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Research Papers

Research Notes

A Comparative Study of the Fingerprints of Pepsin Digests of Gliadins and Glutenins: Non-identity of Comparable Ninhydrin Positive Spots

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Received 12 *June* 1985; *revised* 11 *September* 1985

A comparative study of the fingerprints of the pepsin digests of gliadins and glutenins from four wheat varieties indicated that they were similar. However, comparable pairs of acidic peptides isolated from the gliadin and glutenin of one of the varieties ('Sonalika') differred among themselves int heir amino acid compositions. Thus, the comparable spots in the fingerprints of gliadins and glutenins may not be of identical peptides. The results suggest that gliadins and glutenins are not likely to have common peptides.

Eversince the observation that glutenin is a complex protein of disulphide bonded low molecular weight peptides[1], a relationship between glutenin and gliadin has been sought. Thus, Woychick *et al.*[2] on the basis of starch gel electrophoresis studies suggested that glutenin arises primarily through intermolecular disulphide bonding of gliadin peptides. Subsequently, considerable similarities in the fingerprints of pepsin digests of gliadin and glutenin were observed by Ewart[3]. The similarities were extensive enough for Ewart to suggest identity of a number of gliadin and glutenin peptides. Later studies by other workers[4-10], though they continued to show similarities, also showed differences between gliadins and glutenins. More recently, Bietz and co-workers[11,12] have determined partial N-terminal amino acid sequences of some low molecular weight gliadins and sub-units of high molecular weight gliadin and ethanol soluble sub-units of glutenin. The sequences of the latter two were found to be identical and those of low molecular weight gliadins were found to be different.

A limitation of some of these studies has been that the similar chromatographic or electrophoretic bands were not investigated further for establishing their identity. And in one case[13] where this was attempted the electrophoretically similar γ-gliadin and a glutenin sub-unit were found to be different. This communication gives a comparative study of the gliadins and glutenins of four wheat varieties using fingerprinting technique and the amino acid compositions of six pairs of comparable peptides from the gliadin and glutenin of one variety.

Materials and Methods

The sources of wheat varieties[14] 'Sonalika' (weak) 'HD 1949' (med. strong), 'HD 4530' (med. strong) and 'K 65' (strong) and the methods for the isolation of gliadin and glutenin[15] have been indicated before. Performic acid oxidation was done as described by Ewart[3]. Five hundred mg of gliadin was dissolved in 15 ml of 85 per cent formic acid and cooled in an ice bath. To this was added an ice cold solution of 1.25 ml of 30 per cent H_2O_2 in 30 ml of 85 per cent formic acid and the mixture allowed to stand for 2 hr with occasional shaking. The reaction mixture was then diluted with water, lyophilized, redissolved in water and lyophilized again after dialysis. Glutenin was similarly oxidized. Oxidized gliadin and glutenin were hydrolysed with pepsin[7] and trypsin[16]. For pepsin hydrolysis 25 mg of protein in 5 ml of 0.02 M HCl (pH 1.8) was incubated with 1 mg of the enzyme (24 hr, 37°C). For trypsin hydrolysis a suspension of 20 mg of protein and 0.4 mg of trypsin in 0.1 M NH_4HCO_3 (pH 7.9) was incubated for 24 hr.

Fingerprinting of pepsin digests: The fingerprints were prepared on 46×57 cm Whatman 3 mm paper using electrophoresis in the first direction and chromatography in the second direction. Electrophoresis was done for 50 min at 2,000 V in a Savant upright electrophoresis apparatus using pyridine acetate buffer (pH 6.5)[17,18]. Ascending chromatography (11 hr) was done in pyridine-n-butanol-H_2O (1:1.5:1 by vol.) solvent. Ninhydrin in acetone (0.2 per cent) was used for visualizing the peptides.

Isolation of acidic peptides: A total acidic peptide fraction was isolated from pepsin digests by chromatography on DEAE-cellulose. The digest was adsorbed on a short column (2.4 × 12 cm) in the acetate form. The column was washed with water and the acidic peptides recovered by elution with 2 M pyridine-acetate buffer pH 4.0. The eluate was lyophilized after dilution with H_2O. This fraction was applied as a band on the width of 3 mm paper and subjected to electrophoresis as above. The paper was air dried and the bands on it located by spraying a thin strip cut from the middle of the paper with ninhydrin. The appropriate bands were cut out and eluted with water. The eluates were hydrolysed with 6 M HCl[19]. Amino acid analysis was performed in a Durrum amino acid analyser (Kit MB F).

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE): The trypsin digests of 'Sonalika' gliadin and glutenin were subjected to SDS–PAGE according to the procedure of Weber and Osborn[20], and Orth and Bushuk[21]. The electrophoresis was done for 3 hr in tris borate buffer (pH 8.9. 17.5 per cent gel), and the proteins stained with Coomassie blue.

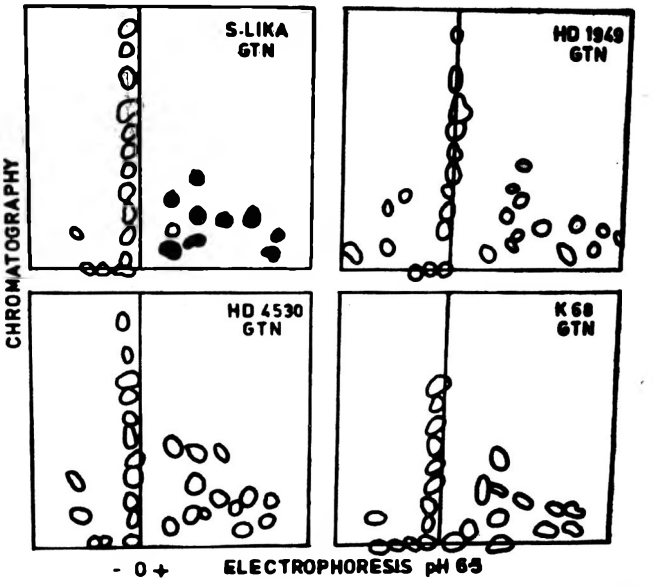

Fig 2. Fingerprints of pepsin digests of glutenins of 'Sonalika' (Weak), HD 1949 (med. strong), HD 4530 (med. strong) and K 65 (strong). Filled spots indicate the peptides isolated for amino acid analyses. See Fig 3.

Results and Discussion

The fingerprints of gliadins from 4 wheat varieties are given in Fig 1. All 4 fingerprints have both common and not-common ninhydrin +ve spots. The same is true for the fingerprints of 4 glutenins (Fig 2). Thus, the fingerprint patterns of gliadins and glutenins are varietal characteristics. Surprisingly, the similarities between gliadin and glutenin of a given variety are more than the varietal similarities amongst gliadins and glutenins themselves (Fig 1 and Fig 2). So much so, that in the case of 'Sonalika' the fingerprints of gliadin and glutenin are almost superimposable. As the purpose in undertaking this study was not only to compare the fingerprints but to further investigate the identity or non-identity of similarly situated spots, six pairs of comparable acidic peptide bands were isolated from 'Sonalika' gliadin and glutenin. The choice of 'Sonalika' was dictated by the fact that in this variety gliadin and glutenin fingerprints are most alike.

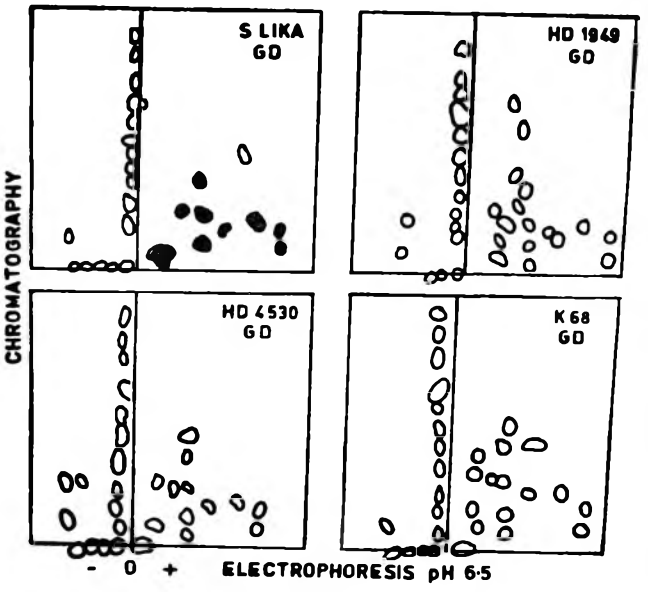

Fig 1. Fingerprints of pepsin digests of gliadins of 'Sonalika' (weak), HD 1949 (med. strong), HD 4530 (med. strong) and K 65 (strong). Filled spots indicate the peptides isolated for amino acid analyses. See Fig 3.

The fingerprints of the total acidic peptides of gliadin and glutenin are shown in Fig 3. These patterns are essentially the same as of the acidic peptides in the respective whole digests as indicated by comparison of Fig 1 and Fig 2 with Fig 3. The dotted lines in Fig 3 indicate the positions where the bands were cut from the preparative electrophoretic run of total acidic peptides. Bands 2, 3, 5 and 6 contain comparable single peptides. Band 1 contains 2 and band 4 contains 3 comparable peptides. The amino acid compositions of these bands (p1 to p6) are given in Table 1. All bands have different amino acid compositions. All are rich in glutamine and in this respect have the characteristic compositions of the parent proteins.

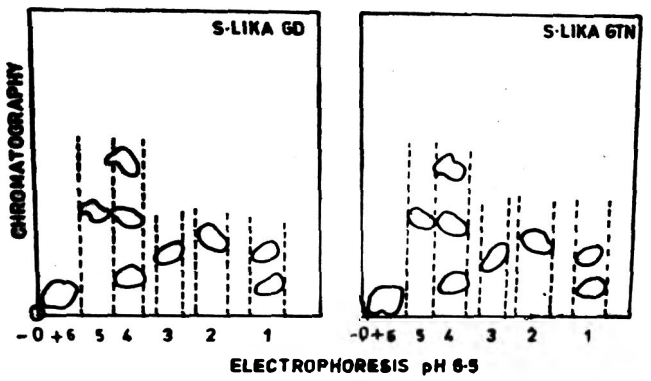

Fig 3. Fingerprints of total acidic peptides of pepsin digests of 'Sonalika' gliadin and glutenin. Dotted lines indicate the peptide bands isolated from preparative electrophoresis. See text.

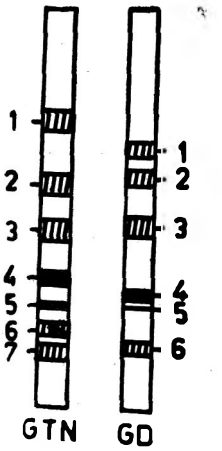

Fig 4. SDS—PAGE of trypsin digests of 'Sonalika' gliadin and glutenin.

The SDS-PAGE patterns of trypsin digests of 'Sonalika' gliadin and glutenin are given in Fig 4. There are 7 bands in glutenin and 6 in gliadin. Of these only the bands 5 are common. Compared to pepsin digestion, trypsin digestion gives larger fragments and therefore the fragments can be expected to be more different.

These studies clearly emphasize the differences between gliadin and glutenin and suggest that gliadin and glutenin are not likely to have common peptides. The high molecular weight gliadin which has been shown to contain peptides identical with the alcohol soluble sub-units of glutenin by Bietz and Wall[12] is structurally more like glutenin and has been designated as low molecular weight glutenin by others[2,7]. Accepting the latter classification would mean that the identical peptides reported by Bietz and Wall[12] belong to two types of glutenin and not to glutenin and gliadin.

Acknowledgement

We thank the Council of Scientific and Industrial Research, for awarding Senior Fellowship to B.P.R.

TABLE 1. AMINO ACID COMPOSITIONS[1] (MOLE %) OF ACIDIC PEPTIDES ISOLATED FROM THE PEPSIN DIGESTS OF 'SONALIKA' GLIADIN (GLI) AND GLUTENIN (GLUT)

	p1[2]		p2		p3		p4		p5		p6	
Amino acids	GLI	GLUT	GLI	GLUT	GLI	GLUT	GLI	GLUT	GLI	GLUT	GLI	GLUT
Asp	13.2	13.2	10.0	9.4	7.5	13.0	6.1	9.2	4.6	5.9	3.3	2.3
Thr	8.3	3.2	3.2	7.0	3.2	7.0	5.8	6.1	3.7	4.1	2.2	1.0
Ser	6.5	1.1	11.9	7.4	2.9	8.8	5.4	4.6	4.4	4.4	4.3	1.9
Glu	28.5	40.8	32.7	40.9	55.9	31.0	47.4	46.8	60.2	54.8	64.0	58.2
Gly	10.2	9.4	5.6	9.7	6.7	8.1	6.5	7.4	5.2	6.4	6.2	8.9
Ala	7.1	5.7	4.7	4.7	4.3	8.2	7.4	5.7	3.5	3.7	2.6	3.0
Val	18.6	7.9	14.2	6.4	8.5	5.0	5.7	4.9	2.9	3.5	3.1	4.1
Met	4.2	4.4	5.0	5.2	6.6	8.6	3.7	1.9	1.2	2.3	1.4	2.6
Ile	1.3	2.7	8.0	2.8	1.3	5.8	2.4	2.1	4.4	4.1	2.8	4.5
Leu	1.8	4.2	2.8	5.9	2.5	1.3	5.2	7.2	6.7	6.8	4.3	5.1
Tyr	—	2.6	2.1	—	—	1.2	1.6	2.2	1.0	1.3	1.7	2.1
Phe	—	3.9	—	—	—	1.1	1.4	1.6	1.8	2.1	3.4	4.7
His	—	0.4	—	—	—	0.4	0.9	0.1	—	0.1	1.0	0.5

1. Average of two determinations. 2. P1 to P6 are peptide bands 1 to 6 of Fig 3.

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Studies on Milling and Noodle Making Quality of Different Extraction Rate Semolinas from *Durum* Wheat

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***Durum* wheat ('Bijaga yellow') was processed to obtain semolina of different extraction rates (50, 60, 70 and 80%) by two milling methods using Polisher-Miag mill and Buehler laboratory *durum* mill separately. Though the semolinas obtained from Polisher-Miag mill had slightly higher ash than the respective extraction rate semolinas from Buehler laboratory *durum* mill, they were comparable in their protein and pigment contents. Semolina from Buehler laboratory *durum* mill were slightly better than those from Polisher-Miag mill in cooking quality as indicated by the tests on dough discs using the stamping test and the visco-elastograph. The extraction rate indicated little difference on the cooking quality. Semolinas from Polisher-Miag mill were comparable to those from Buehler laboratory *durum* mill in noodle making as well as cooking quality.**

In India, *durum* wheats are produced, mainly in the central and peninsular zones to the extent of over 3 million tonnes. Most of the *durum* wheat is processed by disc mills into semolina, which is utilised in the preparation of various sweet and savoury items. The extraction rate of semolina processed in these mills ranges from 40 to 50 per cent; this low extraction is mainly due to inefficient milling techniques. Studies on the high extraction rate semolina is conspicuous by its absence and assumes importance, as it influences the economics of the utilization of *durum* wheats.

Data on the processing of *durum* wheat into semolina of different extraction rates employing two milling techniques, as well as the comparative quality characteristics and noodle-making properties of these semolinas are presented in this paper.

Materials and Methods

A *durum* wheat variety, 'Bijaga yellow', cultivated extensively in Karnataka state of India, was procured from the Agricultural Research Station, Dharwad.

Quality characteristics: Besatz analysis, germinative capacity and pigment content were determined according to Standard methods[1]. Hectoliter weight and total ash content were determined according to AACC procedures[2]. Protein content (N×5.7) was estimated by the micro-Kjeldahl method. Thousand kernel weight was determined by weighing 1000 kernels, in triplicate. Particle size distribution of semolina was determined using a laboratory sifter.

Processing of high extraction rate semolinas by Buehler laboratory durum *mill:* After cleaning, 25 kg batch of wheat was conditioned to 18 per cent moisture level for 12 hr. For obtaining semolinas of different extraction rates, wheat was processed in a Buehler laboratory *durum* mill (MLU 202 D), Miag two layer purifier, grader, reduction system of Buehler laboratory roller mill (MLU 202) and Buehler bran finisher (MLU 302) according to the milling diagram described by Menger and Zwingelberg[3]. The milled products obtained were (*a*) semolina 1 (37.8 per cent) and fine semolina 1 (9.2 per cent) and (*b*) semolina 2 (7.8 per cent) and fine semolina 2 (14.8 per cent) from the first and second grindings respectively. Based on their purity, as indicated by their ash values, these semolinas, and flours from grader (3.3 per cent), reduction system (2.6 per cent) and bran finisher (5.0 per cent) were combined in this sequence in the required proportions for arriving at semolinas of 50, 60, 70 and 80 per cent extraction rates (Table 1).

