEM AND ASM-PROTEIN ISOLATE

	FAO/WHO	CEM	PEM	ASM-protein isolate
Phenylalanine Tyrosine	6.0*	6.4*	7.2*	11.2*
Isoleucine	4.0	3.1	3.1	4.8
Leucine	7.0	6.2	6.2	10.6
Lysine	5.5	6.1	6.1	8.9
Methionine Cystine	3.5*	1.6*	1.6*	1.4*
Threonine	4.0	3.2	3.1	3.3
Valine	5.0	4.3	4.1	3.1
Arginine	—	8.6	7.6	7.9

*Combined value for the two amino acids
Legend for abbreviations as under Table 1.

TABLE 4. COMPOSITION OF THE ESSENTIAL AMINO ACID (EAA) PATTERN (MG/G TOTAL ESSENTIAL AA) OF CASEIN, CEM, PEM AND ASM-PROTEIN ISOLATE

	Casein	CEM	PEM	ASM-protein isolate
Phenylalanine	101	62	72	102
Isoleucine	87	73	74	92
Leucine	173	147	149	197
Lysine	157	144	146	168
Methionine-cystine	71	38	38	26
Valine	110	101	99	75
Threonine	82	75	73	62
Tyrosine	113	90	100	108
Arginine	59	204	183	150
Histidine	47	66	63	20
Total EAA (mg/g N)	3283	2643	2600	3244
Total EAA (Total N)	3.28	2.64	2.60	3.24

Legend for abbreviations as under Table 1.

TABLE 5. NUTRITIONAL EVALUATION OF DIETS FED FOR 28 DAYS CONTAINING CRUDE EUCALYPTUS SEED MEAL (CEM), PROCESSED EUCALYPTUS SEED MEAL (PEM), ASM-PROTEIN ISOLATE, AND SUPPLEMENTATION WITH AMINO ACID

Protein source	Body wt. gain (g)	CP* intake (g)	PER	TD	BV	NPU (ND×BV)
Casein	68.1±2.1	21.6±1.5	2.77±0.21	0.91±0.010	0.88±0.010	0.80±0.009
CEM	33.5±1.6	22.5±0.8	1.49±0.12	0.72±0.005	0.41±0.006	0.29±0.003
PEM	54.2±1.8	24.1±0.9	2.25±0.15	0.87±0.007	0.56±0.008	0.48±0.006
,, +suppl with A*	62.9±1.4	24.6±1.1	2.56±0.17	0.90±0.008	0.62±0.009	0.56±0.008
,, + ,, B	72.7±2.0	25.0±1.2	2.91±0.14	0.90±0.006	0.76±0.009	0.68±0.005
,, + ,, C	76.4±2.2	26.1±1.3	2.93±0.12	0.90±0.009	0.80±0.010	0.72±0.008
ASM-protein isolate	53.0±1.1	24.3±0.9	2.19±0.15	0.88±0.008	0.55±0.006	0.48±0.005
,, +suppl with D*	63.9±1.5	24.8±1.0	2.58±0.18	0.90±0.009	0.64±0.005	0.58±0.007
,, + ,, E	69.1±2.1	25.4±0.8	2.72±0.21	0.90±0.008	0.77±0.008	0.69±0.008
,, + ,, F	72.2±2.0	26.3±1.0	2.75±0.14	0.91±0.009	0.80±0.006	0.72±0.009

*CP=N×6.25

**Amino acid supplements (g/kg diet):

A and D, 4.0 DL-methionine+DL-cystine (2:1 w/w):

B and E, 8.0 DL-methionine+DL-cystine (2:1 w/w);

C, 8.0 DL-methionine+DL-cystine (2:1 w/w), 3.0 DL-leucine, 3.0 DL-isoleucine, 2.0 DL-threonine and 2.5 DL-valine;

F, 8.0 DL-methionine+DL-cystine (2:1 w/w), 3.5 DL-threonine and 3.0 DL-valine.

Values are Mean±S.E.

No. of animals in each group was 6.

N content, the value for ASM-protein isolate (3.24) was higher than those for CEM (2.64) and PEM (2.60) but lower than that for casein (3.28).

The results (Table 5) of feeding trials with rats show that at a level of inclusion of CEM in the diet of 300 g/kg, CP intake, growth, PER, BV and NPU values were comparatively lower than that of PEM diet. This poor performance of CEM may be due to its high tannin content. Similar types of observations have been reported by Chang and Fuller[20] and Gandhi *et al*[21] with the seed meals of sorghum and sal in the diets of rats. Supplementation of PEM diet with essential amino acids improved growth, PER, BV and NPU, indicating a deficiency or an imbalance in the protein provided by the PEM. The PER (2.19) and BV (0.55) values for ASM-protein isolate were lower than that of casein (PER 2.77, BV 0.88) but addition of essential amino acids to ASM-protein isolate diet progressively improved growth, PER, BV and NPU and these values compared well with that of casein. It is interesting to note that addition of methionine and cystine (supplements A, B, D and E) improved the biological values of PEM and ASM-protein isolate markedly while other essential amino acids in supplements C and F had no significant effect so far. It is therefore likely that under the present experimental conditions methionine and cystine were the most limiting amino acids of PEM and ASM-protein isolate.

Values for the biochemical parameters of blood and liver of rats fed diets containing casein, CEM, PEM and ASM-protein isolate are presented in Table 6. The results revealed that CEM in diet affects the biochemical parameters of blood and liver adversely. This may be correlated to its high tannin content. Similar toxic effects of tannins in animal feed are well reported[22,23]. PEM was almost free from tannins and did not reveal any abnormality in evaluations of blood and liver biochemical parameters when given in the diet of rats.

The blood sugar levels were significantly elevated in those animals fed diets containing PEM and ASM-protein isolate, probably due to inhibition of glycolysis by some glycoprotein present therein[5]. ASM-protein isolate and PEM also contain a low amount of saponins, which may have some adverse effects on regulation of insulin from pancreatic β-cells and on blood sugar. Yadav *et al*[24] reported that some Acacia seed protein-supplemented diets can lead to increased blood sugar level in young rats.

It is evident from the the results (Table 6) that the nature of dietary protein influenced the lipid composition

TABLE 6. BIOCHEMICAL PARAMETERS OF BLOOD AND LIVER OF RATS FED DIETS CONTAINING CASEIN, (CEM) CRUDE AND PROCESSED (PEM) EUCALYPTUS SEED MEAL AND ASM-PROTEIN ISOLATE FOR 28 DAYS

Parameters	Casein	CEM	PEM	ASM-protein isolate
		Blood		
Haemoglobin (g/100 ml)	15.7±0.62	13.2±0.81*	15.6±0.58	15.6±0.78
sugar (mg/100 ml)	86.3±2.48	118.8±3.64***	95.5±3.01*	99.7±3.11**
Serum total protein (g/100 ml)	6.1±0.12	4.5±0.21***	6.0±0.32	6.2±0.21
Serum total lipids (mg/100 ml)	268.5±5.12	300.8±6.21**	256.5±5.12	218.2±4.56***
Serum phospholipids (mg/100 ml)	83.1±2.45	84.2±2.01	81.8±2.11	76.5±2.11
Serum cholesterol (mg/100 ml)	66.3±1.65	85.1±2 12***	62.3±2.16	51.1±1.85***
		Liver		
Total protein (mg/g)	141.7±4.12	89.3±3.21***	138.8±4.06	145.3±2.56
Total lipids (mg/g)	165.6±4.21	190.4±5.16**	152.3±3.21*	120.2±3.01***
Phospholipids (mg/g)	78.1±1.58	79.3±2.12	77.2±1.56	71.5±2.21*
Cholesterol (mg/g)	6.1±0.52	7.1±0.48	5.6±0.45	4.1±0.25**

Values were significantly different from those for the corresponding casein group (Student's t-test):
*$P<0.05$; **$P<0.01$; ***$P<0.001$
Values are Mean±S.E. No. of animals in each group was 6.

of serum and liver. It is generally believed that animal proteins show lipid elevating action while plant proteins have some lipid lowering action. Recently, findings by Leelamma *et al.*[25] and Huff and Carroll[26] have shown that the effect on lipid levels depends upon the nature of particular protein rather than its source. Thus, the nature of protein intake affects lipid metabolism but it is not clear how exactly the nature of protein can affect the lipid metabolism. It is possible that this may be related to (*i*) amino acid composition of protein[27], (*ii*) digestibility of different proteins, (*iii*) nature and concentration of non-proteinous materials in prtoeins[28,29], (*iv*) possibility that small glycopeptides may be absorbed, and (*v*) the possibility that absorption of the intact protein may take place for which some evidence has been recently presented[30]. These factors may affect the enzymes involved in the synthesis and catabolism of the lipids. It is in this context that although the lipid levels of blood and liver of rats fed ASM-protein isolate diet varied from those of fed casein diet but still the values were within the normal range of variation. All other biochemical parameters of rats fed diets containing ASM protein isolate did not differ significantly as compared to those fed casein diet.

These observations thus suggest that the eucalyptus seed meal which contains about 6 per cent of tannins should be detoxified prior to its incorporation in animal feedstuffs and ASM-protein isolate may also be used as a source of supplementary protein. However, these unconventional food sources are not well-balanced in their amino acid pattern, hence the incorporation of such products in various food preparations should be carefully planned and the required amino acids added, if necessary.

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Potential Mycotoxigens in Wheat and Wheat Products

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Out of 31 samples of wheat or its milled products, collected from the market and flour mills, 30 were found to harbour storage fungi. A market survey of food products made from wheat, including bread and various pasta products, showed all the 23 samples to be contaminated with fungi. The contamination was particularly heavy in the pasta products, with *Aspergillus flavus* as the most common fungus, eventhough some analysed samples were free from aflatoxin.

Wheat is more susceptible to insect and fungal attacks in storage than rice. Fungi commonly encountered belong to osmophilic species of *Aspergillus* and *Penicillium*, some of which are known to produce mycotoxins.

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Since information on these species of fungi occurring in wheat and its products in India is very scanty, this study was taken up and the results presented here.

Materials and Methods

Samples: The samples, 54 in all, included 20 of whole wheat, 11 of milled products of different grades such as whole meal, *maida*, (refined wheat flour), *rawa* (semolina) and bran, 14 of pasta products such as vermicelli, spaghetti and macaroni, and 9 of bakery products including brown and white bread made in small bakeries and factory-made white bread (Table 1).

Analysis of mycoflora: Storage fungi were selectively isolated from the commodities using standard methods[1] with a few modifications[2]. Czapek-Dox agar with 50 per cent sucrose w/v was used throughout. When bacteria had to be avoided, Streptopenicillin (20 μg/ml) was added to the medium prior to pouring.

In plating whole grains, 100 grains per sample were used. In analysing milled products, direct plating using 10, 50 and 100 mg of the sample, and dilution plating from 10^{-2} and 10^{-3} dilutions were followed, using a total of 10 plates per sample. Pasta products were broken up into bits and the bits picked out at random for plating. Depending on the diameter, 100 (vermicelli), 80 (spaghetti), or 40 (macaroni) bits per sample were plated. In the case of bread, 25 fragments per loaf were cut out aseptically from different regions of the loaf and plated.

Surface sterilization was done using 0.1 per cent mercuric chloride solution which was allowed to act for one minute and washed off repeatedly with 7.5 per cent sodium chloride solution. Whole grains were plated only after surface sterilization, while pasta products were surface sterilized only in some cases.

The plates were incubated in the dark at 30°C and examined after 5 days for enumeration, isolation and identification of fungi. The *Aspergilli* were identified after Raper and Fennell[3].

Fungal infestation was expressed in terms of percentage of infested grains (the term infestation is used in preference to infection which connotes pathogenic fungi), of infested bits of pasta and of infested loaf in the case of whole grains, pasta and bread, respectively and in terms of number per gram in the case of milled products.

A few samples showing an abundance of *Aspergillus flavus* were analysed for aflatoxins using standard methods[4].

Results and Discussion

Storage fungi: Of the 20 samples of whole wheat,

TABLE 1. DETAILS OF SAMPLES USED IN THE STUDY

Commodity/Product	No. of samples	Moisture range (%)
Soft white wheat	4*	8.8– 9.4
,,	3+	9.6–10.0
,,	1*	11.2
,,	1	8.8
Soft amber wheat	1*	10.0
,,	1	10.0
Red wheat (P.V. 18)	1	8.4
Hard wheat (Kalyan)	1*	9.6
,,	1+	8.2
,,	2	7.6– 8.6
Samba wheat (Sarbati)	1+	8.4
,,	3	10.2–11.4
Whole flour (*atta*)	3	9.6–12.6
Extracted flour (*maida*)	3	11.4–12.0
Semolina (*rawa*)	2	12.0
Bran	3	10.2–12.2
Vermicelli	5	—
Spaghetti	4	—
Macaroni	5	—
White bread	3	—
Brown bread	3	—
Factory bread	3	—

—Not done. The samples are collected from *TNCSC godowns; +Co-operative shops; and others are from private shops.

TABLE 2. INFESTATION BY STORAGE FUNGI IN WHOLE WHEAT AND ITS MILLED PRODUCTS, ARRANGED IN THE DESCENDING ORDER OF MAGNITUDE

Whole wheat			Milled products		
Varieties	Moisture content (%)	Extent of Infestation (%)	Grade	Moisture content (%)	No. of propagules/g
Soft white	11.2	100	Bran	12.2	2930
,, ,,	8.8	100	*Maida*	12.0	2280
Samba (Sarbati)	10.2	100	,,	11.8	1830
Soft white	9.8	86	*Rawa*	12.0	1830
Samba/Sarbati)	11.4	77	*Atta*	11.6	1740
Hard (Kalyan)	7.6	60	Bran	10.2	1650
,, ,,	8.2	40	*Maida*	11.4	1200
Samba (Sarbati)	8.4	30	*Atta*	9.6	960
Soft white	9.4	26	Bran	10.8	510
,, ,,	9.0	16	*Atta*	12.6	420
Hard (Kalyan)	9.6	14	*Rawa*	12.0	10
,, ,,	8.6	10			
Soft white	8.8	9			
,, ,,	9.6	8			
Soft amber	10.0	8			
Soft white	9.0	6			
,, ,,	10.0	6			
Red (P. V. 18)	8.4	4			
Samba (Sarbati)	10.6	3			
Soft amber	10.0	0			

19 harboured storage fungi (Table 2). The infestation level varied from 3 to 100 per cent with an average of 35 per cent, but the differences were not correlatable with variety or moisture content.

All the 11 milled samples showed heavy fungal infestation averaging 1800 propagules per gram, a sample of bran showing the highest, and one of *rawa* showing the lowest figures. There was wide variation within each category, but on an average, *maida* and bran recorded the highest and *rawa*, the lowest fungal numbers per gram (Table 2).

Qualitatively, the storage fungi included *Aspergillus glaucus* group comprising *A. amstelodami* (Mangin) Thom & Church, *A. chevalieri* (Mangin) Thom & Church and *A. repens* De Bary, all referred to for convenience as *A. glaucus*.

A. flavus Link, *A. candidus* Link, *A. niger* V. Tiegh, *A. terreus* Thom, *A. sydowi* (Bain & Sart.) Thom & Church, *Penicillium* sp., and *Mucor* sp. *Alternaria* sp., *Isaria* sp. and a few others, rarely seen and referred to as 'other fungi'. Osmophilic bacteria occurred in a few milled products.

Of these fungi, *A. glaucus* was the most abundant in whole wheat as well as in its milled products. Among others, *A. flavus* was more abundant in whole wheat while *A. candidus* and *A. terreus* were more common in the milled products (Table 4).

The pasta products were heavily infested, the level being 100 per cent in spaghetti, 99 per cent in macaroni and 61 per cent in vermicelli. All the samples of bread also showed fungal growth, the level being highest in brown bread (69 per cent average) and lowest in factory-made white bread (12 per cent average)-(Table 3). Qualitatively, the most striking feature was the abundance of *A. flavus* in pasta products as well as in bread. Only vermicelli and factory-made white bread recorded a low occurrence of *A. flavus*. Among the less common fungi, *A. terreus* was not recorded in these food products, while *A. fumigatus* Fresenius and *A. wentii* Wehmer, not recorded in whole wheat or flour, were sometimes found (Table 4).

The occurrence of storage fungi in wheat and wheat flour is well known, and our results show some qualitative and quantitative differences from those of others. Qualitatively, our results for flours are comparable with those of Bothast *et al*[5]. who also reported *A. glaucus* and *A. candidus* as the dominant flora. Basu and Mehrotra[6] on the other hand have reported numerous species of *Aspergilli* and *Penicillia*. In our experience, *A. candidus* usually occurs in the interior of the grain and hence its predominance in the flour.

Mouldiness in bread is an oft-studied phenomenon, but studies on pasta products are relatively rare. Christensen and Kennedy[7] reported *Aspergillus flavus-oryzae*, including toxigenic strains, to be the dominant fungus in most of the 47 pasta samples studied by them.

Aflatoxins: No aflatoxin was detected in any of the 3 samples of spaghetti, 2 of bread or one of macaroni tested (Table 3). However, toxin production in these foods cannot be ruled out, as there are reports of the occurrence of toxin in dry spaghetti[8]; while bread and cake have been found to serve as good media for aflatoxin production[9], the toxin being able to survive the baking process[10].

TABLE 3. INFESTATION BY STORAGE FUNGI IN PROCESSED PRODUCTS OF WHEAT

Sample No.	Product	Infestation (%)		Sample No.	Type of bread/source	Infestation (%)
		Untreated	Surface sterilized			
1	Vermicelli	85.0	—	*1	White/Bakery	40
2	,,	68.0	—	2	,,	40
3	,,	66.0	—	3	,,	36
4	,,	32.5	—	*4	Brown/Bakery	100
5	,,	32.5	—	5	,,	76
6	Spaghetti	100.0	—	6	,,	32
*7	,,	100.0	100.0	7	White/Factory	24
*8	,,	100.0	80.0	8	,,	8
*9	,,	100.0	60.0	9	,,	4
10	Macaroni	100.0	—			
*11	,,	100.0	85.0			
12	,,	99.0	—			
13	,,	97.5	87.5			
14	,,	97.5	72.5			

*Samples analysed for aflatoxin
—Not done

TABLE 4. AVERAGE PATTERN OF DISTRIBUTION OF SPECIES OF STORAGE FUNGI IN WHEAT AND ITS PRODUCTS

Variety/Product	Av. infestation	*A. gl.*	*A. fl.*	*A. cand*	*A. nig*	*A. ter*	*A. syd*	*A. fum*	*A. wen*	*Pen* sp.	*Mucor* sp.	Other fungi	Bacteria
						***Whole wheat** (% infestation)							
Soft white (9)	39.7	20.0	7.7	0.7	9.2	0	0	0	0	0	3.4	3.5	0.1
Soft amber (2)	4.0	1.5	0.5	0	1.0	0	0	0	0	1.0	0	0	0
Sarbati (4)	52.5	19.5	12.3	0.3	3.5	0	0	0	0	0	17.3	0	0
Kalyan (4)	3.10	14.5	7.5	1.8	0	0	1.8	0	0	0	0	5.8	0
PV 18 (1)	4.0	0	0	0	0	0	0	0	0	2.0	0	2.0	0
						Milled products (colonies/g)							
Atta (3)	1040	385	17	539	28	10	2	0	0	52	5	8	80
Maida (3)	1770	510	177	300	11	422	0	0	0	200	153	0	46
Rawa (2)	920	754	5	27	1	2	0	0	0	125	0	7	2
Bran (3)	1700	314	188	579	6	503	0	0	0	49	47	0	823
						*****Pasta (% infestation)							
Vermicelli (5)	61	36.7	14.3	1.0	3.0	0	0	1.0	0	2.2	0	0	0
Spaghetti (4)	100	0	93.0	0	10.1	0	0	0	1.2	0	0	0	0
Macaroni (5)	99	36.5	52.2	4.0	9.9	0	0	0	0	0	4.0	19.0	
						***Bread** (% infestation)							
Bakery, white (3)	39.0	8.0	29.0	0	0	0	0	0	1.3	0	0	0	0
Bakery, brown(3)	69.3	8.0	31.0	0	8.0	0	1.3	0	21.3	0	0	0	0
Factory, white (3)	12.0	2.7	0	0	0	0	0	1.3	0	0	8.0	0	0

*The total of individual species may exceed the percentage of grain infestation, as a single grain frequently produces more than one colony.