Processing of high extraction rate semolinas by Polisher-Miag mill: After cleaning, 25 kg of wheat was conditioned by adding 5 per cent water followed by mixing for 5 min in a hand mixer. After resting for 10 min to facilitate penetration of moisture just into the bran layers, the conditioned wheat was passed

TABLE 1. PROPORTION OF VARIOUS SEMOLINA AND FLOUR STREAMS IN DIFFERENTLY MILLED HIGH EXTRACTION RATE SEMOLINA FROM DURUM WHEAT 'BIJAGA YELLOW'

Semolina extraction rate (%)	Buehler laboratory durum mill process							Polisher-Miag mill process				
	Miag purifier				Reduction grader system*		Bran finisher	Miag purifier			Bran finisher	
	S1 (%)	FS1 (%)	S2 (%)	FS2 (%)	Fl.1 (%)	Fl.2 (%)	Fl.3 (%)	S1 (%)	S2 (%)	S3 (%)	Fl.1 (%)	Fl.2** (%)
50	37.8	9.2	3.0	—	—	—	—	44.0	6.0	—	—	—
60	37.8	9.2	7.8	5.2	—	—	—	44.0	14.5	1.5	—	—
70	37.8	9.2	7.8	14.8	0.4	—	—	44.0	14.5	11.0	0.5	—
80	37.8	9.2	7.8	14.8	3.3	2.6	4.5	44.0	14.5	11.0	3.1	7.4
	% Ash (on moisture free basis)											
	0.67	0.88	0.99	1.06	1.17	1.44	3.67	0.81	0.90	1.21	1.42	2.07

*Buehler laboratory flour mill

**After grinding in Kamas mill

S1—Semolina 1 FS1—Fine semolina 1 Fl.1—Flour 1

S2—Semolina 2 FS2—Fine semolina 2 Fl.2—Flour 2

S3—Semolina 3 Fl.3—Flour 3

through a polisher (Rice huller No. 1: capacity 100 kg per hr) for removal of bran to the maximum extent possible in a single operation[4]. The polished wheat was then processed in a Miag twin variable roller mill, Miag two layer purifier, bran finisher and Kamas mill as shown in the milling diagram (Fig 1).

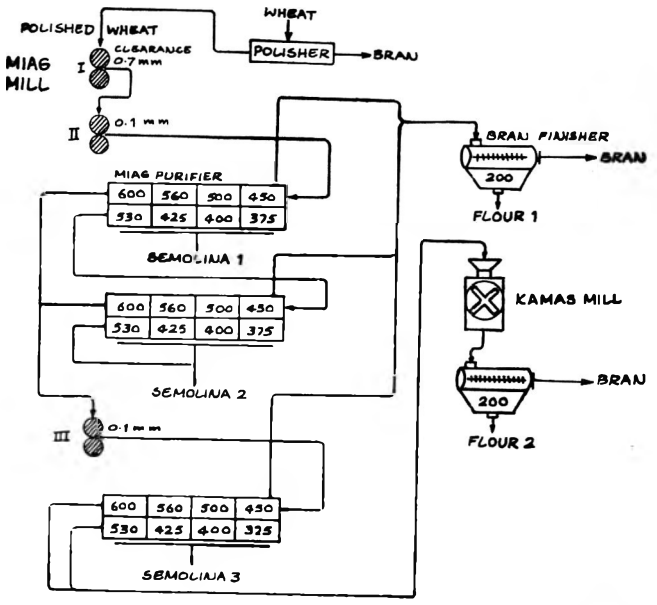

Fig 1. Polisher-Miag mill process flow diagram for milling of wheat into semolinas of different extraction rates

Semolina 1 and semolina 2 were obtained by grinding the polished wheat twice in succession in a Miag mill, and then feeding the stock twice to the Miag purifier. Semolina 3 was recovered by grinding the coarser stock for the third time in a Miag mill, and purifying the same in the Miag purifier. Flour 1 was recovered by passing the purifier siftings through the bran finisher, while flour 2 was recovered by grinding the composite semolina obtained from the purifier in Kamas mill and passing through bran finisher (Fig 1). The different milled products-semolina 1 (44.0 per cent) semolina 2 (14.5 per cent), semolina 3 (11.0 per cent), flour 1 (3.1 per cent) and flour 2 (8.0 per cent) were pooled, preferentially in the sequence mentioned, and in the required proportions to arrive at 50, 60, 70 and 80 per cent extraction rate semolinas. The sequence of milled products used for mixing was determined on the basis of purity, as indicated by the ash values (Table 1).

Stamping test: The method described by Alause[5] and modified by Menger[6] was followed for determining the cooking quality of semolina dough discs.

A 10 g sample of semolina was made into a dough of 34 per cent moisture content, by hand kneading for 3-4 min and allowed to rest for 2 hr. The dough was sheeted to a thickness of approximately 0.95 mm by passing 20 times through two rollers (clearance:0.7 mm) after folding each time to make four laminations.

The sheet was then cut into three discs of 40 mm diameter and subjected to a pressure of 90 kg/cm^2 in a press. These discs were further cut into smaller discs of 7 mm diameter and cooked for 12, 16, 24, 32 and 36 min in boiling water. Cooked discs were separated on a plastic sieve. After resting for one hour each disc was subjected to a 2 kg load for 1 min. The diameter of the discs was measured after removing the load, and allowing the discs to rest for 15 min. The "border cooking time" of semolina dough disc was the time after which the disc diameter exceeded 16 mm due to loss of its visco-elastic properties on the application of load. The scores were given for the border cooking time as follows: zero for less than 16 min; 2 for 16 min; 4 for 24 min; 6 for 32 min and 8 for more than 32 min.

Visco-elastograph test: The absolute elastic recovery of cooked semolina dough discs, prepared and cooked as described earlier for the stamping test, was measured according to the method of Feillet *et al.*[7] in a visco-elastograph.

The first reading (E_1) was the thickness of the cooked disc pressed under a 1.75 kg load for 40 sec while the second reading (E_2) was recorded 20 sec after releasing the load. The elastic recovery of the dough disc was calculated as (E_2-E_1). Based on the elastic recovery, scores were given to different samples according to Table 2.

TABLE 2. ELASTIC RECOVERY (E_2-E_1) IN 0.01 MM

Cooking time (min)			
16	24	32	Score
<10	—	—	0
>10 & <30	—	—	2
>30	<10	—	4
>30	>10 & <30	—	6
>30	>30	<10	8
>30	>30	>10	10

Noodle making quality: Noodles were prepared from semolina of different extraction rates. One kg of semolina was mixed with 250-280 ml of water (depending on the moisture content) for 10 min in a Hobart mixer and extruded as noodles in a Brabender extruder (Type 10 DN). The noodles were dried for 24 hr under controlled conditions over a temperature range of 18-53°C and 60-90 per cent relative humidity.

The cooking quality of noodles was determined as follows: 100 g samples of noodles were cooked in 3 l of boiling water for 12-15 min. Cooking was continued for 2 min after the central core was cooked completely as observed visually by cutting the noodles periodically. The cooked noodles were drained free of water and evaluated by a panel of six judges on the basis of parameters given in Table 3.

Results and Discussion

Quality characteristics of durum *wheat:* 'Bijaga

TABLE 3. EVALUATION OF COOKING QUALITY OF (SCORES 5-1) NOODLES

Quality parameter	Excellent 5	Good 4	Satisfactory 3	Fair 2	Poor 1
Colour	Clear dark yellow	Almost clear yellow to yellowish white	Whitish pale yellow to light brown	Clear brownish	Dark brown
Surface	Smooth closed, bright	Almost smooth, somewhat uneven, almost bright	Somewhat rough/ porous, somewhat swollen, somewhat dull	Rough/porous swoollen, dull	Too rough/ porous, too swollen, too dull
Form	Excellent	Good	Satisfactory	Fair	Poor
Fall of noodles	Discrete	Discrete	Somewhat indiscrete	Clearly indiscrete	Indiscrete
Stickiness	Smooth, not sticky	Almost smooth, hardly sticky	Somewhat rough, somewhat sticky	Rough, clearly sticky	Too rough, too sticky
Consistency bite	Firm, not doughy, not sticky	Firm, somewhat doughy, not sticky	Somewhat firm, somewhat doughy, somewhat sticky	Unevenly firm, doughy, sticky	Hard, doughy, sticky

yellow', had impurities of 4.7 per cent mostly contributed by the grain besatz of 4.3 per cent in the form of discoloured germ grains, infested grains, and brokens. Further, the hectoliter weight (78.7 kg/hl) was comparable and thousand kernel weight (31.2 g) was lesser than the average values (79.4 kg/hl and 43.8 g respectively) reported earlier for Indian *durums* by Haridas Rao *et al*[8]. The germinative capacity (93 per cent) was normal, indicating thereby the soundness of the grains.

Processing of semolinas of high extraction rates in Buehler laboratory durum *mill*: The proportions of various semolina and flour fractions in the semolina of the different extraction rates are presented in Table 1. From the data presented it is evident that semolina of 69.6 per cent extraction rate could be obtained. This consisted of yields of 47.0 and 22.6 per cent of semolinas, from first and second grindings respectively, in a Buehler laboratory *durum* mill used in combination with a Miag purifier. However, 10.4 per cent flour recovered from the remaining milled products by using a grader, reduction system of Buehler laboratory flour mill and bran finisher was required for making up semolina of 80 per cent extraction rate.

The semolina and flour fractions had ash content in the range of 0.67-1.06 and 1.17-3.67 per cent on moisture free basis, respectively.

Matsuo and Dexter[9] described a milling scheme to produce semolina of 70 per cent extraction rate in an Allis Chalmers Laboratory mill. Shuey *et al.*[10] have shown that *durum* semolina of upto 80 per cent extraction rate could be processed in an experimental roller mill, by recovering flour and semolina from dust and dregs.

Processing of semolinas of high extraction rates in a polisher-miag mill: Adopting a new approach of partial bran separation, 9.9 per cent of bran was removed in a single operation by passing *durum* wheat through a polisher.

The proportion of various semolina and flour fractions in the semolina of different extraction rates are shown in Table 1. Semolina of 69.5 per cent extraction rate was obtained from the Miag mill used in combination with the Miag purifier. This included 58.5 per cent yield of semolina from two successive grindings, and 11.0 per cent from the third grinding. To obtain semolina of 80 per cent extraction rate, 10.5 per cent flour recovered from the bran finisher and Kamas mill was combined with semolina of 69.5 per cent extraction rate (Table 1). The ash content of the semolina and flour fractions ranged from 0.81 to 1.21 and 1.42 to 2.07 per cent (on moisture free basis) respectively.

Chemical characteristics of semolinas of high extraction rates: Increases of 0.28 and 0.17 per cent in ash, and 1.0 and 0.9 per cent in protein were observed, as the extraction rate of semolina from a Buehler laboratory *durum* mill and from a Polisher-Miag mill, increased from 50 to 80 per cent respectively. (Table 4). However, the changes in pigment content were only marginal. Statistical analysis carried out on transformed variates (angular transformation) indicated that with respect to total ash, there was significant difference between the two milling methods, and in the case of semolina extraction rate, 80 per cent was significantly different from others, whereas rest of the rates (i. e. 50, 60 and 70 per cent) had no such differences (Table 4).

With regard to protein, there was no significant difference between the two milling methods, but significant differences were found among extraction rates (Table 4).

TABLE 4. EFFECT OF EXTRACTION RATE ON SOME CHEMICAL CHARACTERISTICS* OF DIFFERENTLY PROCESSED SEMOLINA FROM *DURUM* WHEAT-'BIJAGA YELLOW'**

Semolina extraction rate (%)	Total ash		Protein (N×5.7)		Pigments as β-carotene	
	B. lab mill (%)	P. Miag mill (%)	B. lab. mill (%)	P. Miag mill (%)	B. lab. mill (mg%)	P. Miag mill (mg%)
50	0.58	0.71	13.5	13.9	0.24	0.24
60	0.62	0.73	13.9	14.1	0.23	0.23
70	0.69	0.77	14.3	14.2	0.25	0.25
80	0.86	0.88	14.5	14.8	0.26	0.26

*Values expressed on 14% moisture basis

**Total ash, protein and pigments in wheat were 1.40, 15.1 and 0.29 mg% respectively

TABLE 5. PARTICLE SIZE DISTRIBUTION OF DIFFERENTLY PROCESSED HIGH EXTRACTION RATE SEMOLINA OF *DURUM* WHEAT-'BIJAGA YELLOW'

Sieve opening (μ)	% overtailings at indicated semolina extraction rates							
	50		60		70		80	
	B. lab mill	P. Miag mill	B. lab mill	P. Miag mill	B. lab mill	P. Miag mill	B. lab mill	P. Miag mill
500	6.9	10.5	7.3	11.4	6.8	10.6	6.8	9.7
400	21.1	13.3	18.0	15.6	19.2	15.0	15.9	9.4
250	45.0	52.3	40.7	54.9	45.2	47.3	40.7	47.1
160	17.3	20.3	21.8	16.8	19.0	17.4	20.9	18.4
Pan	9.7	3.6	12.2	1.3	9.8	9.7	15.7	15.4

Particle size distribution: It can be observed from the data on sieve analysis, presented in Table 5, that semolina obtained by a comparatively simpler process using Polisher-Miag mill were slightly coarser, when compared with those of corresponding extraction rate semolina obtained from the Buehler laboratory *durum* mill.

Cooking quality of 'dough discs': The quality of semolinas, as determined by stamping test on dough discs, was not affected at 16 min cooking time, either by the extraction rates, or by the milling process adopted. However, the semolina obtained from a Buehler laboratory *durum* mill were significantly better in quality with a higher "border cooking time" of 32 min as compared to 24 min for semolinas from polisher-Miag mill (Table 6). Alary *et al.*[11] also studied the cooking quality of *durum* wheat semolina using a visco-elastograph for evaluating its suitability for making the pasta products. The elastic recovery of dough discs, as measured in the visco-elastograph,

TABLE 6. COOKING QUALITY OF 'DOUGH DISCS' AND NOODLES FROM HIGH EXTRACTION RATE SEMOLINA

Semolina extraction rate (%)	Border cooking time (min)	Stamping test Elastic Score+	Visco-elastograph test recovery* at diff. cooking time (min) (E_2–E_1)			Score‡	Overall noodle cooking quality Score**‡
			16	24	32		
Buehler laboratory *durum* mill							
50	32	6	72	76	8	8	4.6
60	32	6	71	75	39	10	4.6
70	32	6	74	77	10	10	4.5
80	32	6	74	72	12	10	4.5
Polisher and Miag mill							
50	24	4	69	69	9	8	4.4
60	24	4	66	69	12	10	4.4
70	24	4	71	68	9	8	4.3
80	24	4	70	58	9	8	4.3

*Values×0.01 mm

**Average score based on colour, surface, form, stickiness and bite consistency.

+No significant difference among extraction rates but the difference was significant between milling methods ($P<0.05$).

‡No significant difference between milling methods or among extraction rates.

Maximum score: for stamping test=8; Visco-elastic test=10; for overall quality=5.

was comparable with scores of 8 and 10 for semolinas of 50 and 60 per cent extraction rates respectively from the two milling processes. On the other hand, for the same milling process used, extraction rate of 70 or 80 did not affect the score of elastic recovery; semolina from a Buehler laboratory *durum* mill had a better score of 10 as against 8 for semolina from a Polisher-Miag mill. However, the difference in score was not statistically significant. In general, the increase in extraction rate of semolina had very little effect on the elastic recovery of the dough discs (Table 6).

Cooking quality of noodles: The cooking test indicated that irrespective of the milling process adopted, the clear, yellow colour of noodles from semolina of 50 and 60 per cent extraction rates changed to light brown, in case of semolina of 70 and 80 per cent extraction rates. Further, surface characteristics like smooth, nonporous, and bright appearance of noodles from semolina processed in a Buehler laboratory *durum* mill were only slightly better than semolina processed in a Polisher-Miag mill. It is interesting to note that, irrespective of the extraction rate of semolina or the milling process used, the form, as indicated by fall of noodles (Table 3) was excellent and discrete, and the noodles were non-sticky, smooth, further the consistency of bite was firm, smooth, non-doughy and non-sticky. The cooking quality scores obtained for noodles from semolina of different extraction rates were only slightly higher i.e., 4.5-4.6 when processed in a Buehler laboratory *durum* mill as compared to those from 4.3 to 4.4 when processed in a Polisher-Miag mill (Table 6). However, the differences were not statistically significant. Dexter and Matsuo[12] have reported that cooking quality of sphaghetti from semolina of extraction rates in the range of 58 to 76 did not change, except for colour, which became brown and dull, from yellowish or yellow, as the extraction rate of semolina increased. Menger and Zwingelberg[3] have also observed an insignificant effect of extraction rate of upto 80 per cent on the noodle making quality of semolina. Hence, it can be inferred that the noodle making quality of semolina obtained by the two milling processes compared favourably. Further, the extraction rate of semolina had little effect on its noodle making quality.

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Studies on Puffing of Bengal Gram. I

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Processing conditions for puffing one commercial variety of Bengal gram have been standardised. Process parameters in successive steps appear to be rather specific to get maximum puffing, and are as follow: (1) an initial moisture of around 7.8%; (2) preliminary roasting of grains with sand at 170°C for 75 sec; (3) tempering the grains for about 90 min to reach a moisture of about 4.9%; (4) dipping in water for 5 sec to absorb 0.8% additional moisture; (5) final puffing of grains by roasting at 230°C for 30 sec; (6) impact between a roller and a hot plate for dehusking and splitting. Under these conditions, the bulk volume of grains gets doubled by puffing.

Puffing of Bengal gram (*Cicer arietinum*) is extensively practised in India particularly in the South. Puffed gram mixed with oilseeds or popped cereals, and spiced or sweetened are popular snack foods. Puffing improves the flavour, modifies texture and helps in dry or wet grinding of Bengal gram[1].

Traditionally, puffing and toasting of Bengal gram is carried out in houses or by village-level processors. In these methods, the sun-dried gram is toasted with sand in open pans and tempered. The grains are then dipped in water, toasted again with sand at higher temperatures (around 200°C) and beaten in cloth bags when most of the grains puff and dehusk, some of them split. Recently, these operations have been mechanised with the introduction of semi-automatic roasting machines and dehusking rollers.[2] However, the methodology remains largely traditional. The yield of puffed products (whole and split grains) is 62-68 per cent with 5-10 per cent brokens. Unlike in the case of rice where the volume expansion is 8-10 times[3], increase in bulk volume of Bengal gram on puffing is only 1.2 to 1.5 times (20-50 per cent).[1] Moreover, all grains do not expand to the same extent nor all varieties of Bengal gram are used for puffing. The processors show preference to certain varieties of grams grown in certain agro-climatic regions known to give superior products with good aroma[4]. Exploratory studies have shown that moisture conditioning prior to heating helps in good puffing as also some hardening agents like calcium phosphate, calcium caseinate or egg white. Steaming, parboiling or incipient fermentation do not appear to have any beneficial effect on puffing[5].

As the process parameters are vague and empirical, studies were conducted to standardise conditions such as temperature, moisture and duration of operations and the results are reported here.

Materials and Methods

One commercial variety of Bengal gram normally preferred by the processors and readily available in the local market was used. After treatment with insecticides, twenty kg of the grains were cleaned, size-graded and kept in a closed tin for two weeks for moisture equilibration. Required quantities were drawn from time to time for experiments.

The method followed in this region for puffing Bengal gram was adopted as a reference for standardising process conditions and consists of the following operations in sequence:

1. Sun-drying of cleaned, size-graded grains for 8 hr.
2. Preliminary roasting of grain with heated sand at 150-180°C.
3. Bagging and slow aeration for 'sweating' out the moisture and cooling overnight.
4. Dipping grains momentarily (5-10 sec) in water in perforated buckets followed by draining of excess water.
5. Roasting water-treated grains with heated sand at 200-240°C for puffing.
6. Dehusking and splitting in rollers, aspiration of husk and size grading in sieves.

Each sequential step was standardised using the reference method, the grains were then puffed and bulk volume determined using standard 50 ml measuring cylinder. The experiments were done in triplicate.

Effect of moisture content of raw material: Two kilograms of the cleaned size-graded grains (initial moisture being 11.5 per cent) were dried in a through-

flow drier at 50-55°C and lots of 100 g removed from the bulk at different moisture levels (6-11 per cent) as indicated by weight loss. The samples were stored in closed glass jars for equilibration for 2 days and moisture determined by standard method[6]. Twenty grams of the samples were then puffed by the reference method and the bulk volume of the final product determined. Condition at which maximum volume expansion obtained was taken as the optimum one.

Effect of preliminary roasting conditions: Preliminary roasting of 20 g (bulk volume 25 ml) lot of grain was done in a hand operated electrical coffee roaster. The roaster consists of a mild steel drum of 1.5 litre capacity provided with a handle to rotate and electrical heater. The drum and heater are enclosed in a sheet metal cage which can be tilted for charging and discharging material. The grains were heated at sand temperatures varying from 150 to 200°C for time intervals of 30 to 135 sec. The grains were separated from sand by sieving, equilibrated and puffed.

Effect of moisture content of equilibrated grains: In order to determine the influence of moisture reached by grains after tempering, they were stored after preliminary roasting in covered cloth bags for 0 to 4 hr to cool slowly and loose moisture by 'sweating'. The grains were then cooled to room temperature, moisture content determined[6] and puffed.

Effect of water uptake during dipping grains in water: The 'sweated' grains were dipped in water (in wire-mesh baskets) for 0 to 60 sec, excess water drained and removed between folds of filter paper and puffed at (different) absorbed moisture levels.

Effect of temperature and duration of final roasting: Roasting of water-dipped grains was done in the coffee roaster described earlier with sand heated between 200 and 250°C for 20 to 40 sec. After separating the sand by sieving, the bulk volumes of the puffed grains were determined.

Influence of impact on puffing, dehusking and splitting: The effect of impact was assessed by passing the roasted grains through a laboratory model puffing roller coupled with a hot plate (fabricated for the purpose) and in a centrifugal sheller (rice) at different revolutions. Bulk volume of puffed grains/splits from the two machines were determined after aspirating the husk. Bulk volume of dehusked split *dhal* from 20 g grain was also determined for comparison.

Results and Discussion

Increase in bulk volume is of great economic significance in the manufacture and sale of puffed Bengal gram, particularly when/where sale is based on volume measurement. These studies have shown the profound influence of process conditions on the bulk volume of the final product.

TABLE 1. INFLUENCE OF GRAIN MOISTURE ON BULK VOLUME OF PUFFED BENGAL GRAM

Moisture (%)	Bulk vol after puffing (ml)	Increase in bulk vol (%)
11.0	26	4
10.3	28	12
9.2	37	48
7.8	43	72
6.7	40	60
5.9	30	20
5.0	25	Nil

Note: Preliminary roasting at 180°C for 60 sec; tempering time: 90 min, dipping in water: 5 sec and final roasting at 230°C for 28 sec.
Bulk volume of raw grain: 25 ml.

Table 1 shows that an initial moisture of about 7.8 per cent in the raw grain is ideal for obtaining the maximum volume expansion (72 per cent). The moisture content of raw Bengal gram varies with relative humidity of storage atmosphere[7]. Adequate care must therefore be taken to bring the grains to the optimum moisture level of about 7.8 per cent by moistening or drying. Under conditions of preliminary roasting, grains at a moisture content of 7.8 per cent undergo changes necessary for good puffing. Husk of very dry grains usually cracks on roasting.

Effect of temperature and duration of preliminary roasting on the expansion of grains are given in Fig 1.

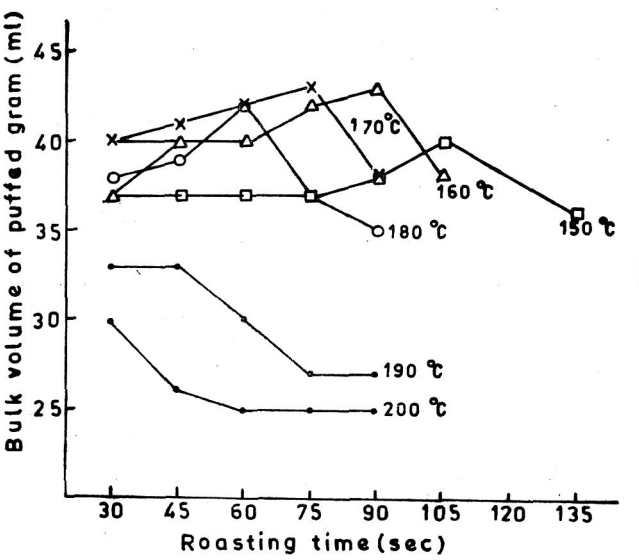

Fig 1. Effect of preliminary roasting treatment on puffing of gram.

Any desired temperature of grain may be reached by roasting the grains at high temperature for short durations or low temperatures for long durations. Among the different temperatures and durations of preliminary roasting tried, raw grains (7.8 per cent moisture) when roasted at 160°C for 90 sec or at 170°C for 75 sec undergo changes that lead to maximum volume expansion on puffing. Under these conditions the grains attain a temperature of 119-122°C.

On slow cooling with gradual aeration (tempering), the heated grains loose moisture. The effect of tempering for varying periods at an ambient relative humidity of 60-65 per cent is given in Table 2. In 30-60 min of tempering, the grains loose the maximum amount of moisture by 'sweating' (final moisture of 4.74 per cent) after which there is a slight increase (4.90 to 4.94 per cent) of moisture, possibly due to absorption of condensed water vapour. Grains with 4.90 per cent moisture content gave the maximum puffing volume. Eventhough the moisture content does not increase further (from 120 to 240 min), there is a gradual decrease in the bulk volume on puffing, indicating an adverse effect of prolonged tempering. When grains are tempered in big gunny bags (a commercial practice) the cooling is more in the peripheral layers than in the interior of the bag. When the grains from the periphery (of bags) are isolated and puffed, the increase in bulk volume was low (less than 20 per cent) compared to those from the interior of the bag: 20-25 per cent unpuffed or partially puffed grains obtained in commercial practices is possibly due to this. Hence the necessity to maintain adequate conditions of moisture equilibration (temperature, moisture and duration) to get good puffing.

TABLE 2. EFFECT OF MOISTURE CONTENT OF EQUILIBRATED GRAINS ON THE BULK VOLUME OF PUFFED BENGAL GRAM

Tempering time (min)	Grain temp. after tempering (°C)	Grain moisture after tempering (%)	Bulk vol after puffing (ml)	Bulk vol increase (%)
0	112	6.85	26	4.0
30	74	4.79	37	48.0
60	55	4.74	42	68.0
90	39	4.90	45	80.0
120	35	4.94	37	48.0
180	29	4.94	36	44.0
240	29	4.94	35	40.0

Note: Initial moisture of grains 7.6%.
Preliminary roasting at 170°C for 75 sec.
Other conditions as in Table 1.

TABLE 3. EFFECT OF WATER UPTAKE DURING DIPPING IN WATER ON BULK VOLUME OF PUFFED BENGAL GRAM

Duration of dipping (sec)	Grain moisture after dipping (%)	Bulk vol after puffing (ml)	Bulk volume increase (%)
0	4.9	39	56
5	5.7	44	76
10	6.0	42	68
15	6.2	40	60
30	6.9	32	28
60	7.3	26	4

Note: 1. Tempering time: 90 min;
2. Other conditions as in Table 2.

The tempered dry grains (moisture 4.9 per cent) can absorb moisture quickly when dipped in water—a property exploited in puffing. Table 3 shows the effect of absorption of varying amounts of water during dipping on volume expansion. When dipping increased from 0 to 60 sec the moisture content of the grains also increased from 4.9 to 7.3 per cent. Maximum bulk volume was observed around a moisture level of 5.7 per cent (an absorption of 0.8 per cent moisture) which is obtained in 5 sec of dipping the grains. In this short time of dipping, moisture absorbed is concentrated in the husk and possibly the peripheral layers of kernel.

Roasting in sand at elevated temperature is the final step in the puffing process. The expansion in bulk volume at different sand temperatures and duration of roasting is given in Fig 2. A good volume expansion (puffing) is obtained at a sand temperature of 230°C and a roasting time of 30 sec. Higher temperatures of sand caused browning and gave unacceptable products, while lower temperatures resulted in low puffing. A quick increase in steam pressure inside the grain, which is necessary to cause maximum volume expansion is obtained by roasting the grains at 230°C for 30 sec. Moreover, browning (or charring) at elevated temperatures of roasting may weaken the seed coat with the result that it cannot hold the steam pressure. Grains with cracked or damaged husk also do not puff due to their inability to hold steam pressure. The puffing expansion is not as much as in cereals possibly due to various factors like starch-protein interaction, quality and quantity of starch, etc.[3,8,9].

The results of studies on the effect of impact on dehusking and splitting using a centrifugal sheller or

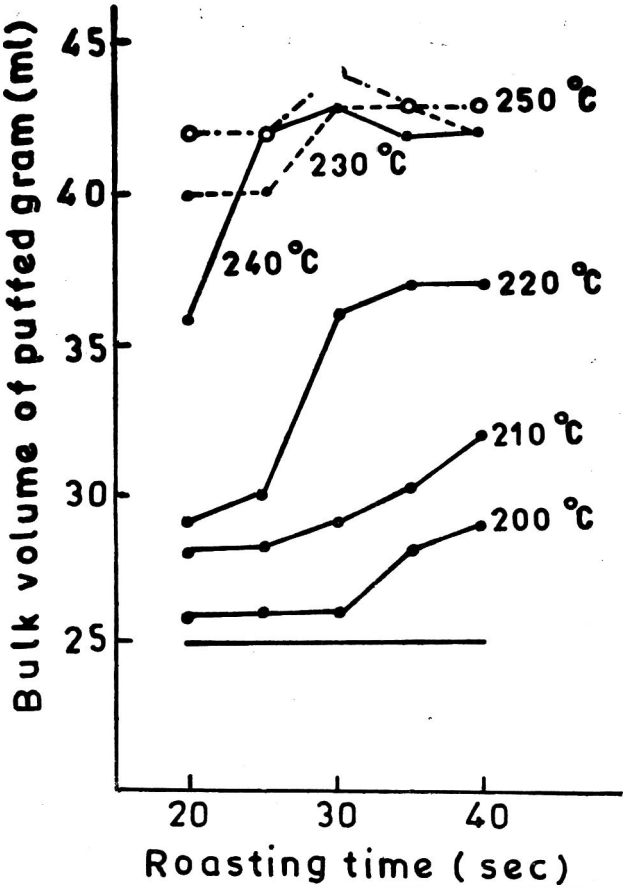

Fig 2. Effect of final roasting treatment on puffing of gram.

a roller are given in Table 4. As the revolutions of the centrifugal sheller were increased from 1,000 to 3,250 per min, there was an increase in the volume expansion of the dehusked and split product reaching a maximum increase of 90 per cent. However, the product was not crisp and lacked in lustre probably due to cooling of grains (in the sheller) compared to the products obtained from roller where cooling is low. A doubling in the bulk volume on puffing could be achieved when the roasted grains were dehusked and split between a hot plate and a roller rotating at peripheral speeds around 300 m/min. In commercial practice, the total bulk volume increase is only 20-50 per cent[1], as process conditions are not strictly maintained.

Although the bulk volume of this variety of Bengal gram could be doubled by adopting the above mentioned conditions, further work is indicated to standardise process conditions for puffing different Bengal gram cultivars. Varietal and agro-climatic factors in addition to chemical and physicochemical properties of the grain, particularly the husk may play a crucial role in puffing of Bengal gram.

TABLE 4. EFFECT OF MECHANICAL IMPACT ON THE BULK VOLUME OF PUFFED BENGAL GRAM

Speed (rpm)	Bulk vol of puffed splits (ml)	Bulk vol increase (%)
Centrifugal throw		
1000	26	30
2000	32	60
2500	33	65
2750	37	85
3000	38	90
3250	38	90
Between knurled roller and hot plate		
700(228)	37	60
800(249)	39	95
900 (283)	40	100
1000(311)	40	100
1100(342)	39	95

Note: 1. Duration of dipping in water: 5 sec. 2. Final roasting at 230°C for 30 sec. 3. All other conditions as in Table 3. 4. Figures in parentheses indicate peripheral speed of roller (m/min) 5. Bulk volume of dehusked splits (*dhal*) from 20 g raw grains was 20 ml.

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Relationship Between Flavour and Oligosaccharide Content of Soybean Varieties

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Organoleptic testing of canned whole soybean indicated that some varieties have more acceptable flavour than others indicating that flavour can be improved. A simple chi-square test did not show any relationship between flavour and oligosaccharide content in soybean, suggesting that oligosaccharide content may not be a suitable criterion for flavour selection in soybean.

Flavour is perhaps the most important factor limiting the use of soybean as a human food especially outside South-East Asia[1,2]. Raw, mature soybean have a "bitter", "beany" and "astringent" taste, unacceptable to many people not accustomed to eating soybean.

The Protein Advisory Group (PAG) of FAO[3] urged plant breeders to improve the quality of legumes, including soybean, for human consumption with major emphasis on flavour, cookability and digestibility. Breeding and selection was seen as one of the most important methods of solving many of the problems associated with flavour. The success of this approach has been limited by lack of genetic variability and objective techniques for evaluating flavour.

The objective of this study was to find out if soybean varieties differ in flavour and whether an objective method of differentiating soybean lines in terms of flavour could be found. The relationship between organoleptic score (flavour) and the oligosaccharides, sucrose, raffinose and stachyose was investigated.

Materials and Methods

Soybean lines from the cross 'Big Jule' × 'Bethel', (designated 'Jeth'), together with some selected germplasm soybean varieties were grown in replicated field plots at Hawkesbury College of Advanced Education, Richmond, Australia. At maturity, each plot was harvested separately and each seed lot was then subjected to analysis for quality characteristics.