A.gl:=*A. glaucus; A.fl*=*A. flavus; A.nd*=*A. candidus; A.nig.*=*A. niger; A.ter*=*A. terreus; A. syd*=*A. sydowi; A.fum*=*A. fumigatus, A.wen*=*A. wentii; Pen.*sp.=*Penicillium* sp.

Figures in parentheses indicate number of samples tested.

Sources of contamination: The high levels of fungi in the flour suggest that the raw material itself might be the source of contamination of these food products.

In the case of the pasta products, those with a thicker diameter had a higher percentage of infestation, mainly with *A. flavus*. This suggested that the enriching additives and the microenvironment of the slow and prolonged drying process might have selectively favoured the growth of *A. flavus* within the sticks. In order to verify that the fungus was present within the product and not as a surface contaminant from spores in the air, we plated some samples of spaghetti and macaroni after washing in a dilute detergent solution (Tween 80, 0.1 per cent aqueous) and after surface sterilization. The washings were plated separately. The washings recorded very low counts and *A. flavus* was not generally found in these, but the washed or surface sterilized bits showed the same fungus as the whole bits. This suggested that the fungus occurred as mycelium within the product, although we could not detect it by microscopic examination (Table 3).

In the case of bread, the high degree of contamination of brown bread, made from whole flour including bran, which usually recorded high fungal numbers, indicated that the flour was the major source. This could act directly by growing in the dough and surviving the baking process, or indirectly by contaminating the bakery atmosphere and settling on the baked bread. The latter possibility was suggested by the results of our limited aeromycological survey of the interior of a bakery (Table 5).

Discussing the presence of toxigenic *Aspergilli* in pasta, Christensen[11] suggests that the fungi enter the dough during or after mixing and grow during drying. Those who study mouldiness in bread, however, usually point

TABLE 5. AEROMYCOLOGY OF A BAKERY

Fungus	Av. colonies (no.) per plate at different processes of bread manufacture*				
	Kneading	Fermentation	Baking	Slicing and packing	Total
Aspergillus fumigatus	3	2	1	2	8
A. niger	1	1	1	1	4
A. candidus	4	0	0	0	4
A. glaucus	1	0	0	2	3
A. flavus	1	0	1	0	2
A. terreus	0	0	0	1	1
A. foetidus	1	0	0	0	1

*Exposure time: 5 to 10 min.

to low-grade flour as the cause[12,13]. Studies are now under way in our laboratory to establish the actual manner of contamination of bread. At this point it may be said that the use of good quality raw materials, and the maintenence of proper environmental hygeine would make these wheat foods less hazardous.

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RESEARCH NOTES

STUDIES ON THE SUITABILITY OF POLYPROPYLENE POUCHES FOR PACKING MANGO PULP*

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Mango pulp of Cv. 'Lucknow Safeda' was stored in polypropylene pouches (200 gauge) with thermal sterilization (T_1) and with 350 (T_2) and 100 (T_3) ppm SO_2, at 32-35°C (ambient) and at 4-6°C. All the samples stored at ambient temperature were spoiled after two months, while those stored at lower temperature were in good condition after 4 months. There was rise in pH, protein content and tannins. There was decrease in total sugars and soluble solids in treatments T_1 and T_3. There was no appreciable loss of moisture or vitamin C. There was almost complete loss of available SO_2 during storage. The treatment T_2 was found to be the best for preserving mango pulp.

The exhorbitant price of tinplates has necessitated the use of polyethylene (HDPE) and other flexible packaging for food material[1,2]. Since HDPE containers cannot withstand high temperatures, bulk preservation of pulp is achieved by sulphitation and subsequent lower temperature processing, which also preserve the delicate mango flavour. However, there are some constraints to sulphite treatment because of the residual off-flavour in the pulp[3]. Shrikhande *et al.*[4] have reported storage of mango pulp for 6 months after thermal process and bulk packaging in HDPE containers, without sulphitation. Recently, polypropylene/aluminium foil/polypropylene pocuhes[2] and asceptic packaging[5] have been introduced in developed countries for packaging of food products and these may be adopted by the industry in our country in the near future.

We have designed an experiment to assess the storage quality of mango pulp in flexible polypropylene pouches. Mango pulp from Cv. 'Lucknow Safeda' was expressed and was filtered to remove fibres. Two hundred and fifty gram of pulp was filled into polypropylene pouches (2/3 capacity), sealed and heated in boiling water for 20 min (T_1). In the second treatment, the pulp was heated to 80°C for 10 min, cooled to about 50°C, mixed with

TABLE 1. CHANGES IN CHEMICAL CHARACTERISTICS IN MANGO PULP (CV. LUCKNOW SAFEDA) DURING STORAGE IN POLYPROPYLENE POUCHES

Treatment	Storage period (months)	Storage temp (°C)	Moisture (%)	pH	Acidity (% citric acid)	TSS (%)	Sugar		Vitamin C (mg %)	Protein (mg %)	Tannins (mg %)	SO_2 (ppm)
							Total (%)	Reducing (%)				
—	Initial		78.2	4.6	0.32	21.4	18.1	4.5	5.8	4.1	102	—
T_1	1	32-35	76.4	5.2	0.13	18.4	10.2	6.7	4.4	4.1	232	Not added
T_1	,,	4-6	77.6	4.7	0.20	19.8	15.7	5.7	4.8	4.2	160	,,
T_1	2	32-35	81.8	5.9	0.15	17.8	11.1	8.7	4.5	4.6	271	,,
T_1	,,	4-6	79.3	5.1	0.26	18.9	13.6	6.8	5.6	4.3	209	,,
T_2	1	32-35	79.5	4.5	0.19	20.2	19.8	5.5	4.5	5.3	226	160
T_2	,,	4-6	79.6	4.8	0.20	21.2	19.4	5.4	6.6	5.6	186	250
T_2	2	32-35	70.8	5.3	0.26	19.4	17.6	7.9	3.5	5.2	292	70
T_2	,,	4-6	78.8	5.0	0.21	20.5	18.3	5.7	6.8	4.3	210	160
T_2	3	32-35	—	—	—	—	—	—	—	—	—	—
T_2	,,	4-6	77.1	5.0	0.31	20.4	16.4	5.6	4.1	5.5	184	64
T_2	4	32-35	—	—	—	—	—	—	—	—	—	—
T_2	,,	4-6	76.2	5.2	0.22	19.8	17.0	6.0	2.6	4.6	176	50
T_3	1	32-25	77.8	5.2	0.20	20.4	14.6	6.7	4.5	5.5	246	64
T_3	,,	4-6	79.6	4.8	0.20	18.8	14.4	4.8	5.7	4.0	178	80
T_3	2	32-35	80.9	5.7	0.25	18.6	11.3	8.7	3.3	6.2	239	nil
T_3	,,	4-6	79.7	5.2	0.11	18.9	13.8	5.8	5.0	4.9	284	64
T_3	3	32-35	—	—	—	—	—	—	—	—	—	—
T_3	,,	4-6	80.0	5.4	0.16	19.3	12.1	6.4	4.3	4.5	195	50
T_3	4	32-35	—	—	—	—	—	—	—	—	—	—
T_3	,,	4-6	79.3	5.3	0.28	15.4	11.7	8.5	3.3	5.9	151	nil

(—) No estimation made due to spoilage
T_1 - T_3=Treatments.

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350 ppm SO_2, filled in pouches and sealed (T_2). Thirdly, the pulp was mixed with 100 ppm SO_2, filled in pouches, sealed and heated in boiling water for 20 min (T_3). Pouches in each treatment were stored at room temperature (32-35°C) and at low temperature (4-6°C). The analysis[6] of various physico-chemical characteristics was carried out at monthly intervals. The initial composition of the pulp is given in the Table 1.

All the treatments under ambient and T_1 in cold conditions, developed mould growth, clots and precipitation after 2-3 months of storage. Sulphur treatment at higher temperature (32-35°C), might have been less effective due to high pH (4.55) of mango pulp. However, these observations were in accordance with the reports of Krishna Murthy *et al.*[7] The material kept well in this treatments for 4 months. Generally, there was no significant loss of moisture during storage. Further, the pulp under ambient conditions exhibited an increase in reducing sugars (from 4 to 8 per cent) and a decrease in total sugars (from 18 to 11 per cent) and TSS (from 21 to 18 per cent) with the exception of T_2. Changes were still less in cold condition. Sulphitation (350 ppm) seems to help in preserving sugars and TSS. There was a slight increase in pH (from 4.55 to 5.20 in cold and to 5.9 at room temperature) of stored pulp, but there was no concurrent decrease in titrable acidity. Very low concentration of vitamin C was present initially in the mango pulp and there was no further loss during storage. Soluble protein increased but more so under ambient temperature. Tannins rose sharply at room temperature but in cold treatment it increased from 102 to 284 mg per cent upto 2 months and then declined to 150-170 mg per cent. There was no deterioration in colour or organoleptic quality of the pulp or the juice prepared from the stored pulp. Determination of available SO_2 content showed meagre amount, 50 ppm in T_2 and absent in T_3 at the end of 4 months.

It may be inferred from this study that flexible polypropylene pouches (200 gauge) may not be suitable for long storage (over 2-3 months) of mango pulp under North Indian conditions of high temperature, even with sulphitation (350 ppm SO_2). Higher levels of SO_2 and acidity may improve the preservation of mango pulp in flexible pouches.

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ON THE INFLUENCE OF EVACUATION ON LIQUID WATER ABSORPTION BY PADDY GRAINS

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Studies on moisture uptake of evacuated and untreated paddy revealed that it was higher in evacuated paddy under different time-temperature conditions. It is indicated that soaking of evacuated paddy may substantially reduce the process time of parboiling.

Soaking of paddy is important during parboiling and its duration decides the total period of the process[1-7]. Duration of soaking mainly depends upon the soaking characteristics and the variety of paddy. Once the soaking characteristics of paddy are known, the conditions of treatment can be easily worked out to attain a desired moisture content of paddy suitable for parboiling, steaming, *poha* (rice flakes) making etc[2-8]. Achieving desired moisture level in short time leads to reduction in total time of parboiling.

Possibility of reducing the soaking time during parboiling by evacuation of paddy prior to soaking was explored.

'Jaya' variety of paddy having 13.2 per cent moisture (d.b.) was used in this experiment. Samples of 150 g each were mixed with 225 ml hot water in a beaker and placed in pre-stabilised waterbath at desired temperature for soaking (Table 1). The initial moisture content of the paddy was determined by standard air oven method[6]. The moisture content of soaked sample(s) was calculated as follows:

$W_1 (100+M_2)=W_2 (100+M_1)$ ..... (1)

Where,

W_1=initial weight of the sample at moisture content M_1, g.

W_2=final weight of the sample at moisture content M_2, g.

M_1=initial moisture content of the sample, per cent, d.b.

M_2=final moisture content of the sample, per cent, d.b.

These values of moisture content of soaked samples were used for analysis.

TABLE 1. MOISTURE OF PADDY (VAR. JAYA) SOAKED UNDER DIFFERENT CONDITIONS**

Soaking period (hr)	Paddy moisture (% d.b.) at diff soaking temp.					
	30°C	40°C	50°C	60°C	70°C	80°C
Under atmospheric pressure						
¼	20.2	21.0	21.9	23.4	25.6	28.9
½	21.0	22.2	23.7	25.0	26.9	32.7
¾	22.1	23.8	26.1	27.6	29.5	36.0
1	22.9	25.1	27.2	29.1	32.8	41.2
2	24.8	27.4	30.1	31.9	37.0	46.6
3	27.1	29.9	32.5	35.0	41.5	62.3
4	28.6	32.1	34.2	36.3	45.2	77.2
5	30.1	33.8	36.0	37.8	49.3	104.1
6	31.7	35.4	38.7	40.5	53.9	118.3
Under vacuum						
¼	30.6	32.2	34.0	35.0	35.7	37.1
½	31.8	33.1	35.3	36.0	37.2	38.5
¾	32.2	34.7	36.8	37.5	38.5	41.2
1	32.7	35.7	37.7	39.3	39.9	43.7
2	35.2	37.5	39.6	42.5	43.5	52.8
3	36.6	38.3	41.9	43.5	46.0	68.5
4	37.0	39.0	42.5	44.5	49.4	90.1
5	38.2	40.6	44.2	46.2	51.6	134.9
6	39.8	41.4	46.1	47.3	54.1	167.2

Initial paddy moisture content: 13.2% (d.b.)

**Average of three replicats.

Another set of paddy samples were subjected to vacuum (–76 cm of Hg, as indicated by the compound gauge) for five minutes and 225 ml hot water was let into the flask soon after. The sample was then transfered to waterbath. Moisture content of sample was calculated (equation 1) after soaking for appropriate period as per schedule (Table 1).

Water uptake: Persual of data in Table 1 reveals that there was sudden rise in moisture content at initial stages of soaking at all the soaking treatments. It has been reported[3] that this phenomenon may be due to (*i*) the filling of void space between the husk and pericarp with water immediately after grains were immersed in water, and (*ii*) capillary action by pores in the hull causing rapid absorption.

According to Becker the initial moisture gain in soaked wheat grains was a measure of the amount of water required for saturation of pericarp. It can be stated supporting the above statement[4], other reported results[3] and the test data (Table 1) that the initial moisture gain was low, in case of direct paddy soaking, as compared to evacuated paddy soaking. This may be due to the presence of air gap between hull and rice kernel in case of direct soaking. The evacuation of paddy prior to soaking might have expelled most of the air from the void spaces and neutralised the resistance to entry of water resulting in high initial moisture gain.

The moisture content values (Table 1) also indicate that the total moisture gain increased with increase in soaking temperature and this was true for all the tests. Two reasons can be put forth for this phenomenon: (*i*) Air present in the micropores might not have been removed completely during evacuation of paddy, which would have expanded with the rise in temperature and quickly replaced by water, (*ii*) water is dipole compound and is regarded as a polymer of hydrogen bond. The bonds are broken when water is heated, which results in the dissociation and entry of water molecules into the weakened starch due to heat.

It was observed that air bubbles were liberated at the surface of evacuated paddy grain during the first 2-5 min of exposure to water. The time of air bubble liberation was inversely proportional to the soaking temperature[7]. This phenomenon confirms the observation on vacuum[4] and also supports the statement indicating the presence of unexpelled air in evacuated paddy.

It is also evident from the moisture content values (Table 1) that the evacuated paddy would attain the desired level of moisture in less soaking time and at lower soaking temperature than direct soaking of paddy. Hence, the evacuated soaking would help in reducing the soaking time and severity of heat treatment to grain.

TABLE 2. RATE OF MOISTURE ABSORPTION, PER CENT DB PER HOUR FOR JAYA PADDY WHEN SOAKED UNDER DIFFERENT CONDITIONS**

Soaking period (hr)	Moisture absorption (% db) at different soaking temp					
	30°C	40°C	50°C	60°C	70°C	80°C
Under atmospheric pressure						
0.125	28.1	31.0	34.8	40.7	49.4	62.6
0.375	3.0	5.0	6.9	6.2	5.2	15.2
0.625	4.6	6.2	9.6	10.3	10.3	13.3
0.875	3.0	5.1	4.6	6.0	13.6	20.8
1.5	1.9	2.4	2.9	2.8	4.2	5.5
2.5	2.3	2.5	2.4	4.1	4.5	15.6
3.5	1.5	2.2	1.7	1.3	3.7	15.0
4.5	1.5	1.7	1.8	1.6	4.1	26.8
5.5	1.5	1.6	2.7	2.7	3.7	14.2
Under vacuum						
0.125	65.9	76.1	82.3	87.2	89.8	95.7
0.375	4.6	3.3	5.0	4.0	6.2	5.4
0.625	2.0	6.5	5.9	5.8	5.2	10.7
0.875	2.5	4.2	3.8	7.5	5.6	10.2
1.5	2.5	1.7	1.9	3.1	3.6	8.6
2.5	1.4	0.9	2.3	1.0	2.5	16.2
3.5	0.4	0.7	0.6	1.1	3.4	31.6
4.5	1.2	1.6	1.7	1.6	2.2	54.8
5.5	1.6	0.7	1.9	1.2	2.5	32.3

Initial moisture content of paddy is 13.2% (d.b.)
**Computed from Table 1.

The rate of moisture absorption: The values in Table 2 indicate the slow rate of moisture absorption up to 50°C for both the tests. In all the cases the moisture absorption rate was high in the initial stage of soaking and decreased with increase in soaking time. However, after some time of soaking (1.5 to 2 hr) at 80°C, it increased in both the cases, due to bursting of grains. Comparison of subsequent (after initial stage) values of rate of moisture absorption indicate low rate in evacuated paddy soaking than in direct atmospheric pressure soaking. This may be due to substantial increase in the moisture content at the initial stage of soaking of evacuated paddy causing decrease in moisture potential (saturation moisture–initial moisture content of sample). Thus, the low rate of moisture absorption in the subsequent soaking is an indication of high total moisture uptake by the sample (Table 1 & 2).

Conclusion: It is obvious that due to high moisture absorption, gelatinization of starch may take place earlier, for evacuated paddy soaking than for direct soaking, at higher (70 and 80°C) temperatures. This phenomenon would be desirable and help reducing the total time of parboiling process.

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BIOLOGICAL EVALUATION OF LOW GLUCOSINOLATE VARIETY OF RAPESEED (*BRASSICA CAMPESTRIS* VAR. *TORIA*) MEAL

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A 4-week trial was conducted with male albino rats to ascertain the biological quality (PER, FE, PU, FU and NPR) of repeseed meal (RSM) having low glucosinolate content. The RSM proved to be an inferior quality protein due to less efficient utilization in contrast to casein. Thus, it is deduced that RSM can be better utilized only after partial or complete removal of crude fibre and toxic components.