Organoleptic taste testing was conducted in air conditioned cubicles using canned soybeans cooked at 116°C for 30 min. Tasters were asked to classify their numbered samples as to whether they were "beany" or "not beany" in comparison with the reference. Each sample was tasted by at least two tasters. The reference used was a seed lot of the line 'Big Jule', which in earlier tasting by various people was indicated to be "liked" by all who tasted it, and was considered "not beany" in flavour.

Sugar content of the seeds was determined using High Performance Liquid Chromatography (HPLC)[4]. Oligosaccharide determination was done on soymeal samples prepared from dry seeds. The soymeal was defatted using hexane in soxhlet extractor for 8-10 hr. Sugars in samples of defatted meal were extracted with 25 ml distilled water to which 0.2 ml sodium azide was added to inhibit bacterial activity and constantly shaken for one hour in a water bath at 30°C. The suspensions were centrifuged at 2,500 rpm for 5 min. Aliquots of the supernatant were centrifuged at 3,000 rpm through Amicon (Massachusetts, U.S.A.) Centriflo ultrafilters (CF-25), which separate proteins and other macromolecules. The clear filtrates were then analysed using a HPLC unit equipped with a Waters Radial Pak (Dextro-Pak) column with estimation from peak heights. The characteristics of the plastic Dextro-Pak columns for separation of carbohydrate oligomers in HPLC have been described by Cheetham *et al.*[5] A standard solution containing 0.5 per cent sucrose, 0.25 per cent raffinose and 0.25 per cent stachyose was used to estimate the contents of these sugars in the unknown samples.

Results and Discussion

A total of 72 soybean varieties were tested in this study. Table 1 shows the organoleptic rating by taste panel members, and oligosaccharide content of some of the tested soybean varieties. Panel testing could

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TABLE 1. ORGANOLEPTIC SCORE AND OLIGOSACCHARIDE CONTENT IN SOME SOYBEAN LINES

Line	Score	Sucrose (%)	Raffinose (%)	Stachyose (%)	Total oligosaccharides (%)
Jeth 1	+*	6.04	1.16	3.64	10.84
Jeth 2	—	4.87	1.10	3.43	9.40
Jeth 3	+	5.29	0.82	3.27	9.38
Jeth 6	±	4.68	0.97	3.00	8.62
Jeth 7	±	4.67	1.41	3.00	9.08
Jeth 8	—	5.17	1.26	2.93	9.36
Jeth 17	+	4.69	0.83	3.55	9.07
Jeth 18	±	5.83	1.42	3.34	9.69
Jeth 19	—	5.07	1.07	3.34	9.48
Jeth 24	—	4.83	1.19	3.66	9.68
Jeth 117	+	5.63	1.39	3.74	10.76
Big Jule (reference)	—	5.05	1.16	3.50	9.71
Bethel	+	4.44	1.10	3.51	9.05
China 409	—	5.77	1.02	3.05	9.82
Sen Nari	+	6.81	0.91	3.54	11.81
Tsuronoka	+	6.37	1.03	3.05	10.45
Chi-square test		2.03	1.71	3.83	1.33

*Organoleptic score + = Beany — = Not beany; ± =Conflicting opinion

To be significant at $P \leq 0.05$ the $x^2_{(1)}$ value has to be ≥ 3.84.

identify soybean lines which were either "beany" or "not beany" in flavour compared to reference samples. The table also shows conflicting opinions among tasters on the flavour of some soybean lines which indicates the problems to be expected when using taste testers in evaluation of soybeans for human consumption.

The results of this study indicate that differences among soybean varieties in flavour acceptability do exist and could be detected by a taste panel, suggesting that a taste panel could be used in selection for improved flavour. However, the use of a taste panel is likely to be costly, and problems are likely to arise when large numbers of lines or varieties are to be evaluated for flavour. Availability of an objective method for evaluating flavour could overcome these problems.

A correlation study between organoleptic score and oligosaccharide content (sucrose, raffinose, stachyose and total sugar content) using chi-square test did not reveal a strong relationship between flavour and oligosaccharide content suggesting that oligosaccharide content cannot be used as a reliable indirect selection criterion for flavour. Nevertheless the success achieved in tomato[6-8] gives hope that it might also be possible in soybean to find a correlation between flavour and the content of a particular compound that could be fruitfully exploited in selection programmes for improved flavour. This calls for more eleaborate research, the success of which will greatly help improve human acceptability of soybeans as a good source of proteins.

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Frozen Storage of Some Indian Green Vegetables

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The quality of some Indian green vegetables like okra, (*Abelmoschus esculentus*), capsicum, (*Capsicum annum* var *grossa*) gherkin (*Coccinia cordifolia*), fenugreek (*Trigonella foenumgraecum*) leaves and coriander (*Coriandrum sativum*) leaves was studied under frozen condition. Effect of blanching and freezing as well as frozen storage at -18°C was evaluated in terms of chlorophyll and ascorbic acid retention, microbiological quality and sensory properties. Quality of frozen vegetables remained excellent during the entire period of storage for three and half months.

The frozen food industry in India is still in its infancy. Most of the cold storages in India are used for potatoes while the rest are used for fish and dairy products. Only a small capacity is used for fruits and vegetables[1]. However, the production of frozen foods has shown an increase in the last decade mainly due to export. In 1979-80, India exported 40,000 tonnes of frozen vegetables worth Rs. 18 crores. The bulk of it consisted of okra and green beans[2]. In view of this trend, the quality of some common vegetables during frozen storage was evaluated.

Materials and Methods

Okra (*Abelmoschus esculentus*), gherkin (*Coccinia cordifolia*), green peppers or giant capsicum (*Capsicum annum* var. *grossa*), coriander (*Coriandrum sativum*) leaves and fenugreek (*Trigonella foenumgraecum*) leaves were obtained from the local market.

The vegetables were sorted and washed under running water. They were blanched in boiling water: okra for 2 min, gherkin for 2.5 min, capsicum for 1.5 min, and fenugreek leaves for 20 sec. Coriander leaves were not blanched. Then the vegetables were cooled by immersing in cold water for 5 min. Adequacy of blanching was determined by peroxidase test[3]. Vegetables (100 g) were packed in polyethylene bags, and frozen by immersing in an alcohol bath kept at -30°C, and stored at -18±2°C for 105 days.

Quality was evaluated by estimating chlorophyll[4,5] and ascorbic acid[6], by microbiological examination (total plate count and *E. coli*)[7,8] and by sensory evaluation. An expert panel of eight judges who used colour, flavour and texture as basis for evaluation on a scale of 1 to 7 indicating bad to excellent quality respectively.

Results and Discussion

The changes in chlorophyll and ascorbic acid content during blanching, freezing and frozen storage are given in Tables 1 and 2. Loss of chlorophyll during blanching ranged from 3 to 7 per cent which is in

Table 1. Chlorophyll (mg/100g) and reduced ascorbic acid (mg/100g) content of vegetables

Vegetable	Before blanching		After blanching and freezing		% retention		Duration of blanching (sec)
	Chlorophyll	Ascorbic acid	Chlorophyll	Ascorbic acid	Chlorophyll	Ascorbic acid	
Okra	8.5	32.5	8.0	30.2	94	93	120
Gherkin	13.4	15	12.5	13.8	93	92	150
Capsicum	22.5	140	20.4	132	94	94	90
Fenugreek leaves	32.0	59	31.0	57.4	97	96	20
Coriander leaves*	38.0	127	—	—	—	—	—

*No blanching was done

TABLE 2. CHLOROPHYLL AND REDUCED ASCORBIC ACID RETENTION (%) OF DIFFERENT VEGETABLES DURING FROZEN STORAGE

Storage period at −18°C (days)	Okra		Gherkin		Capsicum		Coriander leaves		Fenugreek leaves	
	Chlorophyll	Ascorbic acid	Chlorophyll	Ascorbic acid	Chlorophyll	Asocrbic acid	Chlorophyll	Ascorbic acid	Chlorophyll	Ascorbic acid
5	99	99	99	99	98	99	98	98	99	98
15	97	97	97	96	96	97	95	95	97	96
30	94.5	95	94	94	93	95	92	91	95	93.5
45	92	93	91	92	90	92	88	85	93	91
60	89	91	87	90	87	88	81	81	90	88
75	86	87	81	87	83.5	85	74	75	87	85
90	82	85	76	83	78	82	68	69	83	82
105	77.6	81	71	80	72.5	78	63	60	79.5	79

good agreement with the values (5-7 per cent) reported by Brijesh Narain[5] and Talburt *et al.*[9] About 17-24 per cent of the chlorophyll is lost after 3 months of frozen storage. Relatively, the loss was higher in coriander leaves; and this was higher than the values (10 per cent) reported earlier[10].

The loss of ascorbic acid during blanching was about 4-8 per cent which is similar to the values reported by Lee *et al.*[11] In the present study about 63-81 per cent of the initial level of ascorbic acid is retained as compared to 80 per cent retention in frozen okra reported by Dietrich *et al.*[12] and Awoih *et al.*[13]

The microbial load in raw vegetables is mainly dependent on the conditions prevalent during harvesting and transportation. The microbial counts for different raw green vegetables given in Table 3 are comparable to the counts reported previously.[14]

TABLE 3. TOTAL PLATE COUNT AND *E. coli* COUNTS OF DIFFERENT VEGETABLES

Vegetables	Total plate counts (No/g)			*E. coli* count
	Fresh $\times 10^5$	Washed $\times 10^4$	Blanched $\times 10^3$	(No/g) of fresh vegetables
Okra	8.4	2.4	2.4	4.4×10^4
Capsicum	1.0	3.0	1.1	1.2×10^3
Fenugreek leaves	69	19	2.0	3.8×10^5
Gherkin	3.6	1.5	1.8	3.8×10^3
Coriander leaves	90	32	3.0	6.2×10^5

Although the initial counts were high, there was a rapid drop in the microbial count after blanching and during frozen storage (Table 4). *E. coli* was absent

TABLE 4. TOTAL PLATE COUNT AND *E. coli* COUNTS DURING FROZEN STORAGE

	Days of storage						
	5		30		60	90	105
	TPC	*E. coli*	TPC	*E. coli*	TPC	TPC	TPC
Okra	2×10^3	6	860	2	720	720	720
Gherkin	1.7×10^3	6	700	0	550	550	550
Capsicum	1.1×10^3	4	900	1	750	700	700
Fenugreek leaves	2×10^3	8	1×10^3	2	730	730	730
Coriander leaves	3.8×10^4	25	1.48×10^4	8	4.6×10^3	2.8×10^3	2.8×10^3

E. coli count was nil after 60 days.

after two months of frozen storage. Lee *et al.*[15] have also shown that both blanching and frozen storage reduce the microbial counts.

During the entire period of storage (105 days) the frozen vegetables were acceptable to the taste panel members in respect of colour, flavour and texture. In coriander leaves, the colour loss which was appreciable, could be detected after one month storage at -18°C.

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Sources of Fungal Contamination of Bread

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An intensive search was made in three small bakeries for the possible sources of contamination. In all the three cases, it was found that the flour, vegetable fat and sugar, were the primary sources of contamination as the fungi present in these were not totally eliminated in the baking process and no mould inhibitors were used in any of the bakeries. Various objects in the bakery environment served as secondary sources, but the major one was air, and most of the secondary contamination occurred during the brief period when fresh loaves from the oven were left to cool in the open.

During an earlier study[1] it had been found that market samples of bread were usually contaminated with storage fungi both on the surface and the interior. Frequently, *Aspergillus flavus* was the main component of the mycoflora. In view of the reports of possible aflatoxin production in bakery products, it was thought worthwhile to investigate whether the inoculum came from the raw material itself, or from the atmosphere subsequent to baking. The investigation was therefore made by following certain lots of bread through different stages of processing, starting from raw materials up to marketing, in three different bakeries within a radius of 30 km from Madras.

Materials and Methods

Source of samples: The three bakeries were selected on the basis of heaviest contamination in bread, following a preliminary mycological analysis of several market samples. These were small cottage industries, and all operations were done manually. The method of breadmaking was similar in all cases: the flour was kneaded with water, sugar and vegetable fat in a ratio

of 10:5:2:2 and a small amount of yeast, left to ferment for 3 to 4 hr, distributed in tin boxes covered with loose-fitting lids and baked in an oven preheated by burning firewood and charcoal. After 40 to 60 min of baking at about 140°C, the bread boxes were taken out and the bread inverted on to jute sacking (gunny bag), transferred to metal trays and allowed to cool in the open, before being despatched to the shops or sold at the bakery's sales centre. Slicing and packing were done at the time of selling, using a breadknife and old newspaper respectively. No mould inhibitors were used in the bread making process.

Flour, vegetable fat, sugar and water used for bread making were collected at random in sterile sampling tubes from 10 different regions of the stock, and mixed together. The dough samples were taken from different regions of the lump using a cork borer, and placed in polythene bags after verifying the sterility of the latter. Bread for sterile sampling and storage was collected directly from the oven into sterile metal cans or sterile paper bags. The loaves cooled or stored in the bakery were collected in new polythene bags. For sampling environmental sources, surfaces were rubbed with sterile cotton swabs which were then placed in sterile test-tubes. Air mycoflora was quantitatively analysed using an Airborne Bacteria Sampler (MK II: Casella & Co. Ltd, London). Samples of raw materials, dough and the cotton swab smears were placed in an ice box until they were brought to the laboratory for processing.

Processing of the samples: All the samples were processed immediately after being brought to the laboratory. The moisture content of the samples was determined by oven drying. A high-osmotic selective medium, namely Czapek-Dox agar with 50 per cent sucrose w/v was used for plating, as we were investigating only the osmophilic fungi. Petri dishes of 10 cm dia. were used throughout. Direct plating as well as dilution plating methods were used wherever feasible i. e., for flour, dough and bread. In other cases only dilution plating was done. The dough was homogenized in an electrical blender, and the vegetable fat emulsified with a 1.2 per cent solution of sodium citrate, before making dilutions. Cotton swabs were streaked directly on the plates.

Eight replicates were maintained for each sample. The plates were incubated at 30°C and examined from the third day. In the case of direct plating the number of inocula giving off fungi were recorded, and the percentage of infestation was calculated from this, based on the total number plated. In the case of dilution plating, fungal infestation was expressed in most cases as number of propagules (/g) and as number per ml, per cm^2 and per m^3 in the case of water, paper and air respectively. The *Aspergilli* were isolated in slants of normal Czapek-Dox agar (3 per cent sucrose) and identified after Raper and Fennell[2].

Results

Fungal infestation in bread at different stages of processing: The raw materials from all the three bakeries were heavily infested with storage fungi. The number of colonies ranged from 3300 to 4200/g in flour, 1400 to 4700/g in vegetable fat and 1500 to 2000/g in sugar. Water samples had a low level of contamination, from 10 to 20 colonies/ml. *Aspergillus candidus*, *A. amstelodami* and *A. flavus*; *A. versicolor* and *Paecilomyces variotii*; and *A. flavus*, *A. amstelodami* and *Penicillium* sp. in that order were the most common species in flour, vegetable fat and sugar respectively.

Among dough samples, fresh dough had an infestation ranging from 62.5 to 72.5 per cent with 5600 to 9200 propagules(/g). After rising of the dough there was a fall in numbers to 3900 to 5700/g. The qualitative pattern was somewhat similar to that of the flour.

The contamination was very much reduced during baking, but not totally eliminated. Analysis of bread aseptically collected from the oven revealed 8.5 per cent contamination, with 200 to 300 propagules/g. Qualitatively, however, *Mucor* was the most common, followed by *Aspergillus amstelodami*, *A. niger* and rarely others, in low numbers. After 2 days of storage in a sterile can, the percentage of contamination rose to 15 to 21.5 per cent, with 200 to 500 propagules/g. There was visible mycelial growth after 4 days of storage and heavy sporulation after 6 days, of *A. amstelodami*, *A. flavus*, *A. niger*, *Penicillium* and *Mucor*. Hence the bread could not be processed. This excessive fungal growth was attributed to the retention of moisture in the metal can; so the experiment was later repeated in one bakery alone, using sterile kraft paper bags for aseptic storage. In this case fungal numbers rose to 960 and 3950/g after 4 and 6 days respectively. There was only a slight decrease in the moisture content.

When the bread loaves taken out of the oven were left to cool in the open, there was a considerable increase in the level of contamination from 8.5 to 42 per cent after just one hr, the number of propagules ranging from 1100 to 1300/g. These included a wide range of species, the most common being *A. amstelodami*, *A. flavus*, *A. versicolor*, *Paecilomyces variotii* and *Mucor* sp. A sample of the cooled bread sliced at the sales section of the bakery showed an even higher level of contamination of 50 per cent, with 1700 to 2400 fungi/g. The bread loaves stored in the shop for 2 to 6 days registered a slight increase in contamination from 42 to 47 per cent and 55 per cent after 2 and 4 days respectively, beyond which there was a levelling off. The corresponding fungal numbers were 1200 to

TABLE 1. OCCURRENCE OF FUNGI DURING PROCESSING AND STORAGE OF BREAD

Type of sample	No. of colonies/g			
	Mean	Surface (S)	Interior (I)	Ratio (S/I)
Flour	3,830	—	—	—
Vegetable fat	2,570	—	—	—
Sugar	1,830	—	—	—
Water	20	—	—	—
Fresh dough	7,570	—	—	—
Risen dough	5,070	—	—	—
Baked bread	230	300	150	2:1
Bread stored (2 days) in sterile paper bag/metal can	530	810	560	3:2
Bread stored (4 days) in sterile paper bag	960	1,140	790	3:2
Bread stored (6 days) in sterile paper bag	3,950	3,660	4,250	5:6
Cooled (1 hr) bread	1,230	1,970	660	3:1
Cooled sliced bread	2,000	2,590	1,660	5:3
Bread stored (2 days) in shop	1,370	2,210	650	4:1
Bread stored (4 days) in shop	1,570	2,660	670	4:1
Bread stored (6 days) in shop	1,430	2,610	590	4:1

Not done

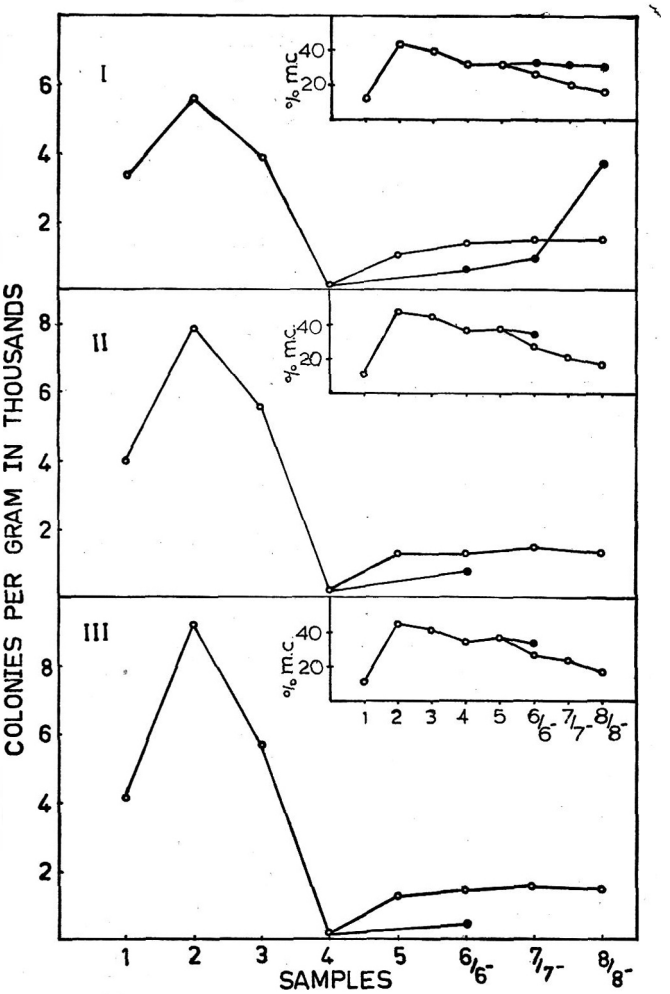

Fig 1. Progress of mycoflora at different stages of bread making and storage, as correlated with the moisture content (mc) of the samples in the three bakeries designated as I, II and III: 1, flour, 2, fresh dough, 3, risen dough, 4, bread immediately after baking (collected aseptically), 5, bread cooled for an hour in the bakery, 6 to 8, bread stored in the open for 2, 4 and 6 days, 6' to 8', beread stored aseptically in paper bag for 2, 4 and 6 days.

O——O, Normal (open) storage; ●——●, Aseptic storage.

1500/g, and 1500 to 1700/g, after 2 and 4 days respectively. There was a continuous decrease in moisture content, the average being 27, 22.5 and 17.5 per cent at 2, 4 and 6 days respectively.

The quantitative pattern of fungi in the different samples is presented in Table 1. In bread, the numbers were usually higher at the surface than in the interior, but the difference was more pronounced in bread left in the open. Only in sliced bread and in bread aseptically stored for 6 days, the numbers were high in the interior also. The progress of mycoflora at different stages of bread making and during open and sterile storage of bread, and its correlation with moisture content, are given in Fig 1.

All the suspected sources tested in the environment of the bakery or shop, yielded storage fungi in varying degrees. Among surfaces, the gunny bags used for collecting the loaves from the oven showed the maximum contamination. Newspaper used for wrapping had as many as 130 to 190 colonies/cm^2. The air within the bakery was more heavily laden with storage fungi than the air outside, while that in the shop had the highest numbers (Table 2).

Qualitative pattern: The species of osmophilic fungi recorded in the three bakeries, in the order of their maximum occurrence were, *Aspergillus amstelodami*, *A. flavus*, *A. versicolor*, *A. niger*, *A. candidus*, *A. chevalieri*, *A. nidulans*, *A. terreus*, *A. fumigatus*, *A. effusus*, *A. sydowi*, *A. repens*, *A. caesiellus*, *A. ochraceus*, *A. wentii*, *Mucor* sp., *Paecilomyces variotii* and *Penicillium* sp. Of these, *A. amstelodami*, *A. flavus*, *A. niger* and *Mucor* sp. occurred in nearly all samples and places including

TABLE 2. STORAGE FUNGI IN THE UTENSILS AND IN THE ENVIRONMENT OF BAKERIES

Bakery	Av. no. of colonies/plate						No/cm²	No/m³		
	Table (T)	Container (C)	Gunny bag (Gb)	Cooling tray	Packing case (Pc)	Knife	News paper wrapper	Air sample		
								Inside bakery	Outside bakery	Inside shop
I	6–10	6–10	11–15	6–10	6–10	6–10	130	1000	700	1600
II	6–10	6–10	11–15	0–5	6–10	6–10	150	1000	900	1200
III	6–10	6–10	6–10	0–5	6–10	0–5	190	1200	1000	1300

T: Kneading table, C: Baking container, Gb: Gunny bag used for collection of bread from oven, Pc: Packing case used for transport

raw materials, bread and the environment. Conversely, some samples such as bread loaves stored in the open and the air within the bakery, contained nearly all the fungi listed. The species developing in aseptically stored bread, on the other hand, were limited in number, and all of them were present in one or more of the raw materials. *A. amstelodami* was the most ubiquitous species in all cases.

Discussion

The results of the present study emphasise the ubiquitous nature of storage fungi. In relation to the contamination of bread, however, it may be said that the raw materials, mainly flour, sugar and vegetable fat in that order are the primary sources of contamination, since the fungi present in these are not totally eliminated in baking. The survival of some species-namely *A. amstelodami*, *A. flavus*, *Mucor* sp. and sometimes *A. niger* and *Paecilomyces variotii*-either indicates their high thermo-resistance, or their survival in minute dry pockets within the dough, where the heat may not penetrate. When stored at high moisture these fungi quickly proliferate and cover the entire loaf within 5 or 6 days.

The main source of secondary contamination is air. While the inoculum from the air comes mainly during open storage, most of it has already settled on the loaves in the short span of an hour required for the bread to cool to room temperature (Table 1). This is natural, as there would be continuous air currents around a hot object while it is cooling. Once it has cooled to room temperature, any further inoculum has to come only through passive settling.

Among other environmental sources, the gunny bag on which the hot loaves are collected is the most likely source of contamination, not only because of its high fungal content but also because of the susceptible state of the loaf. Additional handling in the shop such as slicing and wrapping are likely to add further to the contamination, and it would be wiser for the customers to avoid these.

Although earlier authors suggested that the flour was the main source of mouldiness in bread[3-6], the present trend of thought in the West is that the fungi do not survive the baking process but that the contamination comes after the bread leaves the oven, mainly from the bakery air and the slicers[7,8]. Our studies show that the fungi are not eliminated in baking. Considering the common household practice of storing bread in closed containers at room temperature, and in view of reports of aflatoxin production in bakery products[9], it is imperative that some control measures are enforced. The incorporation of preservatives such as calcium propionate or dimethyl fumarate[10], and setting apart a clean, dust-free area for cooling the loaves might help greatly in reducing fungal contamination. Above all, however, the use of good quality raw materials should go a long way in minimising mould growth in bread, as it is the internal fungi derived from raw materials that would grow more rapidly under favourable conditions than the surface fungi that are mainly derived from the air.

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Growth of *Staphylococcus aureus* in *Khoa*

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The growth of *Staphylococcus aureus* inoculated into *khoa* (an Indian milk product) prepared with 3 levels of moisture (26-28, 38-42 and 45-48%) and stored at room temperature (25-35°C) and under refrigeration (4-5°C) for 2 days was studied. Strains of *S. aureus* inoculated at 1×10^3 CFU/g of *khoa*, grew well reaching 10^{12} CFU/g in 48 hr at room temperature, while the strains failed to grow in *khoa* under refrigeration. The moisture levels did not show any significant effect on staphylococcal growth. Microtome sections of *khoa* revealed the occurrence of staphylococcal clusters which gradually increased in size as the storage period progressed from 24 to 72 hr ultimately resulting in large clumps of cells. Strains of *S. aureus* grown in brain heart infusion and nutrient broths added with autoclaved solutions of casein and lactose showed the presence of staphylococcal clusters each formed of a large number of cells, similar to the one observed in microtome sections of *khoa*.

Khoa, a heat concentrated milk product having a moisture level of 26-28 per cent, is widely used in the preparation of Indian milk sweets. This is often subject to post-processing contamination by *staphylococci*, due to poor hygienic conditions during its manufacture and handling. In most parts of India, *khoa* is usually prepared in rural areas which is then stored for sometime and subsequently transported over long distances to consuming centres without proper cooling facilities. Generally, the time taken between manufacture and consumption varies from 24 to 48 hr. During this period, there can occur a rapid proliferation of cells and production of enterotoxins. *Khoa* has been shown by Bhat *et al.*[1] as a good medium for growth of a variety of microorganisms, growth and viability of the microorganisms being greater in moist samples than in dry samples.

In this study, the growth pattern of *S. aureus* inoculated into *khoa* prepared with 3 levels of moisture and stored under simulated market conditions was evaluated. The appearance of staphylococcal cells in *khoa* was studied through microtome sectioning of *khoa*. In addition, the probable effect of some intermediate substances formed as a result of interaction between casein and lactose during heat processing of *khoa* on growth pattern of *S. aureus* was also evaluated.

Materials and Methods

Test cultures: The test cultures included two wild strains of *S. aureus* (K-283 and K-192) isolated from market *khoa* producing single enterotoxins (SE)-SEA and SEE, respectively and one standard strain (A_{100}) producing SEA, obtained from Dr. M. S. Bergdoll, Food Research Institute, Madison, Wisconsin. These cultures were maintained on yeast extract glucose agar slants and sub-cultured once in a week.

Autoclaved solution of casein and lactose: This was prepared by dissolving 26.5 g of casein in 1N NaOH and the volume made upto 100 ml with glass distilled water. To this was added 20 g of lactose and the pH was adjusted to 7.0 with dilute HCl. The final solution was autoclaved at 15 lb pressure for 15 min.

Preparation of khoa: One litre aliquots of good quality raw cow's whole milk were taken and boiled

(98°C) in a open pan ('Karahi') with continuous stirring and concentrated till it reached a pasty consistency. The final product was stored after cooling in previously sterilised glass bottles. The entire process was carried out by avoiding any contamination. For converting one litre aliquot of milk into *khoa*, the time taken was 15 min. However, during processing, the temperature varied between 75 and 95°C.

Post-processing contamination of khoa: *Khoa* samples were prepared with 26-28 per cent (low), 38-42 per cent (medium) and 45-48 per cent (high) levels of moisture simulating moisture levels of market samples. Moisture levels of 38-42 and 45-48 per cent were adjusted by addition of sterile water. Aliquots of 100 g were inoculated individually with the test strains at levels of 1×10^3 colony forming units (CFU)/g of *khoa*. The suspension of test strains in 1 ml of saline was thoroughly and uniformly mixed with the sample in a previously sterilised mortar with the help of a spatula. Addition of 1 ml inoculum resulted in an increase of 0.1 per cent in the moisture level. Samples were then stored in sterile 250 ml wide mouthed glass stoppered bottles (Corning) at room temperature (RT) of 25-35°C and refrigeration temperature (RFGT) of 4-5°C for 2 days. Samples were analysed for staphylococcal populations at 24 and 48 hr of storage. *Khoa* samples before inoculation with *S. aureus* were analysed for general bacterial counts by pour plate method using tryptone yeast extract dextrose agar to find out the presence of any contaminants. Uninoculated *khoa* samples served as controls

Microtome sectioning of khoa: Microtome sections of 5 μ each of freshly inoculated *khoa* (moisture level 26-28 per cent) samples with the test strain K-283 and the same stored at RT for 24, 48 and 72 hr were taken by pre-fixing the sample in a solution of formaldehyde: ethanol:acetic acid (5:15:1) for 2 hr followed by washing in water for 2 hr and finally immersing in paraffin blocks. Sections were stained with Newman's stain and examined microscopically under oil immersion lens of light microscope and photomicrographs of the same were taken.

Growth in broths added with autoclaved solution of casein and lactose: Twenty five ml each of brain heart infusion (BHI) and nutrient broths and the same supplemented with 2 ml each of autoclaved solution of casein and lactose were inoculated with the test strain K-283 and incubated for 24 hr at 37°C. Incubated broths were analysed for staphylococcal growth. pH of the broth samples before and after incubation were taken. Smears of the incubated broth samples were examined microscopically and photomicrographs of the same were taken.

Enumeration of Staphylococci: Appropriate dilutions of *khoa* samples in 2 per cent sodium citrate buffer solutions were surface plated on the egg yolk-tellurite-glycine-pyruvate agar (ETGPA) of Baird-Parker[2]. Dilutions were plated in replicates. Staphylococcal colonies formed on the incubated plates were counted and the average of the plates were expressed as staphylococcal CFU/g.

Results and Discussion

Growth of S. aureus *during storage of* khoa: The

TABLE 1. GROWTH OF *S. AUREUS* IN *KHOA* DURING STORAGE

S. aureus	Storage temp. (°C)	Storage period (hr)	*S. aureus* (CFU/g) at indicated moisture levels 26–28%	38–42%	45–48%
K-283	25-35	24	6×10^{10}	8×10^{10}	7×10^{10}
	25-35	48	8×10^{12}	8×10^{12}	5×10^{12}
	4-5	24	4×10^{3}	4×10^{3}	8×10^{4}
	4-5	48	4×10^{4}	8×10^{4}	1×10^{5}
K-192	25-35	24	4×10^{10}	6×10^{10}	6×10^{10}
	25-35	48	5×10^{12}	8×10^{12}	4×10^{12}
	4-5	24	2×10^{3}	5×10^{3}	3×10^{2}
	4-5	48	2×10^{4}	4×10^{4}	2×10^{4}
A_{100}	25-35	24	8×10^{7}	6×10^{8}	4×10^{8}
	25-35	48	6×10^{9}	5×10^{10}	6×10^{10}
	4-5	24	2×10^{3}	2×10^{3}	2×10^{3}
	4-5	48	2×10^{3}	8×10^{3}	4×10^{3}

Inoculum of test strains=1×10^3 CFU/g of *khoa*

results presented in Table 1 reveal that the test strains of *S. aureus* grew well during storage of *khoa* at RT for 24 hr. Both the wild strains showed almost a similar growth pattern. In 24 hr of storage, the staphylococcal populations were 10^{10} CFU/g which increased to 10^{12} CFU/g on subsequent storage for another 24 hr. The growth of wild strains were not affected by the 3 levels of moisture in *khoa*. However, staphylococcal populations reached by *S. aureus* A_{100} in *khoa* were slightly less as compared to the wild strains. There was a slight effect of moisture levels on the growth of this strain, wherein, the staphylococcal populations increased from 10^9 to 10^{10} CFU/g with an increase in the moisture level of *khoa*.

There was no appreciable increase in the staphylococcal population of test strains in *khoa* stored at RFGT for 48 hr (Table 1) except for a count of 1×10^5 CFU/g of wild strain K-283 in *khoa* having 45-48 per cent moisture. Control samples on analysis showed the absence of *Staphylococci*.

The appearance of staphylococcal cells in *khoa* may be seen from the photomicrographs of the microtome sections of *khoa* inoculated with *S. aureus* K-283 and stored for 72 hr at RT. In the freshly inoculated *khoa* a few staphylococcal cells were uniformly distributed. On subsequent storage (24 and 48 hr) there was an increase in the number of staphylococcal cells per cluster (Fig 1 (*a*) and (*b*)) which finally (72 hr) resulted in large sized clusters (Fig 1 (*c*)) resembling some of the algal colonies of chlorophytes. Such large clumps of staphylococcal cells can be tentatively termed as "microcolonies". These clusters were formed in different regions of *khoa* depending upon the availability of moisture.