Brassica campestris var. *toria* is an important oil seed crop. Seed residue after expression of oil is mostly being utilized as animal feed or manure[1,2]. Since it is a rich source of protein with well balanced amino acid composition[3,4], this note presents data on its biological quality by evaluating PER, FE, PU, FU and NPR in male albino rats.

Male albino rats (wistar strain) 21-28 days young

TABLE 1. NUTRITIONAL EVALUATION OF RAPESEED MEAL

	Casein (10%)					Rape seed meal (10%)				
	7 days	14 days	21 days	28 days	Total (g/28 days)	7 days	14 days	21 days	28 days	Total (g/28 days)
Food intake (g)	50.00 ±3.00	43.90 ±2.10	45.51 ±3.50	48.50 ±1.75	187.91 ±8.41	31.06 ±3.00	34.60 ±1.50	36.00 ±3.75	30.00 ±2.00	131.66 ±5.84
Protein intake (g)	5.00 ±0.30	4.39 ±0.21	4.55 ±0.35	4.85 ±0.17	18.79 ±0.84	3.10 ±0.30	3.46 ±0.15	3.60 ±0.37	3.00 ±0.20	13.16 ±0.58
Wt. change (g)	32.50 ±6.00	85.05 ±5.00	99.00 ±5.32	107.60 ±6.00	—	64.00 ±4.32	67.00 ±6.72	75.00 ±5.22	82.50 ±4.78	—
Wt. gain (g)	5.50	2.55	13.95	8.60	30.60	-13.00	3.00	8.00	7.50	5.50
PER	1.100	0.580	3.065	1.773	1.628	-4.193	0.867	2.222	2.500	0.417
PU	.909	1.721	0.326	0.563	0.61	0.238	1.153	0.450	0.400	2.39
FE	0.110	9.058	0.306	0.177	0.162	-0.419	0.086	0.222	0.250	0.041
FU	9.09	17.21	3.26	5.63	6.14	2.38	11.53	4.50	4.00	23.92
NPR	6.660	6.913	9.175	7.505	3.10	13.161	8.901	9.944	11.766	2.530

Initial body weight of animals=77.0±5.72 g

Net weight lost by NPG animals=27.80 g

PER=Protein efficiency ratio; PU=Protein utilization, FE=Feed efficiency; FU=Feed utilization NPR=Net protein retention

maintained at H.A.U., Hissar, were housed individually in cages. Standard diet (M/s Hind Lever Ltd., India) was supplied for 5 days to adapt the animals to new environment with *ad libitum* water. The animals were weighed and allocated into two groups (6 rats/group) with similar mean body weight. Each rat was weighed weekly and handled daily for feed intake.

The untreated toria seeds grown in Amritsar district (Punjab) were procured and defatted using successive extraction with petroleum ether for 24 hr. Diets containing casein and RSM (rapeseed meal) at 10 per cent protein level were prepared[5] and fed to respective groups for 28 days. The glucosinolate content of RSM was also determined[6]. Similarly the third group (NPG) was fed protein free diet for the same number of days. The biological indices like PER, FE, PU, FU and NPR were calcualted from food intake and weight change data.

The effect of diets on various biological indices is presented in Table 1. The casein fed animals showed almost a constant intake and progressive growth pattern while RSM depicted a weekly variable intake and gained weight only after an abrupt loss during first week.

The decreased food intake by RSM group during the first week of feeding trial is probably due to poorly acceptable and non-palatable nature of the meal[7,8]. Later, these animals tend to eat more to meet the energy requirements as the RSM contains a high level of non-digestible carbohydrate fraction (13 per cent) and protein (16 per cent) associated with the hull[9,10]. The intake, however, drops to basal value (fourth week) as if to state the inadequacy of RSM to act as a good quality protein. The casein fed animals, depicted a normal consumption. The weight change data (Table 1) reflects a progressive gain by casein in contrast to RSM animals. The loss of weight by NPG was obviously due to active catabolism[11].

The various biological indices indicate that animals fed RSM diet proved inferior. The PER of casein, 2.50, was however not presently achieved and seems to be affected by factors like environmental temperature as well as rat strain differences. The above findings provide support to the earlier reports[12,13].

The values of PU/FU indicate that utilization is a measure of feed required for a unit gain of weight[14], which qualifies RSM to be a poorer quality protein because more food is consumed for a unit gain in contrast to casein. Furthermore, better utilization probably promotes greater retention (NPR) as observed in the casein fed animals (Table 1). On the other hand, RSM

fails to gain proportionally to the amount of food consumed, possibly due to the explanation proposed above.

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EPOXYSTEARIC ACID CONTENT OF SAL (*SHOREA ROBUSTA*) KERNEL FAT

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Sal (*Shorea robusta*) fat samples, extracted in the laboratory from fresh kernels as well as procured from commercial sources, were analysed for epoxystearic acid content by a titrimetric method and hydroxystearic acid by thin layer chromatography. All the samples contained these acids within the limit (3% together). Alkali refining and bleaching did not affect the concentrations of these acids.

Possible presence of epoxystearic acid in the fatty acid profile of sal (*Shorea robusta*) fat is a special feature of the fat[1]. During storage, this epoxy acid or triglycerides containing epoxy acid gradually decrease and give rise to dihyroxystearic acid or triglycerides containing this acid[2]. The Central Committee for Food Standards, Ministry of Health, Government of India, based on the toxicological studies[3] has allowed the use of refined and bleached sal fat in confectionery and bakery products with the proviso that 9,10-epoxy and 9,10-dihydroxystearic acid contents of the sal fat together[4] should not be more than 3 per cent. The review[5] on sal fat contains very little information on oxygenated fatty acids and no data on the oxygenated fatty acid contents *vis a vis* quality of kernel on effect of processing crude sal fat are available.

The present study reports the results of study on (*a*) variation in epoxy acid content of different quality of sal seed kernels from forests of Madhya Pradesh and Orissa and (*b*) effect of processing (alkali refining and bleaching) of sal fat having epoxy acid. In addition, dihydroxystearic acid content along with iodine value and free fatty acid (FFA) of the sal fat are reported in this note.

Samples of fat were extracted in the laboratory from freshly collected sal seeds. Commercially fresh samples both crude and refined and bleached were obtained from M/s K. N. Oil Industries, Mahasamund (M.P.) and M/s Orissa Oil Industry, Sambalpur.

Moisture content of kernel was determined by drying the sample in a vacuum oven at 60±5°C. Fat was extracted using petroleum ether (40-60°C) in a Soxhlet apparatus. Free fatty acids and iodine value estimated according to AOCS methods[6] and oxirane oxygen content was estimated by the titrimetric method[7] using tetraethyl ammonium bromide. Oxirane oxygen was multiplied by factor 18.6 to get the value of epoxy acid

TABLE 1. ANALYSIS OF SAL KERNELS AND THE QUALITY OF EXTRACTED FAT FROM DIFFERENT AREAS

Type of sal seed	Moisture (%)	Fat (%)	FFA (as oleic acid %)	Stearic acid		Total stearic acid (%)
				Epoxy (%)	Dihydroxy (%)	
Mahasamund						
Fresh kernels (3)	6.73±0.67	16.37±1.35	0.83±1.35	1.17±0.51	Nil	—
Commercial fresh	9.8 ±1.20	16.2 ±0.84	1.24±0.84	1.07±0.28	Nil	—
Old samples (7)	8.53±0.10	15.1 ±0.33	4.4 ±0.22	0.37±0.00	0.80±0.17	1.17±0.22
Sambalpur						
Commercial fresh (2)	10.25	18.55	1.45	1.19	Nil	—
Old samples (3)	7.53±0.26	13.67±0.30	4.63±0.26	0.41±0.14	0.76±0.20	1.17±0.10

Figures in parentheses indicate number of samples analysed.

expressed as 9,10-epoxy stearic acid. 9,-10-dihydroxy-stearic acid was estimated by the thin layer chromatography as described by Belavadi *et al.*[8]

It may be seen from Table 1, that the fat content from different sources did not vary significantly. Epoxystearic acid was present in both fresh and old samples. But the old samples had less of epoxystearic acid than the fresh samples and dihydroxystearic acid was present only in the old samples. This is in confirmation with the results obtained by Bringi *et al.*[2] The moisture content varied from 8 to 10 per cent. Equilibrium moisture content of sal kernel corresponding to 70 per cent RH has been reported to be 11 per cent[9]. Fat content of samples ranged from 13.5 to 18.0 per cent. Fresh samples had free fatty acid content ranging from 0.8 to 2.0 per cent whereas old samples from 4.0 to 5.7 per cent. It has been reported[9] that during storage of kernels having moisture content of about 10 per cent at ambient temperature, FFA increases at the rate of 0.5 per cent per month.

Epoxystearic acid content of fresh samples was found to vary from 0.19 to 1.82 per cent. In the old samples the epoxy acid content was less and the presence of dihydroxystearic acid was observed. However, there was no quantitative inverse relationship between epoxy and dihydroxy acids.

Table 2 gives the epoxy acid and dihydroxystearic acid contents in original, refined and bleached sal fat from different areas. It could be seen that refined and bleached sal fat (old samples) had a low epoxy acid content and presence of dihydroxystearic acid was also observed. The dihydroxystearic acid content was a little higher in Sambalpur samples compared to Mahasamund samples. But the total of epoxy-and dihydroxystearic acids did not exceed 3.0 per cent. Iodine values were in the range of 38.5 to 40.2 from both regions and this was within the range reported by others[5]

TABLE 2. ANALYSIS OF ORIGINAL FAT AND REFINED AND BLEACHED SAL FAT FROM DIFFERENT AREAS

Type of fat	FFA (as oleic acid %)	Stearic acid		Total stearic acid (%)
		Epoxy (%)	Dihydroxy (%)	
Mahasamund				
Original fresh (3)	—	1.04±0.3	Nil	—
Refined and bleached fresh (8)	—	1.0 ±0.22	,,	—
Refined and bleached old (2)	1.28	0.22	0.86	1.08
Sambalpur				
Original old (5)	4.1 ±0.89	0.32±0.1	1.22±0.44	1.54±0.39
Refined and bleached old (3)	1.28±0.1	0.2 ±0.0	2.17±0.66	2.36±0.70

Figures in parenthesis indicate number of samples analysed.

and within the limits prescribed by Central Committee for Food Standards.

Presence of epoxy acid in the refined and bleached sal fat showed that the process of alkali refining and bleaching had no effect on it. Similar observation has been made earlier by Krewson and Scott also[10].

Summing up, it was observed that epoxystearic acid is a natural component in the sal fat. As the fat gets aged the epoxy acid content was reduced and dihydroxy-stearic acid appeared. The process of alkali refining and bleaching had no effect on the epoxy acid and dihydroxy-stearic acid.

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ON THE QUALITY OF BREAD CONTAINING DIFFERENTLY PROCESSED POTATO

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Comparative studies carried out on the quality showed that the bread containing cooked potato mash was softer (compressibility: 10.8 kg/V) than those of bread containing drum dried potato flour (compressibility: 15.0 kg/V) or flour from dried chips (compressibility: 12.8 kg/V) at 15% level of incorporation (on equimoisture basis). The result indicate the possibility of reducing the cost of production.

In countries where wheat is in short supply, use of available tubers in bread formulations will help in reducing the wheat imports. Incorporation of potato flour in bread has certain advantages in aiding the fermentation by yeast, improving the flavour, texture and retention of crumb moisture, which in turn helps in delaying the onset of staling[1-4]. Energy required for dehydration of potatoes calls for development of alternate methods.

Sun-drying adopted to get dry potato chips, is dependent on the vagaries of weather and is rather slow and often not a hygienic process.

Comparative studies on the utilisation of cooked potato mash and potato flour in the bread formulation were undertaken and the results are presented in this paper.

Wheat flour: Wheat flour was milled from a commercial variety 'WG-357' grown in Punjab, to an extraction rate of 71 per cent in a Buhler laboratory mill-MLU 202.

Flour from dried potato chips: A locally popular variety 'K. Chandramukhi,' well known for its high specific gravity and hence high yield of flour was processed according to the method of Chandra Shekara and Shurpalekar[5]. The yield of potato flour was about 20 per cent on the basis of raw tuber.

Flour from drum dried potato flakes: Drum dried flakes were processed according to McG McBean[6] from the same potato variety. For improving the spreading and drying characteristics during drum drying, the level of solids was adjusted to an optimum of 12 per cent on the basis of preliminary trials. Drying was carried out on an Escher-Wyss double drum drier—Masc 231 using the following conditions: (*i*) Steam pressure: 5 kg/cm^2, (*ii*) drum clearance: 0.5 mm, and (*iii*) speed: 8 rpm.

The flakes were ground in the Kamas hammer mill-Slaggy 200A using a sieve with an aperture diameter of 0.8 mm.

Cooked potato mash: In the preparation of cooked mash, the steps followed were essentially the same as in the case of drum dried flakes with the elimination of dilution and drum drying operations.

Blending of potato flour/cooked mash with wheat flour: According to Chandra Shekara and Shurpalekar[7], a maximum level of 15 g potato flour from dried chips could be blended with 85 g wheat flour in the preparation of wheat-potato bread. Drum dried potato flour and cooked potato mash were also used for blending at same levels viz., 15.3 and 79.0 g respectively on equimoisture basis.

Bread making quality and evaluation: Bread making trials were conducted according to Malt-Phosphate-Bromate method of Irvine and McMullan[8] with the modifications of using 1 per cent fat in the formulation and a fermentation time of 105 min instead of 165 min. The level of water was 4 per cent less than farinograph water absorption at 500 B.U. in the case of potato flours to avoid stickiness in the dough, while, in case of cooked potato mash, the same level was maintained by adding

TABLE 1. EFFECT OF ADDITION OF 15% SOLIDS FROM DIFFERENTLY PROCESSED POTATO ON THE QUALITY CHARACTERISTICS OF WHEAT BREAD*

Form of potato as	Water added (ml)	Loaf vol. (cc)	Crust colour	Crumb		Overall acceptability
				Colour	Compressibility (kg/V)	
Dried chips flour	77.6	505	Dark brown	Cream	12.8	Satisfactory
Drum dried flake flour	76.5	500	Blackish brown	Brown	15.0	Fair
Cooked mash	20.0**	510	Dark brown	Cream	10.8	Satisfactory
Wheat (control)	65.4	600	Brown	Cream	7.0	Excellent

*All the breads except control had a slightly coarse and somewhat non-uniform crumb grain.
**After taking into consideration the high moisture content.

only 20ml water, after taking into consideration the moisture present in the mash.

Overall acceptability of the breads was arrived at on the basis of a maximum score of 100, using the weightage for different parameters according to Pyler[9]. On the basis of total score obtained, the different breads were graded as follows: Excellent: 91-100, Good: 81-90 Satisfactory: 66-80, Fair: 51-65, Poor: 50 or less.

Crumb texture was studied by recording its compressibility on General Foods Texturometer-GTX using slices of 1.3 cm thickness.

The results presented in Table 1 indicate that, bread based on wheat-cooked potato mash blend was comparable to those based on a blend of wheat and potato flour processed from dried chips. In contrast, the bread containing drum dried potato flour was unacceptable with an excessively dark crust and a somewhat brownish crumb (Fig 1 and 2.). Further, when compared with bread containing potato flours, the bread based on cooked potato mash had a better flavour and softer crumb, as indicated by the values for compressibility (Table 1).

The results have shown the possibilities of avoiding the high cost of dehydration in processing of potato flours, by utilising equivalent quantity of cooked potato mash solids, for obtaining comparable breads with improved crumb softness. This finding highlights the practical importance of using cooked potato mash in the preparation of wheat-potato breads, in view of fast increasing energy costs in several countries.

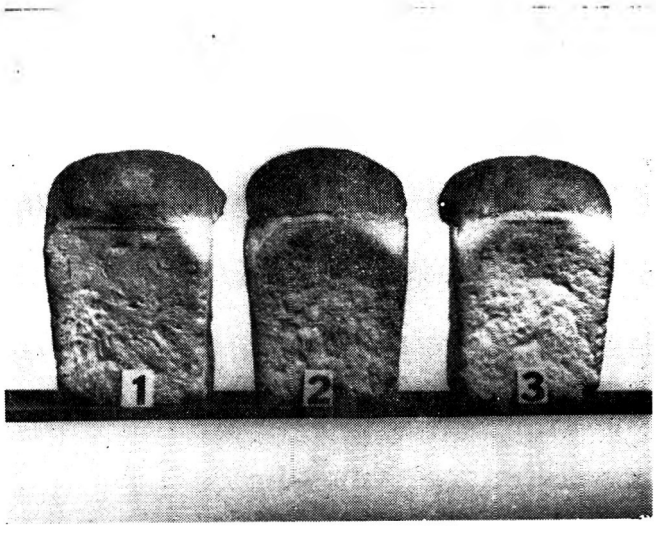

Fig. 1. Crust appearance of breads based on 85:15 blends of wheat flour and differently processed potato

1. Flour from dried chips. 2. Flour from drum dried flakes. 3. Cooked mash.

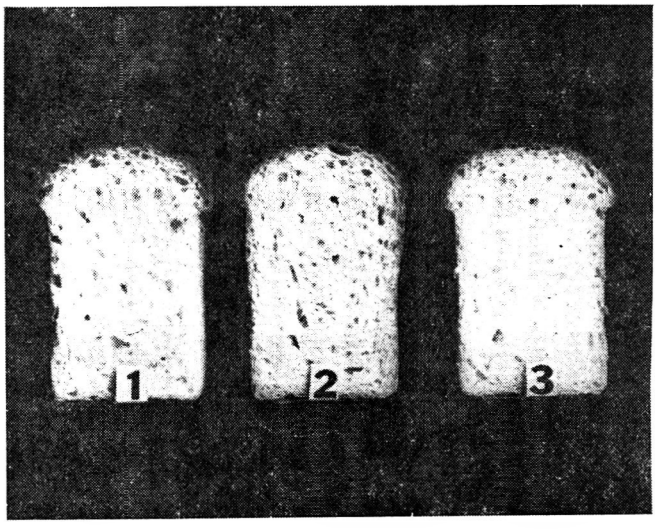

Fig. 2. Crumb appearance of breads based on 85:15 blends of wheat flour and differently processed potato

1. Flour from dried chips. 2. Flour from drum dried flakes. 3. Cooked mash.

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PARTIAL SUBSTITUTION OF MEAT IN *DENDENG GILING* WITH BREADFRUIT AND CORN GRITS

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Preliminary experiments have explored the possibility of substituting breadfruit for meat at levels up to 30% and corn grits for meat at levels up to 20% in *dendeng*, an intermediate moisture beef product of Indonesia. The substitution of a combination of 15% breadfruit and 10% corn grits for meat gave a product with acceptable organoleptic properties.