Bhat *et al.*[1] in their studies have shown that *khoa* serves as an excellent medium for microbial growth. Similarly model experiments with raw and pasteurised milks[3-5] and cheese[6-8] have revealed the growth and production of enterotoxins by *S. aureus*. Invariably, enterotoxin (s) production corresponded with higher cell numbers of *S. aureus*.

The unhygienic practices followed in *khoa* preparation and lack of sanitation offer ample scope for post-processing contamination by *Staphylococci*. Generally the time gap between preparation and consumption of *khoa* is 2-3 days during which time the product is stored with no cooling facilities, thus enabling a rapid staphylococcal growth.

The present study revealed that *S. aureus* occurring as post-processing contaminants in *khoa* grew well and reaching maximum cell populations within 48 hr of storage at normal market conditions. Similarly, Bhat *et al.*[1] observed that growth and viability period of the test organisms introduced into *khoa* were related

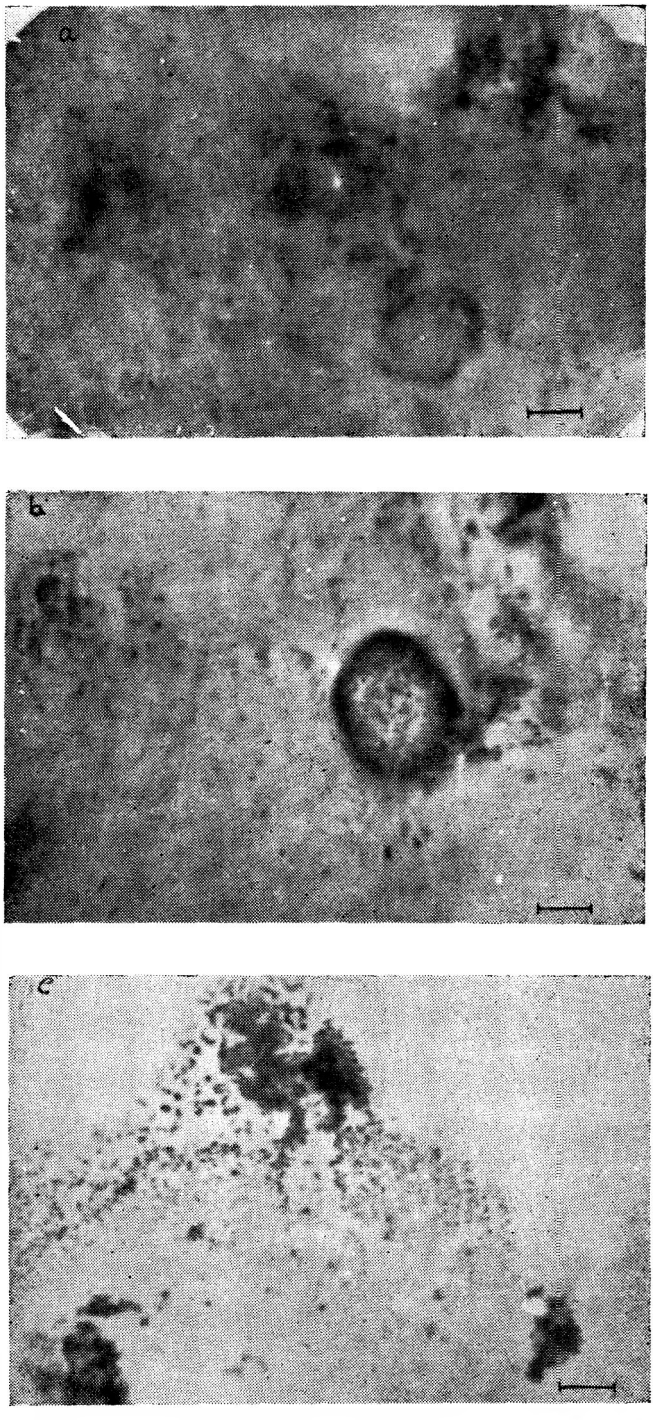

Fig 1. Photomicrographs of Newman's stained 5 μ thick sections each of *khoa* sample inoculated with *S. aureus* K-283 and stored at room temperature (25-35°C). (*a*) 24 hr old, (*b*) 48 hr old, (*c*) 72 hr old. All bars represent 2.7 μ.

to moisture levels. They also found that the growth of organisms were better and their viability period longer in partially sterile samples than in non-sterile samples. *Khoa* used in this study was almost sterile, as the analysis of fresh samples revealed the absence of micro-

organisms. The sterile nature of *khoa* as well as the moisture levels ranging from 26 to 28 per cent have enabled a good growth of staphylococcal organisms.

During processing of *khoa*, the casein gets coagulated and takes up certain amount of moisture, resulting in a micro environment in *khoa* which enables rapid growth and enterotoxin production by *S. aurens*. This can be seen from the microtome sections (Fig 1) wherein the formation of staphylococcal clusters each consisting of a large number of cells in those regions of *khoa* have resulted due to the availability of nutrients aided by moisture. Similar aggregation of cells by starter bacteria, especially by *Streptococcus cremoris* in cheese was observed by Dawson and Feagan[9]; however, the cell numbers were comparatively less. As compared to the texture of cheese which enables a firm embedding of starter bacterial cells, the loose texture of *khoa* would tend towards an easy disintegration of staphylococcal clusters.

Growth of S. aureas K-283 *in broths supplemented with autoclaved solution of casein and lactose:* The results in Table 2 indicate that the growth of *S. aureus* K-283 was better in BHI and nutrient broths supplemented with autoclaved solution of casein and lactose as compared to their growth in unsupplemented broths (control). There was a decrease in the pH levels of both the supplemented broths as well as unsupplemented BHI broth from the initial pH 7.0, whereas in control nutrient broth the pH increased from 7.0 to 7.2.

The growth of the test strain for 24 hr in BHI control broth showed the occurrence of characteristic staphylococcal clusters, while in supplemented BHI broth, the clusters were formed by a comparatively large number of cells. Similarly, when the strain was grown for 24 hr in control nutrient broth, there was the occurrence of small clusters of *Staphylococci* each consisting of 2 to 8 cells per cluster. However, in supplemented nutrient broths the staphylococcal cells were present in large clusters appearing as clumps.

The possibility of certain nutritional factors in *khoa* that would enable a good growth of *Staphylococci* cannot be ruled out, since during preparation there are chances for the formation of certain intermediate compound (s) as a result of interaction between casein and lactose of milk under the influence of heat.

The effect of some nutritional factor (s) of *khoa* on staphylococcal growth can be well evidenced in the studies on the growth of *S. aureus* in BHI and nutrient broths supplemented with autoclaved solution of casein and lactose, wherein there was the occurrence of staphylococcal clusters, each consisting of a large number of cells. These were almost similar to the clusters observed in microtome sections of *khoa*. It appears that this milk product not only provided extra nutrients but also favoured the clumping of staphylococcal cells into large clusters (micro-colonies). Further *khoa* is a semi-solid/solid milk product and bacterial cells multiply by division. It has been observed[10] that there are certain growth promoting factors present in *khoa* enabling an increase in staphylococcal growth and production of thermostable deoxyribonuclease.

The rapid growth of *S. aureus* in *khoa* has to be viewed with serious concern as consumption of such *khoa* results in public health hazards. It is therefore essential for the Public Health Authorities to take necessary steps in strictly enforcing the hygienic concepts which is lacking, so as to avoid staphylococcal contamination at various stages of processing in *khoa* industry and properly organise the processing, storage, handling and transportation of *khoa* to urban centres with cooling facilities and thus ensure a safe milk product for human consumption.

TABLE 2. GROWTH OF *S. AUREUS* K-283 IN BROTHS SUPPLEMENTED WITH AUTOCLAVED SOLUTION OF CASEIN AND LACTOSE (37°C/24 HR.)

Broth medium	Initial pH	pH after incubation	Staphylococcal counts (CFU/ml)
BHI supplemented*	7.0	5.2	4×10^{12}
BHI unsupplemented (control)	7.0	4.6	3×10^{10}
Nutrient supplemented*	7.0	5.8	8×10^{12}
Nutrient unsupplemented (control)	7.0	7.2	7×10^{10}

*25 ml of broth medium + 2 ml of autoclaved solution of casein and lactose.

Inoculum of test strain=1×10^3 CFU/ml.

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Study of the Properties of Frozen Shrimps

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Shrimps were stored under chilled (0°C) and frozen (–18°C) conditions. The properties such as changes in pH, trimethylamine content, extract release volume, swelling and microbial counts were examined. Chilled samples spoiled within a few days. Frozen samples did not spoil even after 6 months. Sensory evaluation of frozen samples showed that though texture deteriorated the appearance and flavour were still very good. Soluble proteins and masked-SH groups showed excellent correlation with texture.

Export of sea food has increased from 52,279 tonnes valued at Rs. 89.51 crores during 1973-74 to 92,671 tonnes valued at Rs. 373.02 crores in 1983-84. Frozen shrimps dominate the export market of seafood, being 58.74 per cent in terms of quantity and 84 per cent in terms of value[1]. Thus, frozen shrimps have an excellent export market especially for the high quality product. The quality depends on several factors like species, size, freezing process and pre-and post-process handling and storage. Some problems encountered in the frozen shrimps are dehydration, toughening, loss of juiciness and drip losses. Shrimps are also subject to microbial spoilage and autolysis. To minimise the problems, prompt cooling to about 0°C and handling under hygienic conditions are required before shrimps are frozen[2]. Some of these aspects especially important for export are discussed by Unnikrishnan[3]. Hebbar and Hiremath[4,5] have studied the freezing of prawns with respect to different quality parameters like drip loss. Drip losses were shown to reduce when additives like polyphosphates, citrate, ascorbate etc. were added[5]. These additives were earlier shown to improve flavour and texture[6] and phosphate was shown to improve bacteriological quality[7]. Quick freezing of packaged shrimp was studied by Albin and others[8]. George[9] showed that shrimps kept frozen for a month and then peeled and deveined before refreezing had poor quality. Seasonal variations were shown to affect the yield of different shrimp varieties[10]. A number of workers[11-15] have also studied cooked frozen shrimps. Cryogenic freezing is becoming an important process for shrimps. Processors are using liquid nitrogen[16] and freon 12[17] to freeze large quantities of shrimps. The present work attempted to study physico-chemical and microbiological changes taking place in shrimps during chilled and frozen storage and their effect on quality.

Materials and Methods

Experimental: Fresh shrimps (*Metapenaeus dobsoni*) were obtained as soon as the fishing vessels unloaded their catch on the docks. They were placed in an ice-box and brought to the laboratory within half an hr. The shrimps were washed, graded, deheaded, deveined. and then packed in plastic bags (net weight 100 g). Freezing was done in an alcohol cooling bath (-35°C) by immersion until the temperature of the centre of the pack reached -20°C. The frozen mass was removed

from the bag and glazed with cold water and stored at -18°C. Some samples were packed fresh in plastic bags after washing, deheading and deveining and stored in the chiller tray of refrigerator at 0°C.

pH measurement: Twenty five grams of peeled shrimps were homogenised with 50 ml water in a blender and the pH was measured using a pH meter.[18]

Trimethylamine (TMA) nitrogen estimation[19]*:* Fifty grams sample of peeled shrimps were minced with 25 g trichloroacetic acid in a porcelain basin. The mixture was allowed to stand for 30 min and filtered under suction using Whatman No. 5 filter paper. TMA-nitrogen of extract was estimated using Conway microdiffusion unit. The bases were titrated against 0.0143N H_2SO_4.

Extract release volume (ERV)[20]*:* Twenty five grams peeled shrimps were homogenised for 2 min in 100ml phthalate buffer (pH 4.5). The homogenate was poured into a funnel with Whatman No. 1 filter paper folded thrice. The extract was collected for 15 min in a 100 ml measuring cylinder and the volume recorded as ERV.

Determination of swelling[20]*:* Twenty five grams of peeled shrimps were added to 100 ml distilled water and homogenised for 2 min. Thirty five grams of homogenate was centrifuged for 15 min at 1500 rpm. The supernatant was collected in a measuring cylinder and volume recorded as S and swelling calculated as per cent SW=400-14.3 S.

Estimation of sulphydryl (-SH) groups[21]: Thirty grams of peeled shrimps were blended with 600 ml 0.5 per cent NaCl containing 0.02M $NaHCO_3$ (pH 7) and 0.02M EDTA. To 3 ml shrimp suspension, 2 ml phosphate buffer (pH 8) and distilled water were added to make the volume to 10 ml. To 3 ml of this, 0.02 ml Ellman reagent (5, 5'-dithiobis-2-nitrobenzoic acid) was added. The lemon yellow colour developed after shaking was measured at 412 nm, after 20 min. The -SH group concentration was calculated in moles/l as $C=(A/E)\times D$ where E=extinction coefficient (13,600/M/cm), D=dilution factor and A=absorbance. This gives the unmasked-SH groups.

Estimation of total -SH groups: To an aliquot of shrimp suspension (0-3 ml) as extracted above by NaCl, 2 ml phosphate buffer and 5 ml 8M urea were added. The volume was made to 10 ml and allowed to stand for 15 min. To 3 ml of this, 0.02 ml of Ellman reagent was added and colour was measured. This gives total-SH groups as calculated above. The masked -SH groups were calculated as the difference between unmasked -SH groups and the total -SH groups estimated after denaturation by urea.

Estimation of soluble protein[22]: Thirty grams of peeled shrimps were extracted with 600 ml 0.5 per cent NaCl solution containing 0.02 M $NaHCO_3$ (pH 7) in a blender for 15 min. The extract was centrifuged. To 1 ml of supernatant, 4 ml Biuret reagent was added and allowed to react for 40 min. Colour was measured at 540 nm. Albumin was used for standard curve.

Microbiological examination: Shrimp samples were examined for total plate counts (TPC), *E. coli* and *Salmonella* counts using standard methods[23].

Sensory evaluation: This was done by a panel of 8 judges. The frozen samples were thawed at room temperature and compared with the fresh samples. A scale of 1-7 from extremely poor to excellent was used for rating the appearance, odour and texture of the shrimp meat.

Results and Discussion

The results on shrimps stored at 0°C and at-18°C are shown in Tables 1 and 2.

At 0°C storage there was a steady rise in pH and after 12 days, it rose to 8.42. Rise in pH indicates bacterial growth[6] and these observations are similar to the report by Fieger and Friloux[24]. Shrimps also developed strong off flavour. The shrimps stored at -18°C showed a decrease in the pH during first 3 weeks. Thereafter there was a slight increase, the final pH reaching 7.4 after 6 months. The initial decrease may have been due to enzymic activity resulting in the production of lactic acid[25].

There was a gradual increase in the TMA value in samples stored at 0°C, reaching a value of 1.4 mg/100g after 12 days. At -18°C however, the increase was marginal. From an initial value of 0.1 mg/100g

Table 1. ANALYSIS OF SHRIMP STORED AT 0°C

Days of storage	pH	TMA-N/ 100 g shrimp	ERV*	% swelling	TPC/g shrimp (×10³)
0	6.86	0.10	48	50	26
2	6.90	0.10	38	68	30
4	7.04	0.30	30	85	39
5	7.10	0.40	29	108	51
6	7.26	0.40	20	160	70
7	7.46	0.60	19	250	76
8	7.74	0.80	18	280	210
10	8.01	1.00	17	306	1000
12	8.42	1.40	16	350	2600
14	8.90	2.10	10	400	6000
15	9.50	2.90	5	480	10000

* Extract release volume

TABLE 2. ANALYSIS OF SHRIMPS STORED AT —18°C

Days of storage	pH	TMA-N/ 100 g shrimp	Extract release vol.	% swelling	TPC/g shrimp
0	6.84	0.100	48	80	26,000
14	6.68	0.100	48	50	6,000
21	6.60	0.100	48	50	850
28	6.60	0.100	48	51	200
35	6.90	0.120	48	51	200
42	7.12	0.120	46	52	200
56	7.14	0.160	46	52	200
70	7.20	0.180	44	52	200
84	7.20	0.180	44	56	200
98	7.20	0.200	44	58	200
112	7.24	0.220	44	59	200
119	7.24	0.220	40	60	200
126	7.26	0.240	38	60	200
150	7.38	0.248	38	61	200
164	7.40	0.250	38	61	200
180	7.40	0.256	38	61	200

in fresh samples, it rose to 0.26 mg/100 g. As the TMA value is also a good indicator of microbial growth, in samples at 0°C there was a good concurrence between the two. However, at -18°C the TMA increases in spite of decrease in microbial count. TMA is formed from the microbial reduction of TMAO[26]. There have been reports that enzymes of certain fish, as well as certain heavy metals can reduce TMAO[27]. This may have increased TMA at -18°C.

There was a substantial decrease in ERV from 48 to 5 after 12 days of storage at 0°C. During the same period swelling increased from 50 to 480 per cent. This compares well with observations of Shelaf and Jay[28]. The increase in the dissociation of the ionisable proteins results in the increase in SW and decrease in ERV[29]. The dissociation will increase repulsion between polypeptide chains causing voids between them which can hold more water. This results in less free water and greater swelling[30] Although dissociation might have been aided by microbial growth[29], increase in pH also might have played a major role in increasing dissociation.

At -18°C, there was a slight decrease in ERV and a slight increase in SW. A slight increase in pH might have caused minor changes. Proteins during frozen storage undergo denaturation,[31-33] which might also contribute to the decrease in ERV and increase in SW.

In the microbiological examination, the total plate count of unwashed whole samples was found to be 64,000 whereas, that of washed whole samples was 38,000 and headless peeled was 26,000. Washed whole shrimps showed a *E. coli* count of 300 but no *Salmonella* was present.

TPC rapidly increased in samples stored at 0°C after 2-4 days in conformity with the report by previous workers[24]. After 12 days, spoilage was evidenced by strong off-flavour. Frozen storage at -18°C, rapidly

TABLE 3. ESTIMATION OF -SH GROUPS AND SOLUBLE PROTEIN

Storage period (days)	Soluble protein (g/100g shrimp)	Total SH groups of shrimp (mg/100g shrimp)	Reactive SH groups of shrimp (mg/100g of shrimp)	Masked SH groups	% soluble protein*	% masked SH groups*
0	10	91.8	3.8	88.0	100	100
18	9.8	90.7	5.6	85.1	98	96.6
30	9.6	89.8	11.0	78.8	96	88.6
60	9.0	88.0	20.9	67.1	90	76.1
74	8.7	90.7	24.5	66.4	87	75.0
90	8.6	91.2	28.4	62.8	86	71.0
104	8.4	91.0	32.0	59.0	84	67.0
120	8.0	90.0	34.0	56.0	80	63.6
134	7.6	90.8	41.0	49.8	76	56.6
150	7.2	90.6	47.6	43.0	72	48.9
164	6.8	90.0	50.2	39.8	68	45.2
180	6.4	89.8	56.7	33.1	64	37.6

*On the basis of initial value.

decreased TPC in 3-4 weeks, reaching a constant value of 200 thereafter.

The changes in soluble proteins and masked -SH groups in shrimps during frozen storage are shown in Table 3. The soluble proteins decreased from 10 to 6 g/100g shrimps. During frozen storage, increase in salt concentration due to decrease in free water, causes denaturation of proteins[34]. This lowers the soluble protein content. Denaturation also effects unfolding of polypeptide chains, so there is a decrease in masked -SH groups. Thus, decrease in masked -SH groups gives an idea of denaturing[35]. Table 3 shows a marked decrease in masked -SH groups during 6 months of frozen storage. Thus the significant decrease in both (Table 3) indicates that there must have been extensive denaturation in proteins of shrimp. Earlier workers used soluble proteins as the indicator of denaturation in prawns and showed that denaturation was more at -12°C than at -18°C during a two year study[32]. Suzuki and Kanna[33] compared krill and shrimp and concluded that during frozen storage at -30 to -40°C proteins in krill underwent denaturation faster than those of shrimps. Khan and Nakamura[36] reported decrease in soluble proteins and masked -SH groups during frozen storage of chicken meat.

The sensory evaluation results of frozen shrimps are shown in Table 4. The appearance and odour were quite satisfactory even after 6 months. However, texture was poor. During the prolonged storage at –18°C it became tough, chewy or rubbery. Texture depends on the extent of denaturation of meat proteins[37]. It was shown above from the soluble proteins and masked -SH groups that there was extensive denaturation of proteins. This must have deteriorated texture. Fig 1 and 2 compare masked -SH groups with soluble proteins and texture score. The

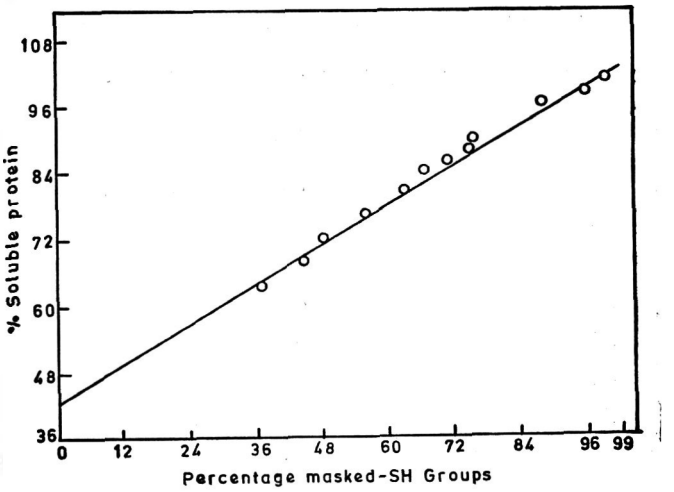

Fig 1. Comparison of masked -SH groups with soluble proteins.

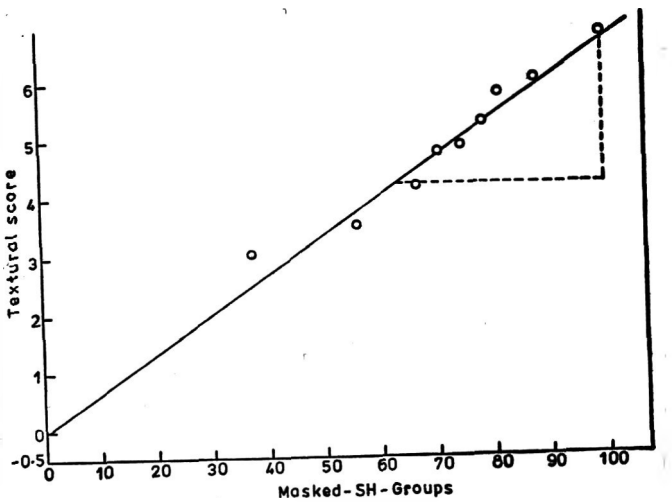

Fig 2. Comparison of masked -SH groups with texture score.

TABLE 4. SENSORY EVALUATION OF FROZEN SHRIMP

Storage at—18°C (days)	Av. scores for indicated parameters		
	Appearance	Odour	Texture
0	7.00	7.00	7.00
30	7.00	7.00	6.00
45	7.00	6.88	5.75
60	6.88	6.88	5.25
75	6.50	6.50	4.88
90	6.50	6.50	4.73
110	6.50	6.25	4.13
140	6.50	6.25	3.50
180	6.25	6.25	3.00

correlation coefficient between masked -SH groups and soluble proteins was found to be 0.99 and the regression equation $Y=42.9+0.5879x$. The coefficient between masked -SH groups and texture was found to be 0.975 and the equation $Y=-0.004+0.06738x$. Thus both have excellent correlation with the masked -SH groups, so the latter can be used to evaluate both soluble proteins and the texture.

It is concluded that during frozen storage of shrimp texture is affected significantly mostly due to protein denaturation. This can be assessed by measuring the soluble proteins or the masked -SH groups. The latter has an excellent correlation with texture and can be used as a rapid method of evaluating textural quality of shrimps.

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Microbiology of Shrimps Handled and Stored in Chilled Sea Water and in Ice

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The microbiological profile of shrimps was followed from the stage of sorting on board the trawler through the stage of freezing after preservation in chilled sea water (CSW) or in ice for 28 hr. Freshly caught shrimps had a predominant Gram positive flora. Preservation of shrimps in CSW for 28 hr encouraged the retention of most of the Gram positive flora present initially, whereas in iced prawns, all the Gram positive flora except *Micrococcus* were completely eliminated. *Pseudomonas* accounted for 67% of the flora in CSW batch and 82% in iced lot. Frozen prawns derived from CSW batch scored better organoleptically as compared with that derived from iced lot.

Shrimps are of great commercial value all over the world and most of the international trade is in the form of frozen products. It is widely recognised that expeditious handling of perishable foods like shrimps is a prime requisite for maintaining quality. In view of this, it is essential that freshly caught shrimps are handled by techniques that ensure maximum preservation of quality. The importance of icing shrimps on board has been demonstrated by Fieger *et al*[1]. The practice of storing shrimp in alternate layers with ice has several disadvantages. Shrimp in lower layers are subjected to crushing and water from upper layers drips down which increases the bacterial load of lower layers[2]. Storage of fish in chilled sea water (CSW) overcomes some of these problems and has the advantage over ice in that the catch is cooled more rapidly and saves labour and effort in stowing and unloading and avoids crushing[3]. Though there are a number of reports to indicate that the storage life of fish in CSW would be longer than that in ice, the difference was considerable in several cases[3,4] whereas, it was only marginal in others[5]. Shewan[6] considered the various reasons for the differential shelf-life of fish stored in ice and in CSW and surmised that a factor (or factors) other than mere bacterial numbers were involved. He suggested that the types of bacteria surviving, growing and operating under semi-anaerobic conditions in chilled sea water could be different from those in well aerated conditions with melting ice. In the present work, the bacteriology of shrimps handled and stored for 28 hr in CSW and in ice was investigated and the quality differences between frozen shrimps derived from these two batches were assessed with a view to gain a better understanding of these two techniques of handling and preserving raw shrimps.

Materials and Methods

Shrimps (*Parapenaeopsis stylifera*, *Penaeus indicus* and *Metapenaeus dobsoni* of which the first one was dominating) were caught off Mangalore coast at a depth of 5 fathoms. After the catch was taken on to the deck from the trawl net shrimps were sorted out from other bycatch and were washed with sea water. A sample of shrimps was put in ice made of sterile distilled water in a sterile container and kept in an insulated container. This sample was used to study the microflora of freshly caught shrimps.

The catch was divided into two batches; one was iced in layers in an insulated box, the ratio of ice to shrimps being 1:2 and the other put into an insulated box containing chilled sea water to give a ratio of sea water:ice:shrimps of 1:3:4. The boxes were brought to the laboratory and stored for 28 hr at room temperature to simulate the delay that occurs while transporation to distant places for processing. After 28 hr the shrimps were processed in "peeled and undeveined" (PUD) style. The processed shrimps were washed with chilled fresh water, wrapped in 250 g quantities in polythene sheets in small waxed cartons and frozen at -28°C for 14 hr. Frozen samples were stored at -18°C.

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Samples for bacteriological analysis were drawn after storage for 28 hr but before processing (BP), after processing (AP) and after freezing (AF). Aerobic plate countings (APC) were performed by the surface spread method of Speck[7] and the plates were incubated at room temperature (28-29°C). To study generic distribution of bacteria in different samples, 100 colonies from Plate Count Agar were chosen at random using random tables, purified and identified upto generic level using schemes of LeChevallier *et al.*[8] Groups of *Pseudomonas* were differentiated as described by Shewan *et al.*[9]

The quality of frozen prawns were evaluated organoleptically in both raw as well as cooked state based on parameters like taste, texture, flavour, odour and colour by a group of trained panelists using a 10 point scale.

Results

Results in Table 1 indicate that Gram positive flora (60.86 per cent) dominate the microflora of freshly caught shrimps. *Bacillus* (42 per cent), *Pseudomonas* (18 per cent) and *Aeromonas* (12 per cent) were the predominant bacterial genera and among *Pseudomonas* except group I, all the other groups were present. Significantly, *Staphylococcus* was also present in this sample of shrimps. Storage of shrimps in CSW brought about significant changes in the microflora. *Pseudomonas*, particularly group II and IV were the most important bacterial group present. However, interestingly, there were quite a good number of Gram positive bacteria represented by *Corynebacterium*, *Micrococcus*, *Arthrobacter* and even *Bacillus*. *Lactobacillus* and *Staphylococcus* which were present initially, were not observed in CSW stored shrimps. Furthermore, other

TABLE 1. GENERIC DISTRIBUTION OF MICROORGANISMS (PER CENT TO THE TOTAL FLORA) IN SHRIMPS AT DIFFERENT STAGES

Sl. No.	Bacteria	On board	CSW stored			Iced		
			Before processing	After processing	After freezing	Before processing	After processing	After freezing
1.	*Acinetobacter*	0	1	0	0	7	1	18.18
2.	*Aeromonas*	11.96	3	49	33.33	2	5	9.10
3.	*Alcaligenes*	0	1	0	0	0	0	4.55
4.	*Enterobacter*	0	0	1	0	0	0	0
5.	*Flavobacterium*	0	1	1	0	1	0	0
6.	*Moraxella*	1.10	1	1	0	1	5	9.1
7.	*Proteus*	7.60	4	8	0	0	0	4.55
8.	*Pseudomonas*							
	Group I	0	0	0	0	0	0	0
	Group II	4.35	28	3	16.70	52	47	4.55
	Group III	5.43	2	2	0	8	1	0
	Group IV	8.70	37	2	0	22	10	31.80
9.	*Vibrio*	0	0	6	0	0	0	0
10.	*Arthrobacter*	6.52	9	0	0	0	5	0
11.	*Bacillus*	42.40	6	15	33.33	0	9	9.1
12.	*Corynebacterium*	0	1	0	0	0	9	0
13.	*Micrococcus*	2.20	6	0	16.70	7	7	4.55
14.	*Lactobacillus*	3.26	0	1	0	0	0	4.55
15.	*Staphylococcus*	6.52	0	11	0	0	4	0
16.	Yeast	0	0	0	0	0	3	0
	No. of colonies identified	92	100	100	12	100	100	22

bacteria like *Corynebacterium*, *Alcaligenes* and *Flavobacterium* appeared at this stage.

Washing and processing of CSW stored shrimps appeared to bring about a drastic reduction of *Pseudomonas* and a significant increase in *Aeromonas*. The percentage contribution of *Pseudomonas* to the total flora decreased from 67 to 7 per cent whereas that of *Aeromonas* increased from 3 to 49 per cent. Gram positive flora were still present to the extent of 27 per cent represented by *Bacillus* and *Staphylococcus*. Freezing brought about a drastic reduction in the microflora and except *Pseudomonas* group II, *Aeromonas*, *Bacillus* and *Micrococcus*, all the other bacteria were eliminated from the CSW batch of shrimps. Interestingly, *Bacillus* and *Aeromonas* were the most dominant group, each accounting for 33.3 per cent of the flora and *Pseudomonas* constituted only 16.7 per cent of the flora.

Storage of shrimps in ice for 28 hr before processing resulted in elimination of all the Gram positive flora with the exception of *Micrococcus*. *Pseudomonas* groups accounted for 82 per cent of the microflora, group II contributing a major portion of this (Table 1). Compared to freshly caught shrimps, the ice stored shrimps had relatively less diverse flora with only six genera of bacteria being present as compared to nine genera in freshly caught shrimps. Among Gram negative bacteria initially present, *Proteus* was eliminated and *Aeromonas*, which accounted for 11.96 per cent of the flora in freshly caught shrimp was suppressed. *Acinetobacter* and *Flavobacterium* were the two groups detected in iced shrimps which were not present in freshly caught samples.

The results also suggest that a number of microorganisms like *Staphylococcus*, *Arthrobacter*, *Bacillus*, *Corynebacterium* and yeasts are introduced into the iced shrimps at the stage of processing (Table 1). Gram positive bacteria accounted for 28 per cent of the flora of iced shrimps after processing and significantly, *Pseudomonas* was present to the extent of 58 per cent. Though freezing of ice stored shrimps brought about a 10-fold reduction in bacterial numbers, representatives of 10 bacterial genera were present in frozen samples.

Results in Table 2 suggest that there was a marginal difference in the mean organoleptic scores for overall acceptability in frozen shrimps derived from CSW and iced batch upto a storage period of 4 months. Except during the second month, the CSW batch scored better than the iced batch.

Discussion

The results suggest that storage of shrimps in ice or CSW for 28 hr brings about significant qualitative and quantitative changes in the bacteriological profile. The observation that Gram positive flora account for about 60 per cent of the initial flora of shrimps is in agreement with that of Cann[10] who recorded the presence of Gram positive bacteria to the extent of over 50 per cent in tropical shrimps. However, the predominant group observed by us was *Bacillus* whereas Cann[10] noted mostly coryneforms and *Micrococcus*. Even Magar and Shaikmahmud[11] demonstrated the dominance of *Bacillus* in Indian shrimps caught off Bombay coast. Horsely[12] attributed the dominance of *Bacillus* in fish to the proximity of the fishing ground to the shore and suspended sediment. In view of this, the dominance of *Bacillus* in shrimps used in the present study is not surprising in as much as trawling was done at only 5 fathoms depth. The presence of *Staphylococcus* in freshly caught shrimps observed by us could be due to contamination on deck during sorting the shrimps from bycatch or washing.