Breadfruit (*Artocarpus altilis* Fosberg, with synonyms *Sitodium atile* Parkinson, *Artocarpus communis* Forse and *Artocarpus incisa* L.F.) is a tree-borne fruit fit only for cooking and is often eaten as a vegetable. It is consumed baked, boiled, roasted or fried and when cut in slices it tastes much like bread or roasted potatoes[1].

Recently, Graham and DeBravo[2] studied the nutrient content of the skin, stem plus heart and the pulp of very immature, immature, mature and very mature breadfruit (*Artocarpus communis* J. R. & G. Forse). The major component and the principal carbohydrate in all three sections and at all stages of maturity was starch, while the protein content varied from 4.6 to 5.9 per cent, 6.0 to 7.6 per cent and 3.8 to 4.1 per cent in the skin, stem plus heart and the pulp, respectively. Compared to other starchy staple foods eaten by natives of the tropics, breadfruit can contribute appreciable amounts of the principal nutrients and is also a relatively good source of iron, calcium, potassium, riboflavin and niacin.

Corn grits are used as a main food in some regions of Indonesia; Lie[3] reported in the National Socio-economic Survey (1969-1970) that the average consumption of maize in Indonesia was 74 g/person/day; they contain 7.2-7.8 per cent protein, 05-1.0 per cent fat and 72.3-75.0 per cent carbohydrate[4].

The preparation of *dendeng*[5] involves slicing of a whole cut of meat, but it may also be made from minced meat in the form of strips, a product known as *dendeng giling*. As meat is quite expensive, attempts were made to use breadfruit and/or corn grits as a partial substitute of meat. *Dendeng giling* contains approximately 25 per cent protein, 9 per cent fat, 40 per cent sugar, 6 per cent salt and 20 per moisture.

The quantities of the ingredients used for 1 kg minced meat (round steak) are 200 g coconut sugar, 55 g cooking salt, 60 g coriander, 7 g garlic, 8 g tamarind and 2 g roots of greater *Galangal*. The flow sheet for *dendeng giling* preparation is given in Fig. 1. The AOAC procedure for the determination of moisture content of meat and meat products was followed[3] and a Vaisala HM 11 humidity meter and probe calibrated against seven saturated salt solutions in the RH range from 52 (sodium bromide) to 97 per cent (potassium sulphate) was used for water activity (a_w) determination. A scoring difference test as recommended by Larmond[7] was used to determine differences between samples due to the

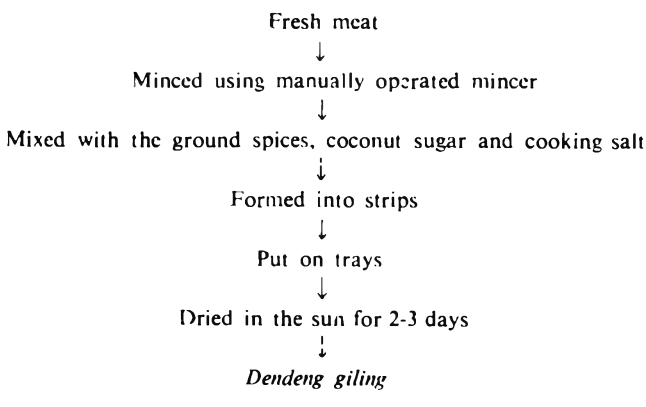

Fig. 1. Flow sheet for preparation of traditional *dendeng giling*.

TABLE 1. A_w, MOISTURE CONTENT AND ORGANOLEPTIC SCORES OF *DENDENG GILING* PARTIALLY SUBSTITUTED WITH BREADFRUIT AND CORN GRITS

Minced meat (%)	Breadfruit* (%)	Corn grits (%)	A_w	Moisture content (%) (wet wt basis)	Organoleptic score+			Overall acceptability
					Colour	Flavour	Texture	
				Experiment I				
100	—	—	0.67	21.7	7	7	6	7
90	10	—	0.62	16.4	6	6	6	6
80	20	—	0.59	18.0	6	5	6	6
70	30	—	0.61	19.9	5	5	5	5
				Experiment II				
100	—	—	0.63	18.8	7	7	7	7
90	—	10	0.61	17.2	6	5	5	5
80	—	20	0.59	17.9	4	5	4	5
70	—	30	0.63	19.6	3	3	4	4
				Experiment III				
100	—	—	0.59	19.1	7	7	7	6
75	15	10	0.59	20.2	6	6	6	6
50	30	20	0.59	19.9	3	3	4	5
25	45	30	0.60	19.5	3	3	4	2

*Samples were sun-dried for 2-3 days; temperature on the trays was in the range of 37° to 57°C

+Mean scores of 60 panelists for rankings on a 9-point hedonic scale, 9=like extremely, 5=neither like nor dislike 1=dislike extremely.

substitution by breadfruit or corn grits. The data obtained was examined by analysis of variance and Duncan's multiple range test to find out if there was a significant difference between samples.

The results shown in Table 1 indicate that increasing the amount of boiled breadfruit decreased the a_w and moisture content compared to the control. The moisture content increased with higher additions of breadfruit which reduced the high evaporation rate and also absorbed water from the meat during drying. Organoleptic tests showed that colour, taste, texture and flavour of *dendeng* substituted with 10, 20 and 30 per cent of breadfruit were not different to that prepared from 100 per cent minced meat.

Dendeng prepared using corn grits substituting for meat at levels of 10, 20 and 30 percent also had a promising shelf life, but organoleptic tests for these products showed that the texture, colour and flavour were affected by the substitution of corn grits ($P<0.01$).

The colour of *dendeng* prepared with 20 and 30 per cent corn grits was different to that containing 10 per cent or no added corn grits, with the yellow corn grits giving a lighter colour compared to traditional *dendeng* which is dark in colour. The texture and flavour of the samples were also affected by the substitution, with an elastic and chewy texture obtained from the gelatinised corn starch produced during cooking of the corn grits.

Dendeng prepared from a combination of breadfruit and corn grits (Table 1) with meat content varying from 100 to 25 per cent was also examined. The combination of substitute agents gave no effect on either a_w or moisture content of *dendeng giling*. The organoleptic tests showed that colour, taste, texture and flavour were again significantly affected by the substitution ($P<0.01$). The texture of 100 per cent minced meat *dendeng* was not different from the *dendeng* prepared from 75 per cent minced meat, 15 per cent breadfruit and 10 per cent corn grits, but was different to samples prepared from 50 per cent minced meat, 30 per cent breadfruit and 20 per cent corn grits and to that from 25 per cent minced meat, 45 per cent breadfruit and 30 per cent corn grits. The substitution of 45 per cent breadfruit and 30 per cent corn grits for some of the meat gave worst product as it showed a light uneven colour, a strong flavour and a taste of corn grits which was unusual in *dendeng*.

Since these products were prepared by sun-drying, no uniform production conditions could be obtained. However, these findings indicate the value of further study on the use of a combination of breadfruit and corn grits as less expensive and readily available local meat substitutes for incorporation into modified *dendengs*

which cost less to manufacture but still contain reasonable levels of animal protein.

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DISSOLVED OXYGEN CONTENT OF COW AND BUFFALO MILK

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Cow and buffalo milks were analysed for dissolved oxygen content by oxygen analyser which worked on polarographic principle. Buffalo whole milk contained significantly higher amounts of dissolved oxygen than that of cow, while in the skim milk, it was similar in both the cases. This may be attributed to higher fat content as well as the nature of triglycerides in buffalo milk, Though dissolved oxygen content of milk decreased with increase in holding temperature, interestingly, milk chilled and held at 5°C showed significantly lower levels of dissolved oxygen than that held at room temperature.

Dissolved oxygen in milk causes oxidative changes in milk and milk products involving autoxidation of lipids[1], destruction of water soluble vitamins[2,3] and influences the bacterial quality of milk[4]. Dissolved oxygen in milk can be determined by simpler devices working on polarographic principle[5]. A comparative study of dissolved oxygen content of cow and buffalo milk and effect of holding temperature on it is reported in this paper.

Milk samples collected directly from five animals, in sterile glass bottles were pooled together and a small quantity from this composite samples was centrifuged at 1500 × g, cooled and the skim milk portion was drained off by puncturing the hardened cream layer. Precautions were taken to obtain the skim milk in asceptic condition, which was then covered with a layer of liquid paraffin to avoid diffusion of oxygen from the atmosphere. The content of dissolved oxygen in milk was determined with the help of "Biochem oxygen analyser" which worked on polarographic principle. The measurements were done at 40°C with previous calibration for air saturated water at 6.6 ppm of dissolved oxygen.

In order to study the effect of holding temperature on the quantity of dissolved oxygen in milk, the asceptically collected pooled raw milk samples were divided into 4 × 200 ml portions and were subjected to different treatments viz., (*i*) immediately chilled to 5°C, (*ii*) heated to 70°C, (*iii*) heated to boiling temperature (96-97°C) and (*iv*) brought down to room temperature (25°C). After the treatments, samples were held at respective temperature for 15 min and were covered on top with a layer of liquid paraffin to prevent exposure to oxygen.

The dissolved oxygen contents of whole milk (soon after milking) and skim milk (soon after separation) of cow and buffalo are given in Table 1. The dissolved

TABLE 1. DISSOLVED OXYGEN (PPM) IN WHOLE AND SKIM MILKS OF COW AND BUFFALO

Sample No.	Cow milk		Buffalo milk	
	Whole	Skimmed	Whole	Skimmed
1	5.3	4.9	6.0	5.2
2	5.3	5.0	6.0	5.2
3	5.1	4.8	5.9	5.4
4	4.9	4.7	6.0	5.2
5	5.4	5.2	5.5	5.0
6	5.2	5.0	5.7	4.9
7	5.7	5.3	5.7	5.0
8	5.3	5.1	5.7	5.1
9	5.6	5.4	6.0	5.0
10	4.7	4.5	5.9	5.0
Mean	5.25	4.98	5.85	5.16
"t" value	2.24*		5.91**	

Significant at *5 per cent and **1 per cent levels.

TABLE 2. EFFECT OF DIFFERENT HOLDING TEMPERATURE ON DISSOLVED OXYGEN CONTENT (PPM) OF COW AND BUFFALO MILK

Sample no.	Temp at which cow milk held for 15 min				Temp at which buffalo milk held for 15 min			
	5°C	25°C	70°C	96-97°C	5°C	25°C	70°C	96-97°C
1	4.7	5.2	4.9	4.6	4.8	5.7	5.4	5.0
2	4.8	5.3	5.1	4.7	4.9	5.8	5.4	4.9
3	4.5	5.1	4.8	4.4	4.8	5.6	5.3	4.9
4	4.8	5.3	5.0	4.7	4.9	5.7	5.4	4.9
5	4.5	4.9	4.7	4.4	4.8	5.8	5.4	5.0
6	4.6	5.2	4.9	4.5	5.0	5.9	5.5	5.2
7	4.8	5.3	5.0	4.6	5.2	6.0	5.6	5.2
8	5.0	5.6	5.2	4.8	5.2	5.9	5.6	5.3
9	4.3	4.7	4.5	4.0	4.7	5.5	5.2	4.8
10	4.7	5.2	4.8	4.5	4.7	7.8	5.3	4.9
Mean	4.67	5.18	4.89	4.52	4.9	5 77	5.41	5.01

oxygen content in whole milk of buffalo was significantly higher ($P<0.05$) than that of cow. However, there was no significant differences in the oxygen content of cow and buffalo skim milk. The dissolved oxygen content of whole milk was significantly higher than that of skim milk in both cow ($P<0.05$) and buffalo ($P<0.01$). Buffalo milk fat is shown to contain significantly higher quantities of dissolved oxygen than cow milk fat[7]. The absorption of oxygen by buffalo milk fat is also shown to be at greater rate than cow milk fat[8]. Hence, it can be concluded that the higher levels of dissolved oxygen noticed in buffalo whole milk but not in skim milk, may be due to the differences in the nature of triglycerides and also higher fat content in buffalo milk than in cow milk.

The effect of temperature on dissolved oxygen level in cow and buffalo milk is shown in Table 2. Analysis of variance of the data showed that the dissolved oxygen content of both cow and buffalo milk decreased significantly with increase in the holding temperature. The decrease in dissolved oxygen with respect to holding temperature was significant between all the treatments.

This may be due to the slow escape of oxygen in milk as the temperature is raised. However, interestingly, milk chilled and held at 5°C also showed significantly lower levels of dissolved oxygen than the unchilled milk held at room temperature. This may be due to greater degree of solidification of fat in chilled milk which may hinder the absorption of oxygen by milk fat and account for lower levels of dissolved oxygen.

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TEST PERIOD FOR THE STUDY OF HYPOCHOLESTEROLEMIC EFFECT OF TEST MATERIAL IN RATS

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Rats with severe hypercholesterolemia were continued with 10% vanaspati or fed with walnut kernel to provide 10% oil or cold pressed unrefined walnut oil. Growth during 4 and 8 week period of this feed intake was normal and there was no difference between treatment means with respect to body weight. During the 4-week period cholesterol in serum decreased from 230 to 125 mg per cent with test fats, and this decrease was not further observed with continued feeding upto 8 week period.

The hypocholesterolemic effect of a test fat was asssesed by the following method. Rats are first made hypercholesterolemic with a diet containing tallow or vanaspati (partially hydrogenated vegetable oils), cholesterol and bile salt. Subsequently, the diet is changed to include the fat to be tested in partial or whole replacement of tallow or vanaspati. Efficacy is judged by

change to lowering of elevated serum cholesterol observed with the test fat. The second period of feeding (test period) is generally of 4-8 weeks duration[1-3].

Longer the test period, more is the time to get the results and in addition, more the requirement of diets, test fat and associated facilities for maintenance of rats. At the same time, the test period should be of such duration that would allow the serum cholesterol to an equilibrium level to enable proper evaluation of the efficacy of the test fat.

In order to find out the optimum period of test feeding, which brings down serum cholesterol level of rats to its equilibrium level, experiments with test periods of 4 and 8 weeks duration was conducted. The test materials used were walnut kernel and its oil. Walnut (*Juglans regia*) kernel contains about 65 per cent of oil of which about 60 per cent is accounted by linoleic acid which has established serum cholesterol lowering effect. Design of the experiment and the composition were same as described by Sen *et al.*[3]

Adult male rats of Wistar strain weighing about 150 g on an average were fed pre-test diet containing 0.5 per cent cholesterol, 0.5 per cent bile salt and 10 per cent vanaspati for a period of 4 weeks (pre-test period) to ensure severe hypercholesterolemia, The rats were divided into six groups by randomised design, each group having 9 rats. Two groups (A & B) were continued to be given pre-test diet i.e., containing 10 per cent vanaspati while in two groups (C & D) vanaspati was completely replaced by walnut kernel to provide 10 per cent oil from it. In remaining two groups (E & F) vanaspati was replaced completely by cold pressed unrefined walnut oil.

Vanaspati of a standard brand and walnut obtained from Kashmir and stored at 2-3°C were utilised in the experiment. Walnut oil was obtained by cold pressing of kernel in a Carvar press with a free fatty acid content of 0.6 per cent as oleic acid and with linoleic acid content of 60.7 per cent on the weight of fatty acids. Kernel had 2.1 per cent moisture, 66.5 per cent crude fat, 67.1 per cent total lipids, 18.3 per cent protein and 2.0 per cent ash. Weight and diet consumption were recorded at weekly interval. At the end of test period, rats were fasted overnight. The animals were anaesthetized and blood collected from heart by cardiac puncture. serum was analysed for total cholesterol according to the method of Sackett[4].

Growth during 4 and 8 weeks period was quite normal and there was no difference between any treatment means either at the end of 4 or 8 weeks period (Table 1).

TABLE 1. FOOD INTAKE AND INCREASE IN BODY WEIGHT DURING 4 AND 8 WEEKS TEST PERIOD

Diet	Mean body weight (g)				Mean food intake (g) in test period	
	Initial (pre-test period)	Initial (test period)	Wt. increase during test period		4 weeks	8 weeks
			4 weeks	8 weeks		
Vanaspati	160.6	229.7	70.3 ± 4.8^a	—	692 ± 9.7^a	—
Vanaspati	154.7	231.8	—	123.4 ± 6.0^a	—	1288 ± 10.7^a
Walnut kernel	155.2	229.0	76.6 ± 4.8^a	—	675 ± 9.7^{ab}	—
- do -	156.4	230.0	—	123.7 ± 6.0^a	—	1289 ± 10.7^a
Walnut oil	157.1	229.9	75.8 ± 4.8^a	—	655 ± 9.7^b	—
- do -	153.3	232.3	—	113.7 ± 6.0^a	—	1265 ± 10.7^a

Means carrying the same superscript in a column are not significantly different ($P<0.05$)
Values are Mean±S.E.

TABLE 2. COMPARISON OF TERMINAL SERUM TOTAL CHOLESTEROL (MG PER CENT) AFTER FEEDING TEST FAT FOR 4 AND 8 WEEKS

Test period (weeks)	Vanaspati (Gr A & B)		Walnut kernel (Gr C & D)		Walnut oil (Gr E & F)	
	Mean±SE	Tr value±SE	Mean±SE	Tr value±SE	Mean±SE	Tr value±SE
4	228±26	2.3566±0.0464	123±30	2.0765±0.1136	125±26	2.0891±0.0939
8	243±24	2.3834±0.0409	119±27	2.0633±0.1116	132±10	2.1207±0.0324
Test of significance*	15	0.0268	4	0.0132	7	0.0316

Tr value: Transformed value

*Results of test of significance by students test (by two tailed test at 5% level); none of the treatment means were significant at $P<0.05$.
Groups A, C and E are 4 weeks test period; and groups B, D and F are 8 weeks test period.

Serum cholesterol conforms to a log normal distribution[5] and so in the present study, the statistical analysis for terminal serum cholesterol values was also carried out on the transformed variate using logarithmic transformation (Table 2). In 4 weeks period, cholesterol level came down from about 230 mg per cent to about 125 mg per cent with test fats and there was no further decrease with continued feeding. Difference between two means for 4 weeks and 8 weeks period for any one of the fats tested, vanasapti (Groups A & B), walnut kernel (Groups C & D) and walnut oil (Groups E & F) was not significant ($P<0.05$).

The results indicate that test period (excluding pretest period) to evaluate a fat for possible hypocholesterolemic effect in cholesterol bile salt stressed rats may be confined to 4 weeks.

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REVIEW PAPER

Chemistry and Technology of Melon Seeds

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The review covers aspects like collection and separation of water melon seeds, processing for oil extraction; physical and chemical composition of seed; composition of seed cake; nutritional quality of melon seeds; storage behaviour of seed oil; enzymes present in seed; and *ogiri*, a food condiment from fermented melon seeds.

Introduction

Melons belong to the family *Cucurbitaceae* which includes musk melon, cantaloupe, water melon, cucumber, pumpkin, and squash. Generally, only fleshy part of the fruit is consumed and the seeds, which are rich in protein and fat are discarded. A small quantity of seed kernels of some melons is used in the bakery, and confectionery products. These seeds are also consumed after roasting in the preparation of beverages. The seeds are a rich source of enzymes, particularly urease and are considered as a diuretic and beneficial in chronic or acute eczema. The seed cake is reported to be free from alkaloids and cynogenic glycosides and can be used as cattle feed[1]. Thus, melon seeds can form an important food article.

In the absence of sufficient information on melon seeds, an attempt has been made in this review to compile and collate the available information on the chemical and bio-chemical aspects of the melon seeds.

Collection and Processing

There is no definite set pattern for collecting melon seeds. Some vendors collect the seeds in small quantities and sell them to shops after washing and drying. Some house-wives also collect them for their own use.

Separation of seeds from the pulp is done customarily by drying the seeds along with the pulp, when they can easily be separated. Another method followed is allowing the seeds with pulp to ferment for 1-2 days, washing and drying in the sun. In the chemical method[2] the pulp is treated with hydrochloric acid and sulphuric acid (C.P.), washed and dried.

A highly economic and labour saving technique has been developed by Kirpal Singh *et al.*[3] wherein the seeds are spread on cemented floor and dried in the sun with occasional turnings. Feel of the seed is an index of proper drying for safe storage. Optimum drying is ascertained emperically by pressing the seeds between fingers.

TABLE 1. DEHULLING OF WATER MELON AND MUSK MELON SEEDS

Product	Water melon[4] Trial 1	Water melon[4] Trial 2	Musk melon[5]
Meats			
Whole and coarse (%)	29.0	20.0	43.15
Powder with contamination of hulls (%)	22.0	32.0	25.28
Total meat fraction (%)	51.0	52.0	68.43
Hulls			
Whole and coarse (%)	39.0	34.0	27.38
Powder with contamination of meats (%)	9.5	14.0	3.50
Total hull fraction (%)	48.5	48.0	30.88

Reports on large scale processing of water melon and musk melon seeds for oil extraction are scanty.[4,5] Cleaned, dehulled seeds are crushed in an expeller to obtain oil. Characteristics of hulled seeds are given in Table 1. However, 45-65 per cent of the kernels are reported to be contaminated with powder and hull.

Physical Composition of Seeds

Data collected at our laboratory, on physical composition of the water melon, musk melon and long melon[6] seeds are recorded in Table 2[6]. Characteristics of 4 varieties of musk melon are given in Table 3[7].

TABLE 2. PHYSICAL COMPOSITION OF MELON SEEDS

Fruit	Yield of seeds (%)	Dry seed composition Kernel (%)	Hull (%)	Kernel/hull ratio	Bulk density (kg/l.) Seed	Hull	Kernel
Water melon	0.80-1.20	42	58	0.73	0.46	0.20	0.50
Musk melon	1.35-2.00	75	25	3.00	0.45	0.10	0.54
Long melon	—	68	32	2.13	0.46	0.12	0.56

Present address: *CFTRI Experiment Station, Lucknow.

TABLE 3. PHYSICAL COMPOSITION OF MUSK MELON SEEDS (DRY) OF DIFFERENT VARIETIES

Variety	Yield of seeds (% dry)	Av. wt. (mg)	Bulk density (kg/l.)	Composition of seed	
				Kernel (%)	Hull (%)
M.F.	1.35	30	0.47	59.1	40.9
Punjab Sunehri	0.90	33	0.51	69.0	31.0
Hara Madhu	2.10	42	0.52	73.0	27.0
Punjab Hybrid	1.50	31	0.52	72.3	27.7

Abdel Akher *et al.*[8] studied some of the physical characteristics and chemical properties of 3 varieties of water melon seeds and reported the mean kernel/shell ratio to be about 0.94, whereas the mean kernel/shell ratio of commercial water melon seeds was found to be 0.73[6].

Rakhimova[9] studied the physical changes in the seeds during maturation. The weight of the seed and the ratio of kernel/seed weight increased with increasing maturation. Also, overall dimensions of melon seeds increased most rapidly at 30-50 days after blossoming. It is also observed that the seeds from the early maturity melons deteriorated qualitatively during storage whereas storage of seeds from the average maturing melon did not seriously damage the seeds. For storage of melon seeds, the recommended moisture level is reported to be less than 6 per cent[2].

Chemical Composition of Seeds

Composition of seeds, kernels and hulls of water melon and musk melon is given in Table 4. Melon seeds are rich in oil, protein and minerals[1,4,5,7-13]. Crude protein content in water melon seed kernels ranges from 24.2 to 37.0 per cent. The fat content in water melon seed kernels ranges from 44.4 to 57.0 per cent. In musk melon seed kernels, crude protein ranges from 31.0 to 35.8 per cent and oil ranges from 42.02 to 47.0 per cent.

Chemical composition of water melon seeds from various parts of the fruit[16], during maturation of the fruit;[9] carbohydrate content during post-harvest period[15] and

TABLE 4. CHEMICAL COMPOSITION OF MELON SEEDS, KERNELS AND HULLS

	Moisture (%)	Crude protein (%)	Fat (%)	FFA of oil (% oleic acid)	Carbohydrates (%)	Crude fibre (%)	Ash (%)	Acid insoluble ash (%)
Water melon								
Kernels[1]	7.1	34.3	44.4	—	4.8	6.7	2.7	—
Seeds[8]	4.85	18.98	26.70	—	—	—	2.4	—
Kernels[10]	—	31.37	54.57	—	—	—	—	—
Kernels[11]	2.7	24.2	54.8	—	11.6	3.0	3.7	—
Kernels[11]	3.1	28.8	48.4	—	9.3	6.8	4.2	—
Seeds[12]	—	31.02	22.2	—	—	—	—	—
Seeds[4]	5.4	19.7	27.4	0.4	12.3	32.9	2.3	0.10
Kernels[4]	4.9	34.2	51.9	0.7	4.4	1.7	2.9	0.11
Hull[4]	8.5	2.2	2.3	5.4	23.6	61.9	1.5	0.31
Musk melon								
Kernels[1]	—	35.8	44.6	—	—	—	5.6	—
Kernels[7]	5.37	—	45.10	—	—	—	4.79	—
Kernels[7]	4.57	—	45.90	—	—	—	4.59	—
Kernels[7]	4.51	—	42.02	—	—	—	4.94	—
Kernels[7]	4.75	—	43.40	—	—	—	4.10	—
Seeds[5]*	6.8	21.0	33.0	—	5.2	30.0	4.0	Nil
Kernels[5]	6.2	31.0	47.0	—	7.2	3.3	5.3	Nil
Hulls[5]	8.6	2.2	2.7	—	6.1	79.0	1.4	Nil

*The analysis is of sound seed washed in water to free it from pulp and adhering soil, and wiped with filter paper and air dried.

Superscript indicates reference numbers.

the mineral content[10,17] have been studied. Proteins, fat and carbohdyrates increased between 20 and 60 days in melon seeds during maturation of the fruit and then decreased. Artificially matured fruits exhibited lower content of carbohydrates, protein and fat in the seeds. Seeds from the various parts of the fruit differed in weight, moisture and composition while the seeds from the central portion had the best reproduction character. This character deteriorated on storage.

Datta *et al.*[18] studied the oil distribution in different parts of some cucurbit kernels. Water melon and pumpkin which had the highest 1000 kernel weight (42-91 g) also had the highest endosperm ratio (12-13 per cent) and highest oil content (50.8-51.8 per cent).

From Table 5 it is seen that the whole seed has got excellent storage stability[4]. The oil content, free fatty acid and protein contents of whole seed do not change even after 6 months of storage. Coarse kernels cannot be stored for more than 2 months without allowing free fatty acids to rise beyond permissible level. Kernel powder keeps very badly; its free fatty acids rising rapidly. It is, therefore, recommended that dehulling of water melon seed be done only when required for continuous crushing[4].

Protein: Musk melon seeds contain all the essential amino acids, although tryptophan occurred in low amounts (0.3-0.4 g/16 g nitrogen)[19].

Akpapunam *et al.*[11] explored the possibilities of protein supplementation of cow peas with water melon seed protein. Amino acid composition reported is given in Table 6. Nitrogeneous matter[1] is presented in Table 7.

TABLE 5. STORAGE BEHAVIOUR OF WATER MELON SEED, KERNEL, KERNEL POWDER AND CAKE[4]

Characteristics	Storage period in days						
	Initial	30	60	90	120	150	180
Moisture (%)							
Whole seed	5.7	4.8	5.6	6.1	7.2	7.0	7.9
Kernel	3.4	4.0	7 9	4.6	5.3	5.3	6.4
Kernel powder	3.4	4.8	3 8	4.8	5.4	5.4	7.7
Cake	3.8	6.1	5.9	7.0	8.1	7.7	9.3
Oil (%)							
Whole seed	27.5	28.5	28 3	—	28.5	28.5	27.6
Kernel	52.9	53.1	—	53.1	51.5	53.0	53.0
Kernel powder	46.9	46.0	46.2	45.5	45.5	45.7	44.0
Cake	8.8	8.8	8 2	8.6	8.0	8.7	8.0
Total protein (%)							
Whole seed	19.7	21.4	19.7	19.4	19.5	19.8	19 5
Kernel	36.0	36.0	—	—	35.0	32.6	34.2
Cake	42.4	41.8	42 8	—	38.5	39.0	39.0
Free fatty acids							
Whole seed	0.0	—	—	—	0.8	1.0	1.2
Kernel	0.91	1.47	2.7	3.5	5.3	6.4	6.6
Kernel powder	2.3	5.0	12.1	17.7	23.6	30.5	36.8
Cake	2.9	2.9	3 5	3.8	4.0	5.2	—
Peroxide value							
Whole seed	1.9	4.5	3 6	6.3	3.8	9.1	2.8
Kernel	3.3	2.2	1 8	11.1	4.5	9.3	6.7
Kernel powder	3.3	5.2	6 1	3.3	3.8	4.3	3.7
Kreis colour of oil in red units in 10 mm cell							
Whole seed	3.4	9.6	9.1	—	6.0	4.8	10.0
Kernel	1.8	4.8	9.1	—	3.3	3.5	7.6
Kernel powder	2.6	5.7	4 9	—	3.4	4.1	4.2

TABLE 6. AMINO ACID (G PER 16 G NITROGEN) COMPOSITION OF WATER MELON SEEDS[11]

Amino acids	Water melon seeds		Scoring pattern
	Nigerian var	American var	FAO/WHO/ 1973
Isolcucine	3.5	3.3	4.0
Leucine	6.6	6.4	7.0
Lysine	2.9	2.7	5.5
Methionine	2.4	2.5	3.5
Cystine	1.0	1.1	—
Phenylalanine	5.0	4.9	4.0
Tyrosine	2.6	3.0	—
Threonine	3.9	3.8	4.0
Tryptophan	1.8	1.9	1.0
Valine	4.6	4.2	5.0
Alanine	5.5	5.4	—
Arginine	12.0	12.2	—
Aspartic acid	7.4	7.8	—
Glutamic acid	13.8	14.2	—
Glycine	4.8	4.4	—
Histidine	1.8	1.8	—
Proline	2.7	2.6	—
Serine	4.4	4.2	—

TABLE 7. NITROGENOUS MATTER IN WATER MELON SEEDS[1]

Component	Per cent
Glutelin	9.4
Globulin	73.4
Water soluble protein	6.3
Proteoses	3.5
Peptones	1.1
Arginine	Traces
Canavanine	Nil
Citrulline	Nil

Globulin has been isolated in crystalline form. The seeds do not contain canavanine or citrulline fractions either in free or combined form and only contain traces of free arginine.

Morgue *et al.*[20-25] studied in detail the soluble and insoluble proteins of water melon seeds. Euglobulin prepared from seeds of water melon was a mixture of 3 proteins, one of which was most abundant. The 3 fractions resembled those of *Cucurbita maxima* seeds. The molecular weight of the largest fraction was 343,000[20].

All the 3 fractions were rich in glutamic acid and arginine; and a regular decrease in the amount of glutamic acid from fraction 1 to fraction 3 was noticed. Arginine was predominant while valine was low in fraction 2. The concentration of aspartic acid was also high. Cystine and methionine were present in small amounts (only traces in fraction 1). Methionine, histidine and arginine were lowest and lysine and glutamic acid highest in fraction 1[21]. Soluble protein components of water melon seeds were detected and the conditions studied[22-24].

O' Kennedy *et al.*[26] observed that the percentages of globulin from cotyledons of dormant seeds of 8 species of the *Cucurbitaceae* family including musk melon seeds were similar. After 4 days of germination, the globulin fraction decreased with a concomitant increase in the water soluble protein fraction. Small changes in total protein occurred[26].

Amino acids were determined in defatted musk melon seed cake in an amino acid analyser. The defatted seed contained 62 per cent protein. The amino acid analysis and calculated chemical score indicate that this seed is adequate in furnishing essential amino acids in the human diet. Leucine and phenylalanine values are high and the supply of lysine, methionine and tryptophan is good. The limiting amino acid in the seed appear to be the S-amino acids (cystine and methionine) which together have a chemical score of 58. The melon (egusi) seed is particularly high in arginine, aspartic acid and glutamic acid[27].

Stafford *et al.*[28] determined protein in water melon seeds extracted with alkali and salt followed by precipitation at the isoelectric pH. The protein in partially defatted meal was 21 per cent. Water melon protein isolate contained high amount of crude protein (82.5 per cent) and virtually all the nitrogen was protein nitrogen. The principal bands of electrophoresis of the isolates corresponded to the molecular weights 21.200 and 32,600. Further, it was observed that lysine was the limiting amino acid in water melon with chemical score of 57. However, the poor methionine content could be partially overcome if the protein isolate concentration were used as additive to cereals in which lysine rather than methionine is limiting.

Alekseeva[29] studied the composition of the protein complexes in the axial part of the embryo and cotyledons of water melon seeds by Sephadex G-200 gel-filtration and gradient column extraction. Aleurones were isolated from cotyledon flour. A difference was shown in the ratio of primary and secondary globulin components of proteins of the axial part of the embryo and cotyledons which corresponded to the observed differences in these seed parts. Differences were noted in the content of secondary components of the total salt soluble protein, in particular nucleo protein, in the axial part of the embryo and in the cotyledons.

Composition of Seed Cake

Water content of cake ranged from 6.5-11.56 per cent, protein 28.2 to 69.78 per cent, fat 0.8 to 7.0 per cent, carbohydrates 8.3 to 27.7 per cent and ash 3.9 to 9.12 per cent[1,10,11] (Table 8). The protein and amino acid composition of defatted seed meal of *C. vulgaris*, *C. melo*, *C. melo utilissimus* and *L. vulgaris* were determined by Bhatnagar *et al.*[30] All plants exhibited high protein contents which ranged from 40.57 per cent in *C. vulgaris* to 57.07 per cent in *C. melo utilissimus*. Of essential amino acids, tryptophan was absent.

Nutritional Qualities of Melon Seeds

Oyenuga *et al.*[10] studied the nutritive value of the water melon seeds. In feeding experiments with rats, raw and fried full fat and defatted melon seed meals had true digestibility in the range 91-93 per cent and protein efficiency ratio, net protein retention, net protein utilisation and biological value which were significantly inferior to those of soyabean meal and hen's whole egg; deficiency in lysine and methionine and threonine being marginal.

TABLE 8. COMPOSITION OF MELON SEED CAKE

Constituent	Water melon				Musk melon seed kernel
	Wealth of India[1]	Oyenuga *et al.*[10]	Akpapunam *et al.*[11] Nigerian	Akpapunam *et al.*[11] American	
Water (%)	8.6-10.1	—	6.5	6.6	11.56
Protein (%) *	28.2-58.8	69.78	51.4	51.5	66.0
Fat (%)	7.0	1.2-2.7	4.0	0.8	4.03
Carbohydrates (%)	8.3-14.3	—	27.7 (by diff.)	27.7 (by diff.)	9.29
Fibre (%)	12.2-39.2	—	2.6	5.6	—
Ash (%)	3.9- 4.9	—	7.8	7.9	9.12

*% N×5.3

TABLE 9. COMPARATIVE NUTRITIONAL CHARACTERISTICS OF WATER MELON SEEDS WITH OTHER LEGUMES AND NUTS

	Protein efficiency ratio			Digestibility			B.V.	N.P.U.
	Akpapunam *et al.*[11]	Umoh *et al.*[32]	Stafford *et al.*[33]	Oyenuga *et al.*[10]	Stafford *et al.*[33]	Umoh *et al.*[32]	Umoh *et al.*[32]	Umoh *et al.*[32]
Water melon	1.07	1.23-1.61	2.4	91-93	88.1	87-99	71-83	69-81
Cowpeas	1.71	—	—	—	—	—	—	—
Sesame	1.22	—	—	—	—	—	—	—
Walnut	—	—	9	—	87.2	—	—	—
Locust bean	—	0.88-1.20	1.81	—	96.6	87-99	73-75	59-65
Irvingia gaebonensis (African mango kernel)	—	0.17-0.49	—	—	—	87-99	73-75	59-65

B.V. = Biological value; N.P.U. = Net protein utilization.

Oke[13] reported water melon with net dietary protein per cent of calories as 12.8. Amino acid analysis showed that lysine is the first limiting amino acid in water melon. The chemical score is 63.1.

Vigo *et al.*[31] have determined the essential amino acid composition, nutritive value, true digestibility value, biological value, protein efficiency ratio, and net protein efficiency of melon seeds.

Umoh *et al.*[32] have studied the nutritive value of water melon seeds and concluded that water melon fruit is nutritious. Staffard *et al.*[33] have also ascertained the nutritive value of water melon seeds and concluded that lysine was the limiting amino acid. The comparative nutritive characteristics of water melon seeds with other legumes and nuts are presented in Table 9.

Fat: The seeds from different varieties and locations contain fat in varying percentages. The oil is yellowish or greenish in colour with a pleasant odour and taste and is used for cooking and as illuminant[1]. Many authors have reported the characteristics of the extracted oil[1,10,34,36] (Table 10) and its fatty acid composition[1,10,34,36] (Table 11). Bhatia *et al.*[37] found similar fatty acid pattern in seeds of water melon, musk melon, pumpkin and cucumber. Among palmitic, stearic, oleic, linoleic and linolenic acids, oleic and linoleic acids together constituted more than 80 per cent of the fatty acid content of the oil. In another study Bhatia *et al.*[38] identified eight classes of polar lipids. Tandon *et al.*[36] reported an yield of 35.6 per cent oil from the seeds of ripe melon. Abdel Akher *et al*[34]. studied the stability

TABLE 10. CHARACTERISTICS OF MELON SEED OIL

	Specific gravity	Sapon. value	Iodine value	Acid value	Unsaponifiable matter (%)	Refractive index
			Musk melon			
Wealth of India[1]	0.9174 (23.5°C)	294.3	125.5	3.9	0.6	—
Ramakrishna *et al.*[5]	0.9170 (34°C)	193	128 (wijs)	2.6	0.9	1.4682 (40°C)
			Water melon			
Wealth of India[1]	0.914-0.923 (15°C)	190-198	115-125	—	0.7-1.3	1.463-1.467
Lakshminarayana *et al.*[4]	0.9166 (30°C)	193	123 (wijs)	3	1.6	1.4668 (40°C)
Oyenuga *et al.*[10]	—	192.8-194.6	11.3-1-118.7	—	—	—
O'Kennedy *et al.*[26]	0.9225	170.1	112.4	1.75	1.31	1.4723
Badami *et al.*[35]	—	278.3	126.6	—	1.1	1.4682 (30°C)

TABLE 11. FATTY ACID COMPOSITION OF THE MELON SEED OIL

Fatty acids	Water melon			Musk melon		
	Wealth of India[1]	Oyenuga *et al*[10]	Abdel-Akher *et al*[34]	Badami *et al*[35]	Wealth of India[1] (Sarda var)	(Punjab var)
Caprylic	0.2	—	—	—	—	2.0
Capric	1.1	—	—	1.20	—	—
Lauric	0.8	—	—	3.26	—	—
Myristic	0.2	—	—	0.74	2.0	1.1
Palmitic	7.6	11.1 18.6	—	13.24	3.2	7.3
Stearic	6.1	13.6-16.8	—	0 74	5.4	0.2
Oleic	35.5	13.6-21.7	15.64	47 77	32.7	43.1
Linoleic	48.7	52.3-57.9	57.05	25.40	55.2	45.1
Arachidic	—	—	—	—	0.9	—
Caproic	—	—	—	—	—	1.0
Hexadecenoic	—	—	—	0 45	—	—

of oil extracted from two varieties 'Kongo chillian black' and 'Giza I' of water melon seeds and reported 9-12 and 6-9 days stability, respectively at 62°C and 72-76 and 48-72 days stability, respectively at room temperature (33°C). Potato chips fried in Kongo seed oil had the same quality as those fried in cotton seed oil.

Gumus[39] separated the seed lipids of *C. melo* by TLC and found that they contained 60-70 per cent linoleic acid; lecithin, cephalin and cerebroside were also identified.

Storage quality of seed oils

From autoxidation point of view, crude oil is the stablest, followed by refined and bleached oils in the decreasing order of stability. The peroxide value rises steadily in crude, refined and bleached oils during storage. Free fatty acids of all categories of oils remained more or less the same as the respective initial values the entire period of 6 months storage[4] (Table 12).

In the case of musk melon seed oil, it is seen that on storage, the free fatty acid contents rise very slowly in 120 days. Rise of peroxide value is also not considerable in the case of raw oil. The increase of oxidative rancidity is in the following decreasing order, refined, bleached and raw[5] (Table 13).

It is observed in these studies that the peroxide values

TABLE 12. STORAGE BEHAVIOUR OF CRUDE, REFINED AND BLEACHED WATER MELON SEED OILS[4]

Storage period (days)	Free fatty acids			Peroxide value			Kreis colour (red units in 10 mm cell)			Lovibond colour (Y+10R) units in 1" cell		
	Cr.	Re.	Bl.	Cr.	Re.	Bl.	Cr.	Re.	Bl.	Cr.	Re.	Bl.
0	1.35	0.14	0.15	2.6	6.2	4.1	6	9	5	22.3	13.4	4.0
30	1.59	0.24	0.18	11.2	14.5	13.1	10	14	7	26.0	15.5	4.2
60	1.37	0.10	0.09	17.2	24.1	28.9	14	15	11	18.8	11.3	2.2
90	1.57	0.11	0.13	25.4	32.0	48.4	—	—	—	—	—	—
120	1.50	0.12	0.13	28.2	36.5	53.1	12	13	12	24.0	12.2	4.2
150	1.51	0.15	0.16	35.7	46.5	79.2	14	14	14	23.0	7.4	3.5
180	1.54	0.14	0.16	44.8	54.3	89.4	15	15	14	21.0	9.0	3.1

Cr: Crude; Re: Refined, Bl: Bleached

TABLE 13. STORAGE BEHAVIOUR OF CRUDE, REFINED, BLEACHED MUSK MELON SEED OILS[5]

Storage period (days)	Free fatty acids			Peroxide value			Kreis test red colour			Lovibond colour in 1" cell Yellow+Red		
	Cr.	Re.	Bl.	Cr.	Re.	Bl.	Cr.	Re.	Bl.	Cr.	Re.	Bl.
0	1.2	0.08	0.07	5.8	—	6.5	20	16	12	42+8.2	18+1.8	4.6+0.4
30	1.25	0.09	0.08	9.8	23.2	18.8	19	17	14	37+7.8	15+1.6	4.1+0.3
60	1.3	0.1	0.09	18.8	43.0	32.7	23	21	15	35+5.2	10.5+1.3	2.7+0.2
90	1.4	0.11	0.1	21.2	48.0	37.8	25	22	16	33+4.2	7.8+1.1	2.3+0.1
120	1.45	0.12	0.11	25.4	53.0	42.7	26	22	17	30+5.0	—	2+0.5

Cr: Crude; Re: Refined; Bl: Bleached

of refined oils are higher than those of bleached oils, while in water melon seed oil, the peroxide values of refined and bleached oils were more or less same upto 60 days, after which the values of bleached increased faster than those of refined oil. The decreasing order for Kreis test is crude, refined and bleached oil. The Lovibond colours decrease perceptibly with storage in the case of crude, refined as well as bleached oils.

Enzymes

Melon seeds are rich source of enzymes and mainly among them is urease. Tnis enzyme has been isolated and characterised from melon seeds.

The enzyme urease has been isolated, charterised[40-44] and kinetics studied[45]. The enzyme derived from seeds of water melon appears to be more potent than those of Jack and soyabean in the determination of blood urea. One g fresh seed from water melon had urease activity of 2030 μ moles ammonia/min. The pH optima for urease was 7.4 and the Km 35 mM.

Dikhtyarev *et al*[46]. in their studies to search for raw material containing urease, determined urease activity in seeds of 33 native and 30 foreign water melon varieties. Highest urease activity was found in seeds of 'Jubike' and 'Klondike R 57' (22,500 and 18,600 units/kg seeds respectively). Some varieties tested had no urease activity.

Malhotra *et al*.[47] isolated a urease inhibitor from melon seeds as an amorphous powder. It inhibits urease of *Cafanus indicus* and of jackbeans non-competetively with almost Ki values 0.64 and 0.45 mg/ml respectively. The inhibitor did not give any colour with ninhydrin by itself, but did so after treatment with trypsin. It moved as a single spot on paper chromatography in a Ph OH-H_2O mixture (4:1 vol/vol. R_f=0.78) but failed to move in a Bu OH-HOAC-H_2O (4:1:1 vol/vol) mixture.

Acid phosphatase

Seeds of 11 plant species including melon seeds were studied with the object to observe the relation between the acid phosphatase contents and the germinating capacity[48]. In the dormant state, seeds contained varying amounts of acid phosphotase, which shared no relation to the contents of sugar, proteins and lipids. Acid phosphatase content found in seeds which failed to germinate relative to those in inhibition stage was the same in wheat and sunflower, higher in chick pea, flax pea and melon and lower in corn, lentil, lupine, kidney bean and soybean[48].

Lipase

Seeds of musk melon from *garma* contain significant lipolytic activity. The lipase in both the resting and germinated seeds exhibits 2 pH optima i.e. 4.0 and 6.0. Lipase activity, however, increase considerably on germination; from 3.4 units/g at pH 6.0 in the resting stage, the activity increased to 11.1 units/g after germination to a primary root length of 3 cm. Lipases having pH optima 4.0 and 6.0 showed different stabilities at pH 6.0 and 40°C which further suggests the presence of 2 different lipases in the seeds of musk melon[49].

The optimum temperature for the enzyme activity under the assay conditions was 40°C and the enzyme was rapidly inactivated at temperatures above 40°C. The enzyme was activated by low concentrations of Ca^{2+} and Na^{+}. The lipase hydrolysed ricinoleic acid glycerides of castor oil faster than the glycerides of mustard oil or soybean oil. The order of activity in the decreasing order of the organic solvent system was Et, Me ketone, di isopropyl ether, heptane, cyclohexanol more than or equal to cyclo hexane[49].

***Ogiri* a food condiment from fermented melon seeds**

Odunfa[50] studied the microbiology and amino acid composition of *Ogiri*—a food condiment from fermented melon seeds. The traditional method for preparing *Ogiri* is described. Micro organisms associated with the various stages of fermentation were mainly bacteria; the genera frequently isolated are *Pediococcus*, *Proteus*, *Klebsiella*, *Escherichia* and *Bacillus*. The predominent amino acids are alanine, lysine and glutamic acid. Arginine and proline are present in low quantities. Threonine is completely absent[50].

Concluding remarks

It is apparent from the foregoing review that melon seeds are rich in proein, fatty acids, and minerals, Hence, the various possible ways of utilising the seeds as food adjunct and additive in sweets, snacks and other food items, need immediate exploration. However, at present the only commercial utilisation of seeds in India is as an adjunct in some of the sweets and snacks. The drawbacks, probably, are lack of organised collection of seeds and tedious manual dehulling. Some work on mechanical dehulling of melon seeds, prior to oil extraction, has been reported[4,5]. But 45-65 per cent kernels obtained were contaminated with powder and hull. However, recent[6] trials on mechanical dehulling have resulted in pure kernel fraction, without any powder and hull.

The need to develop varieties of melon fruits rich in seeds without adversely affecting the quality and quantity of the fleshy portion and yield is apparent from the review. Vaganov *et al*[51]. worked on the role of trace elements in increasing seed productivity of water melon and squashes. Seed treatment with 0.1 per cent trace elements (Cu, Li, Mo, Co, B, Mn or Zn) solution increased the yield of water melon and squash fruits and seeds.

Deoiled melon seed cake, though not available commercially at present, is a good source of protein and can be used as protein suppliment.

Most of the work reported so far is mainly on watermelon seeds and very little on musk melon seeds. No work is reported on the rest of the melon seeds.

Apart from the mechnical dehulling of melon seeds, studies on the following areas may be useful for a better/integrated utilisation of melon seeds: (*i*) hull composition and utilisation, (*ii*) changes in kernel quality during storage, (*iii*) difference in quality of musk melon kernel obtained by traditional and chemical methods of separation of seed from pulp, and (*iv*) level of fat soluble vitamins in the kernels.

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Manufacture of Whey-soy Beverages: A Review

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Soybean and milk whey in combination provide a low cost nutritious beverage which can be utilized in institutional feeding programmes of developing countries like India. Manufacturing aspects such as types of initial ingredients, processing, fortification, flavouring, drying and reconstitution have been reviewed in this article.

Introduction

Beverages, both alcoholic and nonalcoholic, constitute one of the largest group of food products. Nonalcoholic beverages include carbonated, noncarbonated, fruit-based and milk-based drinks, and also drinks such as coffee and tea. The use of beverages of one kind or other naturally constitutes a part of food habit all over the world. This has resulted in the development of certain types of beverages. Among these the protein enriched beverages are intended to furnish nutrient requirements for the target population in nutrition oriented programmes. In recent years, soybean protein, which ranks highest among vegetable proteins has been in use in many protein food products especially protein rich beverages. Similarly, cheese whey, a dairy byproduct can be used as a fluid base for beverage preparations. One important consideration in combining whey with soybean is that soy protein is known to have a balanced amino acid profile[1], although, methionine, a sulphur amino acid, is the limiting amino acid in it[2], while whey protein, is rich in sulphur amino acids[3,4]. A combination of the

two proteins could, therefore, yield a mixture of exceptional nutritional value[5-9].

A 'milk like' whey-soy beverage was produced by Sasaki and Tsugo[10] as early as in 1953 in Japan. However, the whey-soy combination came to light only in the late sixties. Holsinger and co-workers[11], have briefly reviewed literature on protein rich whey-soy beverages. Subsequently, the growing interest in whey-soy drinks and/or whey-soy 'milk extenders' developed in the recent past warrants an in-depth treatment of this subject. In the present paper, an attempt has been made to highlight the developments that have taken place in the area of beverage production from whey-soybean protein mixtures.

Type of whey and whey products

Sweet cheese whey has generally been used for the preparation of whey-soy beverages presumably owing to its sweet (non-acid) flavour. Holsinger *et al*[12]. observed that replacement of sweet cheese whey with cottage cheese whey in their beverage formula based on full fat soy flour[13], adversely affected the flavour of the product. The beverage prepared by Tsugo[14] was based on neutralized acid whey. Cottage cheese whey has also been used along with soybean for the preparation of a drink having the taste of egg nog[15]. Cottage cheese whey can preferably be utilized in citrus type beverages as has been demonstrated by Guy *et al.*[16] These beverages contained 2.7 per cent protein and 16.5 per cent total solids.

The lactose content of whey-soy drinks may sometimes make these less suitable for lactose intolerant persons[17]. Lactose can be substantially reduced either through hydrolysis or by its removal from the system. Holsinger and Roberts[18] used whey obtained from lactose hydrolysed milk. The resulting whey-soy beverage had a flavour comparable to the one prepared from normal cheese whey. An improvement in the flavour of whey-soy beverage was reported by Holsinger *et al*[19]. when whey was subjected to β-galactosidase pretreatment. Patil and Gupta[20] used lactose reduced condensed whey in their formulation of a high protein (4 per cent) beverage. The lactose level in the above preparation was decreased by partially removing crystallized lactose from condensed Cheddar cheese whey.

Form of soybean material

The soybean material has predominantly been used in the form of whole beans, or fat free flour or flakes for whey-soy beverage production. While defatted soy products offer an advantage of utilization of the by-product of the soybean oil industry, full fat soybean material has often been used on account of its fat content and for improvement of flavour.

The conventional soy milk of the Orient is essentially a heat treated water extract of soaked soybeans. Sasaki and Tsugo[10] used hot whey to extract soybean in order to produce a beverage. Mixing soy-milk with whey before processing, is another way of preparing a whey-soy beverage. A product resembling evaporated milk was made by combining soy milk with whey, followed by condensing the mixture[14]. The procedure described by Badui[21] also involved mixing soy milk with whey. Recently, Hodel *et al*[22]. patented a process for preparing a very fine aqueous dispersion of soybean which could be mixed with whey to produce a cow milk substitute.

Sometimes soybeans are boiled or blanched before grinding in whey as is the case with the process for production of a whole milk simulate[23]. The process for the manufacture of the whey-soy beverage developed at this Institute[20] involved mixing of dehulled bicarbonate blanched soybeans[24] with condensed whey before homogenizing the mix in two stages at 208 kg/cm^2 (3000 psi) and at 35 kg/cm^2 (500 psi). Nevertheless, the advantage of boiling cracked, dehulled and soaked soybeans in whey before grinding as indicated by Peng[25] is difficult to comprehend, since such a treatment might lead to lipoxygenase-induced off-flavour besides destroying certain heat labile vitamins contained in whey.

Certain procedures have been based on full fat soy-flour. The research carried out at the Eastern Utilization Research and Development Division, Washington, DC and at the Eastern Regional Research Centre, Philadelphia, U.S.A., is significant in this regard. Guy *et al.*[26] prepared nutritious beverage (protein efficiency ratio, PER, 2.42) by stirring full fat soy flour (containing 20 per cent fat, 42 per cent protein) into whey to obtain a total solids content of 10 per cent. The mix was pasteurized, homogenized and condensed prior to drying. The above workers[16] have also used full fat soy flour for preparation of citrus fruit beverage. Holsinger *et al*[13]. obtained a protein-rich whey-soy drink by pasteurizing and homogenizing a mixture of full fat soy flour, sweet cheese whey, soybean oil and corn syrup solids. A similar beverage formulation from Foremost Foods Company[27], U.S.A., consisted of 41 per cent sweet whey solids, 37 per cent full fat soy flour, 12 per cent soybean oil and 9 per cent corn syrup solids. A beverage intended for the Agency for International Development Programmes also contained 36.5 per cent full fat soy flour[28].

Both the oil and protein constituents of soybean have been used in a concentrated form as has been reported by Patil[29]. The protein-lipid concentrate obtained by acid precipitation of soy milk, gave a whey-soy beverage of acceptable flavour and lower viscosity compared

to a product in which the untreated soy milk was used.

Defatted meal, flour and flakes have also found their places, in combination with whey, in whey-soy beverages. Arndt[30] described the use of 11-25 per cent aqueous slurry of neutralized fat-free soybean meal/flakes along with 89-75 per cent sweet cheese whey. The above blend was adjusted to pH 6.6-7.0 and a whitening agent (not specified in the report) was added to it before drying for the production of simulated dry skim milk. The use of defatted soy flour for preparation of beverage has also been reported by Badui[21]. The colour of the resulting product may favour the use of fat-free soy flour as has been observed by Holsinger[31] who compared several commercial samples of whey-soy drink mixes.

Isolated soybean protein may be used to minimize the unwanted flavours often associated with other forms of soy protein material. By virtue of its excellent functional characteristics[32], the soy protein isolate is expected to improve the physical properties such as viscosity, emulsification, suspension stability, reconstitutability, etc. of the beverage. Nonetheless, comparatively high costs seem to forbid the application of the protein isolate in beverage production. The method for the manufacture of simulated milk described in another patent[33] consisted of heating and vapour flash treating the mixture of soy protein isolate and sweet whey followed by addition of vegetable oil and readjustment of pH and homogenization of the mix which can be dried subsequently.

Heat processing

During the processing of soybeans, adequate heat treatment is an essential prerequisite to ensure destruction anti-nutritional factors such as trypsin inhibitors[34]. Heat treatment also inactivates lipoxygenase, an enzyme which has been implicated in the development of 'beany' flavour in soybean products[35]. In the context of manufacture of soy-whey beverages, the soybean material, unlike whey, requires a somewhat severe heat treatment and is usually processed separately before mixing it with whey. Thus, the final mix needs only a minimum pasteurization treatment. Nevertheless, unheated soy ingredients have often been mixed with whey, and this mixture is finally heat treated as in the procedure suggested by Peng[25] wherein the mixture of untreated soybean and whey was boiled. In the process patented by British Arkady Co. Ltd[36], the mixture of soy ingredients and whey was heated at 1 kg/cm^2 for 10 min. Arndt[33] heated the whey-soy protein mixture to 141 to 150°C before subjecting it to a vapour-flash treatment. Such intense heat treatment is likely to cause substantial losses in vitamins in whey and may also decrease its protein value.

Fortification of beverage mixes

Enrichment of whey-soy simulates of whole milk with oil or fat[37] becomes necessary, since soybean products, particularly defatted flour, flakes and meal are low in oil content. Soybean oil is the major oil used in 'engineered milks' of soybean and whey origin[13,27,38]. Holsinger *et al.*[39] substituted soybean oil with an oil fraction of edible beef tallow in a whey-soy beverage mix containing 19.2 per cent oil, and no significant change in flavour was noticed. The addition of oil would not only raise the calorie content of the drink but also enhance its protein quality. Guy *et al.*[26] noted that the PER of a whey-soy flour-corn oil blend was 2.64 as against a PER of 2.42 for the whey-soy flour mixture.

Despite the presence of appreciable amounts of B-complex vitamins in whey, the overall vitamin content is inadequate in the whey-soy blends. Thus, the beverage requires to be suitably fortified with vitamins. Fortification of the whey-soy beverage with proper amounts of certain minerals would also be desirable to upgrade the nutritional quality of the product which is expected to match that of milk. Hence, vitamin-mineral premixes are frequently incorporated in whey-soy beverage formulations,[13, 20, 27] not withstanding a slight increase in cost of production. The vitamin-mineral premix is generally dry blended with the powdered product so as to keep their losses to a minimum which otherwise are considerable (sometimes of the order of 80 per cent) during processing as has been observed by Holsinger and co-workers[19].

Apparently, the whey-soy protein mixtures have good amino acid balance[40] since the whey protein plays a notable complimentary role. However, fortification of the mixture with methionine has been found to further enhance the protein quality[8]. Enrichment of the beverage developed by Patil and Gupta[9] with 0.75 and 1.13 per cent (of total protein) DL-methionine raised the PER from 2.3 to 2.8 and 3.1 respectively. It may be pointed out that addition of methionine had an adverse effect on the flavour of the product when it was subjected to heat treatment[41]. However, mixing methionine with the powdered beverage can be successfully employed, since the use of such ready-to-reconstitute product involves only addition of cold water to obtain a cold drink.

Additives

Certain additives are often employed to improve the physical and/or sensory characteristics of beverage. Guy *et al.*[16] showed that citrus beverage could be substantially improved by addition of certain stabilizers. Sodium citrate, reported to ameliorate the dispersibility of a whey-soy blend[42], has been shown to be a valuable

additive. The whey-soy beverage powder developed by Patil and Gupta[20] contained 0.25 per cent sodium citrate. While whey-soy protein in the mixture itself would impart considerable turbidity to the drink, whitening agents may be incorporated to improve the appearance of the product as has been reported by Arndt[37].

Corn syrup solids (CSS) is another additive that has been used in the preparation of whey-soy drinks[13,27]. Holsinger *et al.*[43] observed no impairment of the flavour of the beverage when 42 DE CSS was replaced by 28 DE CSS or 10 DE hydrolyzed cereal solids. The nutritional quality of the beverage also remained almost unaffected, the PER and nitrogen digestibility being 2.35 and 83.5 per cent for the 28 DE product, and 2.38 and 83.3 per cent for the 42 DE product, respectively.

Flavour of whey-soy beverages

The flavour of a whey-soy drink is the most important among all the sensory characteristics which decide its acceptability. The type of the soybean and the method of processing are of utmost significance for the flavour of the resulting product. While it is not within the purview of this paper to go into the details of soy milk flavour, it would be pertinent to include processing aspects of whey-soy beverage which have a definite bearing on its flavour.

The heat treatment during processing of whey-soy beverages has been discussed earlier. Depending on the nature of the initial soybean material, a minimum heat treatment is necessary to prevent off-flavour development or to eliminate objectionable flavours present in the whey-soy mix. Vacuum treatment of the mix is another notable point in the processing of the soy based beverages. Concentration of the liquid mix under vacuum not only facilitates subsequent drying, but also substantially improves its flavour. Therefore, a number of proceses for making whey-soy beverages have been reported which include vacuum condensing of the mix[13,14,16,26,44]. The vacuum treatment has often been combined with heat treatment. Arndt[30,33,37] termed the patented vacuum-heat treatment a 'physico-thermo-vapour' flash treatment that was capable of removing objectionable flavours from the product. Holsinger *et al.*[18] found that a 'vacuum flash' deodorizer procedure was as effective as vacuum evaporation in reducing the 'beany' flavour in the whey-soy mix.

It is needless to point out here that in places other than those wherein the consumers are traditionally accustomed to the soybean taste, the soy products must be suitably flavoured to make them more palatable. Synthetic flavourings are thus very valuable in enhancing the acceptability of whey-soy drinks. Guy and co-workers[26] reported that while the consumers expressed a slight dislike for whey-soy mixtures, incorporation of flavours like cherry-vanilla and chocolate greatly improved their acceptability. Strawberry flavour was preferred more than either pineapple or vanilla flavour in a sugared whey-soy beverage developed by Patil[29]. The sugared, citrated and flavoured whey-soy beverage formulated by Guy *et al.*[16] scored 6.0-6.5 on a 9-point hedonic scale. It is thus clear that whey-soy drinks, when suitably flavoured are quite appealing to the consumers. This point has also been amply proved by studies conducted on whey-soy beverage acceptability to mothers and children in several developing countries[38].

Drying and reconstitution

Many beverages have been converted into a concentrate or powder form in order to provide convenience in their handling and use, and whey-soy beverages are no exception[25,36]. Enhanced storage stability of dried beverages add to the value of drying[12,26,45].

Spray drying is usually prefered to other methods of converting whey-soy beverages into powder[13,20,23,26,44,46]. This can easily be ascribed to the well established advantages of the spray dried product with respect to its functional properties and sensory characteristics over the roller dried product. The lactose content and sometimes the sugar content in these beverages have necessitated the use of a relatively low drying temperature. Holsinger *et al.*[12] spray dried a mixture of soy flour and cottage cheese whey at an inlet air temperature of 146°C. Higher drying temperatures are reported to have a deleterious effect on the colour of the product[31]. However, as high a temperature as 190°C has been used by Patil[29] who dry mixed sugar with the powdered product. Since the whey-soy beverage usually contains an appreciable amount of oil that is susceptible to autoxidation, a milder heat treatment during drying would be more desirable for better keeping quality of the finished product.

Other methods such as foam spray drying and vacuum shelf drying have also been adopted though only to a limited extent. Guy *et al.*[16] vacuum shelf dried citrus beverage, while foam spray drying procedure was successfully employed by Holsinger *et al.*[13] Another beverage formulation of Guy *et al.*[26] was converted into powder by mixing the cold mix concentrate with nitrogen, spreading it in thin films on trays followed by drying under vacuum in a shelf dryer. They suggested that concentrated blends of whey and soy flour can easily be spray dried or foam dried under vacuum. However, the foam spray drying technique must be used for such products wherein the solids content is above 45 per cent. According to Holsinger *et al.*[19] when lactose is hydrolyzed, foam spray drying with nitrogen injection is necessary.

Different methods of drying may result in a product

with varying physico-chemical properties which may, in turn, affect its reconstitutability and storage stability. Although not much information is available on this subject, the work of Guy *et al.*[26] deserves brief mention. These workers found that the vacuum shelf dried product has substantially higher amounts of free fat (13.8 to 18.5 per cent of the total fat) than the spray process product (0.9 to 1.3 per cent). With the foam spray dried whey-soy drink, the bulk density increased (from 0.24 to 0.33 g/ml) on decreasing the amount of air injection.

The structure of the beverage powder particles largely determined by the method and conditions of drying, is the prime factor influencing the reconstitutability of the beverage. A recent study using light and scanning electron microscopy showed the presence of slightly collapsed spherical particles with a smooth surface and some aggregates in a spray dried whey-soy beverage[47]. Reduction in the inlet air temperature coupled with increased total solids in the feed caused an increase in the number of the aggregates, many of which were coated with fat droplets. The hydrophobic surface was stated to inhibit the dispersal of these aggregates during rehydration, thereby enhancing phase separation after reconstitution.

Since the whey-soy beverages are, in essence, blends of whey solids and soy solids in one form or another, practical considerations may favour mixing the two ingredients in a dry form. Guy *et al.*[44] reported that the dried whey-soy flour mixture reconstituted more easily and had a greater physical stability than a dry blend of whey powder and soy flour. Holsinger *et al.*[19] found that the solubility index of spray dried whey-soy drink mix increased when reconstituted medium heat, sweet whey solids replaced fresh, fluid sweet whey in the mix before drying.

The nutritional significance of the reconstitution technique was demonstrated by Dellamonica and co-workers[48] who investigated losses in vitamins A and C on hot water reconstitution of a whey-soy beverage. A loss of 50 per cent of the vitamins was noticed after mixing the powder in boiling water for one minute, while 5 min of boiling resulted in 65 per cent loss of the vitamins. These workers have recommended reconstitution by blending the dried product in boiling water for 30 sec followed by cooling at ambient temperature so as to minimize vitamin losses in the product.

Conclusion

The whey-soybean combination seems to hold good promise towards utilizing these valued food materials in the manufacture of nutritious beverages. Such beverages have been found to be highly acceptable as flavoured drinks. However, as milk analogues, whey-soy beverages need to undergo substantial improvement particularly in respect of their flavour. Cheddar cheese whey or sweet cheese whey has generally been preferred to acid whey. Limited attempts have been made in the direction of using lactose-reduced whey obtained through enzyme hydrolysis or removal of crystallized lactose. The enzyme treatment of whey deserves greater consideration in view of the fact that it offers an added advantage in increasing sweetness and thereby enhancing the taste appeal of the product.

Soybean is processed as whole beans or converted into a flour or protein isolate for its incorporation into whey. The method of soybean processing in the first instance and the treatment of the whey-soy blend later, largely determine the flavour of the finished product. In spite of the 'improved' or 'acceptable' flavour attributed to certain beverage formulations, the problem of 'cereal-like' flavour inherent even in properly processed soybeans continues to be a challenge for the food technologists. Undoubtedly, synthetic flavourings have been used with commendable success in raising the flavour quality of whey-soy beverages.

Frequently, whey-soy formulations have been fortified with fat, vitamins and minreals in order to make the nourishing drinks more wholesome. While methionine fortification is not so desirable owing to the off-flavour resulting from heat treatment of the fortified mixture, incorporation of this amino acid into the dried beverage can be of distinct value when the reconstituted drink is intended to be consumed in cold condition. Additives like sodium citrate and corn syrup solids have been employed to enhance the reconstitution or sensory characteristics of the whey-soy beverages. Spray dried beverage powders appear to have a great potential for use in certain international food programmes.

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BOOK REVIEWS

Physical Properties of Foods: Edited by Ronald Towitt, Felix Escher, Bengt Hallstrom, Hans F. Th. Meffert, Walter E. L. Spiess and Gilbert Vos, Applied Science Publishers Ltd, London and New York, 1983; pp. 425; £46.00.

This book is the proceedings of a seminar held under the auspices of COST 90 (European Cooperation in Scientific and Technical Research) to mark the conclusions of the COST 90 project on the physical properties of foods at the Catholic University of Leuven, Belgium. Knowledge of physical properties of food such as water activity, rheological and thermal properties is essential for process development and control. The book comprises four parts and deals with important physical properties of food, development and standardisation of simple methods of measurement and correlation of quality of food in relation to physical properties.

Part one of the book containing seven papers discusses at length the water activity of foods, practical applications of sorption data, water sorption of biological materials, GAB model of constructing sorption isotherms, and also survey on existing sorption data results of the COST 90 project on water activity and finally the future needs in water sorption in foodstuffs.

Part two containing seven papers deals with food rheology, its relevance to food processing, use of rheological data on structure analysis and their application in food engineering; guide lines to measurement of rheological properties of food stuffs and use of emiting data. One paper deals with relationship of these properties on the type of flow and its implication on food rheology. Comments of COST 90 project on rheological properties of liquid foods are also included.

Part three contains five papers on history, aims, results and future of thermo-physical properties work within COST 90. Calculation, measurement of thermophysical properties of foods and their critical evaluation are outlined. Information and prediction of physical properties of nonfood materials are also touched upon.

Part four containing three papers details some observations on COST 90 and presents general conclusions and recommendations of the project. While evaluating the above properties, need for assessing parameters such as accuracy, precision, repeatability and reproduceability of results, methodology and reference standards, relating of experimental method to practical situations and particular versus general useability of data are highlighted.

One important feature of the book is the inclusion of active discussion of each paper after presentation. It also gives in the beginning a list of contributors to the COST 90 project and at the end gives list of participants of the seminar. The editors have attempted to bring out concise informations and opinion of scientific workers on three important physical properties of food and their relation to food processing, preservation and packaging. Proceedings of the seminar is aimed to provide useful first hand information on the subject. The topics covered are broad enough to draw the attention of the specialists in the field of food science and technology whose chief concern is food processing and preservation.

N. BALASUBRAHMANIAM
C.F.T.R.I., MYSORE

Yeast Genetics—Fundamental and Applied Aspects: Edited by J.F.T. Spencer, D. M. Spencer and A. R. W. Smith, Springer-Verlag Postfach, Heidel-berger Platz 3, D-1000, Berlin 33, 1983; pp 533; US $ 57.50.

There has been remarkable growth in the field of molecular biology during the past few decades. Advances made in the field have helped us to understand the molecular mechanisms used by nature for replicating, distributing and modifying biological information. Great deal of information has been gathered about the chemical and physical nature of macromolecules, nucleic acids and proteins and the manner in which carbohydrates, lipids and smaller molecules work together to provide the molecular setting of living systems. Springer-Verlag is bringing out a series of monographs on molecular biology and this book deals with fundamental and applied aspect of yeast genetics. The book contains 15 chapters grouped into four sections. This volume opens with a series preface followed by preface to this particular book which gives an overall review of the contents which is quite extensive, highly informative and critical.

The first chapter by Carter and others deals with the control of cell proliferation. Genes involved in cell division, products of genes involved, organisation of cell cycle events, conditions necessary to start the cell cycle such as nutrients and size, role of hormones in mating, development of alternatives to mitosis are discussed.

Sexual cycle in *Saccharomyces cerevisiae*, in the normal as well as in adverse conditions, are reported in the second chapter. While outlining the sporulation in *S. cerevisiae*, the author discusses the life cycle of heterothallic yeast, morphology and physiological and bio-

chemical changes. Special genetic techniques, gene function and expression during sporulation and factors responsible for mitosis and meiosis are also presented in this chapter.

The phenomenon describing the genetic behaviour of yeast, the fundamental nature of the recombination and genetic alterations are presented in the third chapter by Fogel and his associates. Value of recombination as a tool for strain improvement in yeasts has been detailed, covering all aspects of the technology. The topic of meiotic gene conversion deals with (*a*) mechanistic overview of the phenomenon (*b*) examination of a specific recombination model (*c*) properties and attributes of meiotic gene conversion based on genetic studies in fungi and (*d*) analysis of the newer knowledge obtained from the recombinant DNA structures. Mitotic recombination and yeast cytology does not get attention in the article.

The next chapter concerns the radiation sensitivity in yeast and repair of genetic damage by UV and X-ray irradiation. Game gives the otulines of earlier studies on radiation biology of *Saccharomyces cerevisiae*, major classes of radiation-sensitive mutants that have been isolated and discuss their contribution in understanding the genetics of repair in yeast. This chapter also describes the recent work on the genes that control repair of ionizing radiation damage which are also required for normal meiosis, as well as meitotic and mitotic recombination in yeast. Role of X-ray sensitive mutants in current research in these areas have also been projected.

Strain improvement through breeding is one of the methods of providing better microbial strains for fermentation industry. Till recently morphological criteria and physiological tests were being used in the classification and identification of yeast. It was based on the ability of the isolate to ferment sugars and utilize certain nitrogenous compounds. Now sophisticated immunological test and determination of the composition of the DNA and ultimate criterion of the structure of DNA itself are made use of in the yeast taxonomy. In the fifth chapter, Kurtzman *et al.* review the work carried out in the past 15 years wherein the possibility of whole genome comparisons through DNA reassociation which is now being used to define species. The chapter gives details on nucleic acid isolation and purification, DNA basic composition, DNA relatedness and concludes with an emphasis on molecular taxonomy as an aid to genetic research.

Sixth chapter is devoted to a review on the genetic approaches to the study of protease function and proteolysis in *Saccharomyces cerevisiae*. Dr. Jones discusses the role of proteinase, peptides, and inhibitors in the functioning of the cell and stresses the importance of the recent findings.

Flocculation is one of the most important desirable character of yeasts, whether in brewing, enology or industrial alcohol fermentation. The term is used to denote aggregation of dispersed yeast cells to flocs eventhough there are other forms of aggregation namely, 'clumpy growth' and 'chain formation'. Eventhough 'flocculation' is a cell surface phenomenon, it is also reported to be genetically controlled. It is a property associated with structure of the cell wall and most likely with cell wall proteins. In the seventh chapter of the book, Johnson and Reader present the methodology for the measurement of flocculation, origin of strains, phenotypic variation, cell wall differences, stability of flocculence and industrial applications.

The nature and function of plasma membrane is one of the important factors in studying the physiology and matabolism of the organism. The membrane contains proteins, lipids and carbohydrates, of which the the lipids have received much attention. Rank and Robertson review the role of protein and lipids in material and energy transport and the genetic control of their function, in the eighth chapter. The authors report the procedures employed in the isolation of plasma membrane, cytological purity of membrane preparations and describe the wide range of genetic and physiological manipulations possible to study the structural and functional relationship of the membrane.

Somatic hybridization technique has been adopted for strain improvement in fungi and yeasts. Most of the industrial strains of *Saccharomyces cerevisiae* used in brewing, baking and alcoholic fermentation multiply principally by budding only; sexual activity is seldom observed. Attempts have been made to stimulate them to behave like laboratory strains. The use of protoplast as tools of genetic recommendation in yeasts had proved fruitful. Hybrids have been obtained between *S. cerevisiae* and *Saccharomycopsis lipolytica*. Spencer *et al.* have also produced both intergeneric and interspecific somatic hybrids. The ninth chapter by Freeman and Peperdy describe the technology of protoplast isolation, fusion and reversion and the consequences of protoplast fusion.

The tenth chapter by Wilkie is devoted to genetic and functional aspects of mitochondria. Wilkie briefly describes the mitochondrial genome recombination, replication and transmission together with some aspects of biogenesis and function of the organalle. He emphasises that yeast cell makes a useful model of the eukoryotic cells to study the problems relating to the activity of antimitochondrial drugs because they have some important functions in cellular processes apart from providing ATP.

Evens presents an exhaustive and highly informative review on molecular genetics of yeast mitochondria in

chapter eleven. Clear evidence for DNA in mitochondria was first established in 1962 and since then intense research activity is being carried out on mitochondrial DNA (mt DNA). Mitochondrial DNA of different organisms vary in form, size and base composition even though there are some broad similarities in major phylogenetic group. Size and conformation of mt DNA of protozoa, myxomycetes, fungi-both yeast and filamentous, metazoa, algae and some plants are given in a tabular form. The topics discussed in this chapter are: DNA sequencing of mt DNA, yeast mitochondrial genome, variations in the size of different mt genomes of different yeast strains, base composition of yeast mt DNA, mapping yeast mt DNA, genetic mapping, genes on yeast mt DNA, genes of ribosomal RNA, tRNA genes and the mitochondrial genetic code, simple protein-coding mitochondrial genes, complex protein-coding mitochondrial genes, unassgined reading, frames and introns, mitochondrial transcription, mitochondrial protein synthesis, the petite mutation and mt DNA replication and the evolutionary aspects have also been presented in detail.

The twelfth chapter is devoted to Sc V 'Killer' virus in yeast. Certain strains of *Saccharomyces cerevisiae* (and also of other genera) secrete a proteinaceous toxin, the killer toxin, which is lethal to other strains of the same species. Toxin producing strains are termed killers and the susceptible strains are termed sensitives. Killer strains possess two species of ds RNA, the L and M genomes which are separately encapsulated in identical protein coats as the Sc V-L and ScV-M viruses, and hence can be refered to as virus-like particles. These two particles possess all the attributes of a segmented virus except infectivity. Mitchall and Beavan have dealt in detail various characteristics of these particles like electron microscopy, physical properties and also the molecular studies of the genomes. Details of the killer toxin are also presented.

Genetic analysis and breeding of brewer's yeast is the title of the chapter thirteen. Techniques used in the genetic improvement of brewing yeast strains are discussed in an expert manner. The next chapter is devoted to the improvement of wine yeasts. After tracing the origin of wine yeast, Snow describes the life cycle and ploidy and various methods followed in yeast improvement. Desirable characteristics of the wine yeast and the ways of incorporating it in the newly developed strain are also discussed.

Since several thousands of years, species of the genus *Saccharomyces* have been utilized to produce ethanol both for potable and industrial purposes. Yeasts are the oldest cultivated plants and convert sugars, in substances such as grape and other fruit juices, malted grain extracts, milk etc., into alcohol efficiently and rapidly. The main deficiency of these alcohol producing yeasts is their inability to ferment pentose sugars and polysaccharides. However, a few yeasts capable of utilizing pentoses, dextrin, starch and cellulose have been identified. Attempts are being made to develop new strains of yeasts which can produce alcohol from the unconventional substrates. The review will be of great help to those research workers engaged in this task.

In recent times, yeasts are assuming greater importance in genetic engineering and molecular biology over *E. coli* as the former have established their safety as non pathogenic. This book will serve as a useful reference book to all those who are concerned with modern biotechnology.

K. R. SREEKANTIAH
C.F.T.R.I., MYSORE

Nutritional Standards: by Lewis J. Minor, AVI, Publishing Company Inc., Westport, Connecticut, 1983, pp. 281, $ 20.75.

This book is aimed at helping to explain the basic factors that influence the wholesomeness, nutritive value, flavour, colour, texture, appearance, and preservation of food products. Just as important, it also provides guidelines used to set quality standards for institutional and commercial dietary services. It emphasises that the study of food science and nutrition is critical to our ability to provide healthy diets to future generations. Food has been defined as a complex subject and its proper focus embraces the physical, chemical, biological and social sciences. It has made a concerted attempt to identify quality parameters, according to which quality food-services should operate. Although sources of standards may vary they emanate from professional, consumer, governmental and other concerns and further they all have several things in common, namely,

(1) Food service standards identify goals and represent performance indicators that can and should be utilized to structure and evaluate operational performance.
(2) Food service standards provide a foundation of information that students of hospitality must acquire in order to improve our food industry.
(3) Exemplary standards of excellence must be known so as to be utilised.

This book has 9 chapters dealing with introduction to the study of food, products standards, food additives standards, a primer on monosodium glutamate, food flavour standards, standards in nutrition, new food product development standards, laboratory

guide for food flavour evaluation-both theory and practice.

The first main chapter emphasises the basic knowledge of food, the culinary arts and the sciences of preserving foods important to food service managers. Acceptance of food is determined by such factors as age, socio economic background, travel experiences, family size and make up including activity patterns which are, of course, different for each individual.

The 2nd chapter on food products standards emphasises that these are essential in the marketing of foods and relate to quantity, weight, quality and value of the food product. It deals with standards of egg products, processed eggs, poultry, meat, milk and milk products, fruit and vegetable products, cereal products including spices and tea in depth.

The food additives standards are dealt in chapter 3 where food analysis, consumer protection, sources and limits for food additives, including additive control by legislation with bibliography have been highlighted.

The 4th chapter deals with monosodium glutamate indicating its history, its role as a food flavour enhancer, including use levels as well as safety aspects.

Food flavour standards including laboratory code for flavour evaluation-both theory and practice-have been highlighted in chapters 5, 8 and 9. These deal mainly with the importance of flavours and odours; studies including odour determination and taste evaluation, especially with regard to temperature and blending effects.

Chapters 6 and 7 highlight the food service nutrition standards. Various nutrients used for fortification as well as emulsifying agents, need of carbohydrates, proteins, lipids, fat soluble vitamins, water soluble vitamins, minerals, their digestion, absorption and nutrient utilisation including nutritional labelling.

New food product development standards with regard to the steps involved in the development of new products have been reviewed in chapter 7.

The food service industry is vast and complex and the scope of its activities and job-related knowledge required to operate within it, is very broad.

This book will be a useful guide for professionals, consumers, nutritional experts, government agencies and manufacturers who strive to achieve high quality food service standards by developing and marketing on modern lines the food manufacture/food service system.

O. P. KAPOOR
C.F.T.R.I., MYSORE

Developments in Food Preservation:—2. Edited by Stuart Thorne, Applied Science Publishers, London, 1983; pp. 184; £ 24.00.

Developments in Food Preservation-2 comprises six chapters covering a wide range of aspects of preservation of fresh and processed foods. The first chapter which deals with controlled atmosphere (CA) storage of fruits and vegetables discusses briefly the benefits and limitations; recommended atmospheres for various horticultural crops; and the potential in the transportation of certain fruits and vegetables. Construction and operational aspects of CA stores are dealt in a little more detail. Although the usefulness of CA in conjunction with intermediate temperatures has been found to be beneficial for the storage of chilling sensitive cultivars, the commercial application is mostly limited to apples and to a lesser extent to pears and cabbages. Since CA storage is effective mostly in conjunction with refrigeration, economics is often the limiting factor in the wider application of this method.

Chapter 2 on food irradiation discusses briefly the intensive research and developmental work undertaken in several countries during the last three decades and provides an insight into the factors that have hindered the practical adoption of this new preservation method despite its many proven applications. Since safety of irradiated food has been the major concern for its acceptance by the industry and the consumer, the author has devoted more attention to the massive scientific data generated on toxicological, nutritional, chemical and microbiological aspects of irradiated foods, which finally led to the FAO/WHO IAEA Joint Expert Committee in 1980 to conclude that irradiation of any food, upto a dose of 10 kGy, presents no toxicological hazard. Commercial food irradiation facilities currently in operation in some countries and the outlook for future application of this new technology are discussed briefly.

The physical background to heat and mass transport in solid food stuffs of homogenous, heterogeneous and porous materials and the relevant thermal processes, essential information for the design of any processing operation, are described in chapter 3. Convective heating by means of air is analysed in detail and equations are given for the transfer of heat and mass in different zones of the product during drying operation.

Until recently, in spray drying of food stuffs, meeting powder quality requirements was considered more important than fuel consumption. With the changed situation, however, much developmental activity is currently taking place into ways of reducing energy costs through improved waste heat utilization and energy saving options. These aspects as well as the practical aspects and basic principles of spray drying food stuffs

are described in chapter 4. Developments in product quality improvement and operation of spray driers to meet environmental standards are briefly discussed.

Chapter 5 deals with the use of computers in food processing operations. The different digital systems in use are described in very general terms and their possible applications in various food processing operations are briefly outlined. The reader may not find much information on the basic principles but may get an idea as to how computers can be put to use to improve and maintain standards of product quality and to reduce wastage of material and energy.

Ethylene, a gas at physiological temperature and pressure, is recognized as an endogenous plant hormone controlling various physiological processes of plants particularly maturation and ripening of fruits. Ethylene is also released into the atmosphere as a byproduct of plant metabolism and its presence can cause serious problems of commercial importance in the refrigerated or controlled atmosphere storage of fresh fruits and vegetables. The biosynthesis and production of ethylene in different fruits and vegetables, its role in the ripening of fruits, storage disorders, and recent developments in the methodology for the removal of ethylene from the storage atmospheres are discussed in the last chapter.

This book though provides an overview of the developments taking place in the above mentioned areas of food preservation, lacks an indepth coverage of the topics.

PAUL THOMAS
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Tomato Production, Processing and Quality Evaluation: Wilbur A. Gould, 2nd Edn. AVI Publishing Co. Inc., Westport Connecticut, 1983; pp. 445; $ 57.50.

The book comprises three parts, (*a*) production (*b*) processing and (*c*) technology. Further each part is subdivided into several chapters.

The first part on production is made up of seven chapters. The first chapter covers introduction and history of tomato industry, which traces the origin of tomatoes, importance of tomatoes among the vegetables and the history of tomato processing industry in USA starting from 1847 to date. In the second chapter, field selection, climatic conditions, application of fertilisers, cultivars, disease control, irrigation etc. have been discussed. Genetics and breeding of processing tomatoes forms the subject matter in the third chapter. Fourth chapter explains characteristics required, problems encountered and costs involved in mechanical harvesting of tomatoes. The fifth chapter touches upon various containers used for transportation of harvested tomatoes from the field to the processing plant. Recent development of using water tanks for transporting is of particular interest. Four basic systems of grading followed in USA along with instruments used for evaluating the same subjectively and objectively are described in chapter 6. The seventh chapter elaborates the various unit operations involved in preparation of tomatoes for processing.

The second part on processing comprises five chapters from 8-12 dealing with the processing aspects of tomatoes. Importance of proper filling to get drained weights required under the USA grades A, B and C, firming agents, process time and temperature, necessity of acidification have been discussed in the 8th chapter. In chapter 9, under tomato juice manufacture in addition to preparatory methods and equipments involved, four in-can processing and two bulk processing methods have been described with emphasis on thermal inactivation of flat sour spoilage organisms. Chapter on tomato pulp and paste manufacture describes the definitions under the Food, Drug and Cosmetic Act for the above along with various concentration systems used. Bulk storage methods of aspetic, freezing and acidified storage are of special interest here. Defintion of tomato catsup, method of manufacture, ingredients and quality, forms the subject matter of chapter 11. In chapter 12, formulas and methods of preparation for tomato soup, cream of mushroom soup and vegetable beef soup have been described.

The third part on technology is the largest part in the book comprising ten chapters from 13-22, covering comprehensively quality control and evaluation methods. The chapter 13 deals with quality definition, standards, methods of determining and purposes and bases of quality assurance programme. Standards and procedures for determining the fill of a container for processed tomato products are described in chapter 14. Factors contributing to tomato colour, colour perception and various systems and instruments used in colour measurement forms the subject matter of chapter 15. Methods and instruments used for measuring viscosity have been described in chapter 16. Procedures of determining acidity and pH and the various methods available for evaluation of flavour in tomato products have been described in chapters 17 and 18. Drosophila life cycle, habits, its detrimental effects on tomatoes and its products, its detection and control have been described in chapter 19. Chapter 20 deals with methods of mould counting, genera of moulds in tomato products and their characteristics, causes of flat sour spoilage in tomato juice, its characteristics and prevention along with nature of spoilage encountered in canned tomatoes and catsups are described in chapter 21. Composition

of tomatoes and its products along with factors affecting the retention of nutrients have been described in the last chapter.

The book also contains three appendices, A, B and C. Appendix A gives U.S. standards for identity and grades, fill of a container, factors of quality, definitions, inspection and score sheets. Appendix B describes "Food and Drug Administration part 53—Tomato products". Appendix C gives quality control and evaluation forms.

The book gives an excellent, exhaustive and upto date basic information (supported by illustrations, charts and excellent photographs of the equipments used) on the main factors involved in production, processing and quality control and evaluation of tomato and tomato products. One lacuna which can be pointed out is that information on and developments in the manufacture of tomato juice powder has not been covered in the book. Also it would have been more appropriate if the part I-7 is shifted to part II and part III-22 to part I. The book seems to have been written specially to cater to the needs of manufacturers of tomato products in USA. However, it would be extremely useful as a good reference book to food scientists, fruit technology students and technologists working in the food industries as well as experts dealing with quality assurance programme in the country.

G. RADHAKRISHNAIAH SETTY
C.F.T.R.I., MYSORE

Drying Technology, Vol. 1, *No.* 1, 1983–84: Published by Marcel Dekker Inc., New York, subscription/ annum: Institutions $ 95.00; Individuals $ 47.50.

This is a new journal in the field of dehydration. It is proposed to publish original research papers, review articles pertaining to drying technology covering agriculture, food products, minerals and waste consolidation.

For the year 1983–84 two volumes are being brought out, volume one having two independent numbers and volume two with four numbers i.e. six numbers for the year 1983–84. Vol. 1, No. 1, contains five research papers of fundamental and applied nature covering (*a*) drying granular porous media; (*b*) droplet-gas interaction in counter-current spray dryers; (*c*) water removal from mine slimes and sludge using direct current (applied res. paper); (*d*) drying of small wood particles; and (*e*) temperature distributions and heat transfer during the drying of lumber.

This new Journal also proposes to publish review of books.

B. S. RAMACHANDRA
C.F.T.R.I., MYSORE

Toxicology Data Profile on Saccharin: by R. R. Khan and Gomathy Iyer, The Lucknow Publishing House, Lucknow, India, 1983; pp. 56; Price not mentioned.

At the time when there was a dilemma to ban the use of saccharin, the release of this publication has not only led to the complete understanding of the toxicological data on this non-nutritive sweetener, but also cleared several doubts that existed till now on its use. The book is a compact compilation of all the data such as chemistry, properties, manufacturing processes and manufacturers, metabolism, toxicology, carcinogenicity, mutagencity, embryotoxicity, teratogenicity, immunotoxicology, epidemiology and legal status available on saccharin. Recommendations of American and European committees on the safety of saccharin, references and classified bibliography from 1927 to 1982 are also included at the end. It is an useful guide to all food toxicologists and biochemists in general as well as nutritionists in particular.

Saccharin is extensively used in the manufacture of syrups, medicines, soft drinks and foods. It is rapidly metabolized and excreted mostly in urine and a little in faeces in most of the mammalian species. It has been found to be weakly carcinogenic in urinary bladder of male at high levels. The intake of saccharin by woman in the child bearing years and children should be actively discouraged unless they have a medical condition for which saccharin has a definite health benefit.

Among three habits in USA, cigarette smoking, coffee drinking and the use of saccharin, the artificial sweetener appears to contribute the lowest risk for the development of bladder cancer. The benefits of saccharin to diabetic patients are certainly more than without taking it. It is used in the control of obesity, dental caries and hyperkinesis. The epidemiological data provide no clear evidence that saccharin alone or in combination with cyclamates causes urinary bladder cancer. It is stated that banning saccharin may, therefore, cause great harm to many citizens while protecting a theoretical few.

S. C. BASAPPA
C.F.T.R.I., MYSORE

ASSOCIATION NEWS

Palayamkottai Chapter

The Annual General Body Meeting of the above Chapter was held on 27th July 1984. It was decided to continue the following office bearers for 1984–85 also.

President:	Sri N. Jayaprakasam
Vice-President:	Prof. S. Narasimhan
Treasurer:	Prof. S. Somasundaram
Secretary:	Sri. V. Theetharappan

ICFOST '85

ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA)

Theme: FOOD INDUSTRY OUTLOOK—Emerging Scenario in Developing and Developed World.

Venue: Vigyan Bhavan, New Delhi, April 3-5, 1985.

The Fifth Indian Convention of Food Scientists and Technologists—ICFOST'85 will be held in April 3-5, 1985. in New Delhi. It will be the biggest convention of food processors, scientists, technologists, project managers, R & D and QC personnel, government officials and representatives of national and international developmental agencies. Key speakers include representatives from developed and developing countries as well as international organisations. Technical sessions will include key-note/lead paper presentation as well as contributed papers in diverse areas of food processing. Current R & D work in different areas of food science and technology will be represented in poster sessions. An exhibition of catalogues highlighting the wide range of food ingredients, products, process equipment and machinery, packaging systems, analytical instruments, books and journals will also be a part of the convention.

If you are a food scientist, technologist, processor, supplier of raw materials and ingredients, equipments and machinery including packaging systems and materials, you cannot afford to miss ICFOST'85. Plan now to be in New Delhi for ICFOST'85.

ICFOST'85 is being co-sponsored by the Ministry of Food and Civil Supplies, Ministry of Industry, Government of India, Central Food Technological Research Institute, and All India Food Preservers Association.

Write for full particulars to: Mr. Laljeet Singh, President, AFST(I), Modern Food Industries (India) Ltd., Palika Bhavan, 3rd Floor, R. K. Puram, Sector XIII, New Delhi-110 066, India.

INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (*in triplicate*) should be typewritten in double space on one side of bond paper. The manuscripts should be complete and in final form, since only minor corrections are allowed at the proof stage. The paper submitted should not have been published or data communicated for publication anywhere else. Review papers are also accepted.

2. Short communications in the nature of Research Notes should clearly indicate the scope of the investigation and the salient features of the results.

3. Names of chemical compounds and not their formulae should be used in the text. Superscripts and subscripts should be legibly and carefully placed. Foot notes especially for text should be avoided as far as possible.

4. **Abstract:** The abstract should indicate the principal findings of the paper. It should be about 200 words. It should be in such a form that abstracting periodicals can readily use it.

5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. They should be typed on separate paper. Nil results should be indicated and distinguished clearly from absence of data, which is indicated by '—' sign. Tables should not have more than nine columns.

6. **Illustrations:** Graphs and other line drawings should be drawn in *Indian ink* on tracing paper or white drawing paper preferably art paper. The lettering should be in double the size of printed letters. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size 16cms (ox axis) × 20cms (oy axis); photographs must be on glossy paper and must have good contrast; *three copies* should be sent.

7. Abbreviations of the titles of all scientific periodicals should strictly conform to those cited in the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.

8. **References:** Names of all the authors along with title of the paper should be cited completely in each reference. Abbreviations such as *et al.*, *ibid. idem*, should be avoided.

   The list of references should be included at the end of the article in serial order and the respective serial number should be indicated in the text as superscript.

   Citation of references in the list should be in the following manner:

   (*a*) *Research Paper:* Jadhav, S. S. and Kulkarni, P. R., Presser amines in foods, *J. Fd Sci. Technol.*, 1981, **18**, 156.

   (*b*) *Book:* Venkataraman, K., The Chemistry of Synthetic Dyes, Academic Press, Inc., New York, 1952, Vol. II, 966.

   (*c*) *References to article in a book:* Joshi, S. V., in the Chemistry of Synthetic Dyes, by Venkataraman, K., Academic Press, Inc., New York, 1952, Vol. II, 966.

   (*d*) *Proceedings, Conferences and Symposia Papers:* Nambudiri, E. S. and Lewis, Y. S., Cocoa in confectionery, *Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India*, Mysore, May 1979, 27.

   (*e*) *Thesis:* Sathyanarayan, Y., Phytosociological Studies on the Calcicolous Plants of Bombay, 1953, Ph.D. Thesis, Bombay University.

   (*f*) *Unpublished Work:* Rao, G., unpublished, Central Food Technological Research Institute Mysore, India.

9. Consult the latest copy of the *Journal* for guidance.

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Research Papers

Research Notes

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