The results suggest that handling and storage of

TABLE 2. MEAN PANEL SCORES OF FROZEN PRAWNS

Frozen storage period (months)	Appearance		Colour		Odour		Taste		Texture		Overall acceptability	
	CSW	Ice	CSW	Ice	CSW	Ice	CSW	Ice	CSW	Ice	CSW	Ice
1	7.20	5.91	7.18	6.18	6.73	6.18	7.14	6.00	7.00	6.43	7.00	6.18
2	7.14	7.00	7.00	6.57	6.43	6.29	5.33	5.67	6.33	7.17	6.17	6.43
3	7.43	6.29	7.29	5.71	7.33	6.83	7.20	6.20	7.10	6.40	7.17	5.83
4	7.39	7.28	7.00	7.11	7.11	7.00	7.75	7.00	7.12	7.00	7.25	6.87

Excellent, 9-10; Good, 7-8; Fair, 5-6; Acceptable, 3-4; Not acceptable, 1-2; Spoilt, 0.

shrimps in ice and CSW have different effects on the type of bacteria. In CSW samples a diverse microflora characterised by the presence of a number of Gram positive flora could be observed. This is highly significant in as much as Farber and Lerke[12] demonstrated a positive correlation between diversity of microflora and freshness of seafoods. Representatives of 11 bacterial genera were present in CSW batch of shrimps as compared to the occurrence of only 6 genera in ice stored shrimps (Table 1). Shewan[6] suggested that the qualitative difference in the bacteriology of CSW stored fish and ice stored fish could be due to the semianaerobic conditions prevailing in the former medium as against the well aerated conditions of the latter. He proposed that highly aerobic bacteria like *Pseudomonas* might be suppressed in CSW resulting in proliferation of facultatively anaerobic bacteria. Even in the present study it was observed that though *Pseudomonas* constituted the major portion of the flora in both CSW and iced shrimps, they accounted for only 67 per cent of the flora in the former as compared to 82 per cent in the latter.

The two storage media appeared to have different influences on the three groups of *Pseudomonas*. In iced shrimps, group II was the major group accounting for 52 per cent of the flora while this group constituted only 28 per cent of the flora in CSW stored shrimps. This is significant in as much as Shewan *et al.*[9] demonstrated that wherever *Pseudomonas* spoilage of shrimps occurred, over 50 per cent of the strains belonged to group II. Even Van Spreekens[14] demonstrated that "Dextrose-oxidative *Pseudomonas* spp." (group I and II) could produce strong off odours on fish flesh and on boiled shrimp and are therefore important from the point of view of spoilage of seafoods. The suppression of this oxidative *Pseudomonas* under the semianaerobic conditions of a CSW system is understandable and might be favourable for retaining the freshness of CSW stored shrimps.

Peeling of shrimps can be expected to alter the microflora since those on the surface could be lost during washing and bacteria associated with human body, table top, etc. can be expected to be added. This is evident from the results of the present study. The proportion of *Pseudomonas* has been reduced significantly in CSW samples. Whereas before processing, *Pseudomonas* accounted for 67 per cent of the flora, their contribution came down to a mere 7 per cent after processing. This perhaps suggests that *Pseudomonas* was mainly surface associated in CSW stored shrimps and therefore got washed off during washing and processing. It is possible that nutrients like non-protein nitrogenous substances might leach out into the suspending medium and the surface of shrimps encouraging bacterial growth at the outer surface. This might also explain the observations of Lee and Kolbe[15] that *Pseudomonas* constituted 12.5 per cent of the microflora of refrigerated sea water (RSW) stored shrimps while RSW contained 70.9 per cent *Pseudomonas*. In iced shrimp, perhaps, the melting ice might be washing away the surface nutrients as also the surface associated bacteria, thereby encouraging them to get deeper into the tissue. This is indicated by the presence of *Pseudomonas*, particularly group II in large numbers in the iced lot of shrimps. Contamination with Gram positive and human associated bacteria like *Staphylococcus* was evident in both CSW and iced lots. Cann[10] also presented evidence that bacteria associated with human body were introduced into shrimps during peeling. Lee and Pfeifer[16] noticed elimination of *Moraxella* from peeled shrimp. In the present study, however, no such elimination of *Moraxella* or other specific groups were noticed.

Microflora of frozen prawns might comprise those which have survived freezing and those which have been introduced by the packaging material. This might perhaps explain the differences noticed in the generic distribution of bacteria in frozen shrimps derived from iced and CSW batches.

Results of organoleptic analysis (Table 2) suggest that it might be advantageous to handle and preserve raw shrimp in CSW; also the diversity of microflora and suppression of *Pseudomonas*, particularly, group II, observed in CSW stored shrimps might have an influence on their organoleptic quality.

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Effect of Water Activity on Autoxidation of Methyl Linoleate

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Methyl linoleate supported on cellulose was autoxidized at different water activities (a_w) for 22 days at 30°C and the formation of peroxides and carbonyls was followed. The peroxide values (PV) were considerably higher at high a_ws (325 and 108 at 0.79 and 0.92 a_ws respectively) and were comparatively low at other a_ws (43-69 between a_ws 0.02 and 0.71). The total carbonyls, dicarbonyls and monocarbonyls were high at low a_ws 0.02 and 0.32 as compared to other a_ws although the PV range was almost similar. The keto and semialdehyde esters were low at all a_ws. The study indicates that the rates of peroxide formation and scission are not similar at any a_w. The course of the secondary reactions also varies with the a_w of the system.

Information on the effect of water activity on secondary reactions of lipid oxidation is fragmentary although its effect on autoxidation of lipids in foods has been established[1]. In an earlier communication[2], it was shown that water activity of the system affected the formation of secondary reaction products of oxidation namely carbonyls in walnut oil. This prompted further studies in simpler model systems and in this paper the effect of water activity on carbonyls formation in oxidizing methyl linoleate is reported.

Materials and Methods

The chemicals and the methods used for purification of solvents in this study were same as those reported earlier.[2]

Methyl linoleate: Methyl linoleate was prepared from safflower oil using the procedure described by Westerfeld.[3] It was distilled at 1-2 mm pressure using a 18 cm long Vigreux fractionating column and the centre cut portion boiling between 158 and 162°C collected. This colourless fraction was free from per-

oxides as examined by TLC[4] and analysed 99.6 per cent by gas chromatography.

Preparation of methyl linoleate-cellulose powder model system: To 300 g of methyl linoleate in a 2 l beaker, 300 g of cellulose powder (moisture content 2 per cent) were added in small portions mixing thoroughly after each addition using a glass rod. The uniformity of mixing was checked by estimating the methyl linoleate content in aliquots.

Autoxidation of the methyl linoleate-cellulose powder model system at different water activities: Methyl linoleate-cellulose mix (30g) in duplicate was placed in the main compartment of a 350 ml Warburg flask. Then 15 ml of saturated salt solution of known water activity[5] was placed in the side arm (100 ml capacity) of the Warburg flask. The flask was stoppered and left at 30°C in an incubator and allowed to oxidize for 22 days. Subsequently, the samples were taken in duplicate for peroxide and carbonyl analysis.

Peroxide value: The method Cd 8-53 of A.O.C.S.[6] was followed for the determination of peroxide value (PV) using 1 ± 0.1 g of the methyl linoleate-cellulose mix. The average value of duplicates is reported in Table 1.

Extraction and conversion of carbonyls into 2, 4-dinitrophenyl hydrazone derivatives: The methyl linoleate-cellulose mix (20 g) was mixed with an equal quantity of Hyflo-supercel, packed dry in a glass column and eluted with 500 ml of carbonyl free hexane. The carbonyls in the hexane effluent were converted into 2, 4-dinitro phenyl hydrazones (DNPHS) on the 2, 4-dinitrophenyl hydrazine-phosphoric acid-celite reaction (DNP) column as reported by Schwartz *et al.*[7] The total concentration of carbonyl derivatives was calculated using molar extinction coefficient of 2, 4-dinitrophenyl hydrazones from the literature[7] (E= 22500). The average carbonyl value of duplicates is reported.

Isolation of DNP hydrazones and class separation of monocarbonyl derivatives: The DNPHS in the hexane effluent from the DNP column were separated into dicarbonyls, keto and semialdehyde esters, monocarbonyls and their classes as described previously.[2]

Separation of individual components within a class: The separation of the 2-alkanones, alkanals, 2-alkenals and 2, 4-alkadienals into individual carbonyls was carried out according to the TLC procedure described by Badings and Wassink[8], using methyl cyclohexane

TABLE 1. EFFECT OF WATER ACTIVITY ON THE FORMATION OF CARBONYL COMPOUNDS IN METHYL LINOLEATE SUPPORTED ON CELLULOSE POWDER[a]

		μ moles/mole linoleate										
a_w	PV[b]	Total carbonyls A	A-78 / PV[c]	Carbonyls recovered[d] B	Dicarbonyls Qty (A-B) D	Dicarbonyls % of total	Dicarbonyls D-42 / PV	Keto and semialdehyde esters Qty (B-C) E	Keto and semialdehyde esters % of total	Monocarbonyls[e] Qty C	Monocarbonyls % of total	Monocarbonyls C-3 / PV
0.02	53	418	6	38	380	91	6.4	9	2	29	7	0.5
0.11	43	190	2	37	153	81	2.6	21	11	16	8	0.3
0.22	59	181	2	24	157	87	2.0	15	8	9	5	0.1
0.32	49	603	11	54	549	91	10.4	22	4	32	5	0.6
0.43	49	169	2	20	149	88	2.2	7	4	13	7	0.2
0.51	62	200	2	26	174	87	2.1	18	9	8	4	0.1
0.71	69	200	2	48	152	76	1.6	33	17	15	8	0.2
0.79	325	1882	6	182	1700	90	5.1	155	8	27	1	0.1
0.92	108	267	2	25	242	91	1.9	16	6	9	3	0.1
Lino[f]	0	78	—	36	42	54	—	33	42	3	4	—

[a]Storage period 22 days at 30°C, mild rancid odours were perceptible at the time of analysis in all samples.

[b]Peroxide value (meq O_2/kg linoleate) when taken for carbonyls analysis.

[c]Total carbonyls per unit peroxide value

[d]Carbonyls recovered from the defatting column.

[e]Recovered from alumina column

[f]Fresh linoleate

as the developing solvent. The chromatoplates were scanned using a Camag TLC scanner equipped with a Turner fluorometer model 111 primary filter 110-811 (350-400nm) secondary filter 110-823 (10 per cent neutral density). Relative concentration of each component within the class was calculated by triangulation of the peak area. A linear calibration curve was obtained when known concentrations of authentic 2, 4-dinitrophenyl hydrazones of carbonyl compounds were plotted against peak area of the scanned spots.

Results and Discussion

Peroxides: The peroxide value of methyl linoleate after 22 days of storage at different a_ws is given in Table 1. The PV varied in a limited range of 43-69 between a_ws of 0.02 and 0.71. At a_ws 0.79 and 0.92 the PV were 325 and 108 respectively indicating either acceleration of autoxidation or accumulation of peroxides at those a_ws. Though the pattern of PV was similar to the generally accepted trend of autoxidation of lipids, the peroxide content was comparatively low at 0.02 a_w. One of the possible reasons for this is faster decomposition of peroxides at this a_w. The reason for a higher PV at a_ws 0.79 and 0.92 may be due to the stabilizing effect of water on the peroxides formed[9].

Carbonyls: The total carbonyl value (Table 1) showed no particular trend but there were quantitative differences from one a_w to another. However, the highest carbonyl value was recorded at 0.79 a_w, which also recorded the highest PV. Likewise at 0.43 a_w a low PV was attended by a low carbonyl value. At other a_ws similar correlation was not observed. The total carbonyls formed per meq of peroxide oxygen varied from 2-11 at different a_ws, being highest at 0.32 a_w. At a_ws 0.02 and 0.79, the ratio was 6 while it was uniformly same at other a_ws. This showed that the secondary reactions that follow peroxide scission as indicated by the amount of carbonyls formed could vary although the samples record similar PV. This is evident from a value of 11 for 0.32 a_w sample whose PV was almost similar to that at 0.02, 0.11, 0.22 and 0.43 a_ws.

The dicarbonyls were the major class of carbonyl compounds formed at all a_ws and accounted for 76-91 per cent of the total carbonyls. The ratio of dicarbonyls to PV showed the same trend as that for the total carbonyls, i. e., high values at 0.02, 0.32 and 0.79 a_ws compared to the values at other a_ws.

TABLE 2. EFFECT OF WATER ACTIVITY ON THE DISTRIBUTION OF MONOCARBONYL CLASSES IN METHYL LINOLEATE SUPPORTED ON CELLULOSE POWDER[a]

a_w	Total monocarbonyls (TM)	Monocarbonyls class[bc]							
		2-Alkanones		Alkenals		2-Alkenals		2,4-Alkadienals	
		Qty[d]	% of TM	Qty	% of TM	Qty[d]	% of TM	Qty	% of TM
0.02	29	29	89	3	12	2	—	4	4
0.11	16	A	—	7	54	7	8	7	31
0.22	9	2	—	1	17	5	—	9	100
0.32	32	18	41	A	—	5	—	11	28
0.43	13	4	—	2	20	2	—	8	63
0.51	8	4	—	1	20	1	—	11	—
0.71	15	3	—	3	25	2	—	15	100
0.79	27	4	—	4	17	26	83	5	8
0.92	9	6	—	A	—	A	—	8	83
Lino[f]	3	6	—	A	—	6	—	A	—

[a]Quantity expressed as μ moles/mole linoleate

[b]% of total monocarbonyls (TM) of different classes was calculated as follows: Qty at any a_w—qty of fresh linoleate×100/(TM at any a_w—TM of fresh linoleate)

[c]The monocarbony class do not total to 100% TM as the UV absorption of the classes and of the TM was measured at different wavelengths

[d]Apparent loss of the constituents when compared with that of fresh linoleate. A=Absent.

Lino[f]=Fresh linoleate

The keto and semialdehyde esters varied with a_w (Table 1). Their concentration decreased from the initial values suggesting their participation in autoxidation reaction.

The quantity of monocarbonyls and the ratio of monocarbonyl to peroxide changed appreciably with a_w. The monocarbonyl value was very low at 0.22 and 0.92 a_ws and moderate at 0.11, 0.43 and 0.67 a_ws and high at 0.02, 0.32 and 0.79 a_ws. Although the total carbonyls were the highest at 0.79 a_w, the total quantity of monocarbonyls was comparatively low suggesting two possibilities: (*a*) decreased formation of monocarbonyl compounds at 0.79 a_w with virtually no cleavage of alkoxy radical to a monocarbonyl and a free radical, (*b*) faster conversion of monocarbonyls formed to other compounds like short chain acids or hydroxyl compounds. The formation of such compounds from monocarbonyls is reported in the literature[10].

Separation of the monocarbonyls into classes (Table 2) showed that 2-alkanones were formed only at 0.02 and 0.32 a_ws, whereas at other a_ws their concentration was lower than the initial value. The alkanals were formed to varying extent at different a_ws and were absent at 0.32 and 0.92 a_ws. The 2-alkenals class was present to an appreciable extent only at 0.79 a_w while 2, 4-alkadienals was the major monocarbonyl class at 0.22, 0.43, 0.71 and 0.92 a_ws.

The relative concentration of the individual alkanals varied with a_w (Table 3). For eg., at a_ws 0.02, 0.11, 0.43, 0.51, 0.71 and 0.79 the quantity of ethanal was more than that of other alkanals and its relative concentration differed with a_w. Octanal was found in high concentration at a_w 0.22 while dodecanal was considerably higher at a_w 0.32.

TABLE 3. PATTERN OF ALKANALS ISOLATED FROM OXIDIZING METHYL LINOLEATE SUPPORTED ON CELLULOSE POWDER AT DIFFERENT WATER ACTIVITIES[a]

Spot no.	R_f X100	Identity carbon no.	Relative concn of individual members at indicated a_w							
			0.02	0.11	0.22	0.32	0.43	0.51	0.71	0.79
1	13	C_2	46	60	15	12	66	33	44	60
2	24	C_3	A	A	A	5	A	A	A	A
3	36	C_4	A	37	36	3	6	15	A	2
4	42	C_5	7	A	A	2	A	9	8	8
5	56	C_6	6	A	1	1	5	7	4	19
6	65	C_7	A	A	A	2	A	5	A	2
7	76	C_8	8	A	44	17	1	10	28	A
8	86	C_9	20	A	A	6	A	6	17	A
9	96	C_{10}	A	A	5	A	3	5	A	A
10	100	C_{12}	12	4	A	53	20	10	A	9

[a]In fresh methyl linoleate and sample autoxidized at 0.92 a_w alkanal class was absent. A=Absent.

TABLE 4. PATTERN OF 2-ALKENALS ISOLATED FROM OXIDIZING METHYL LINOLEATE SUPPORTED ON CELLULOSE POWDER AT DIFFERENT WATER ACTIVITIES

Spot no.	R_f X100	Identity carbon no.	Relative concn of individual members at indicated a_w						
			0.02	0.11	0.22	0.32	0.43	0.51	0.71
1	24	C_4	22	27	42	32	63	53	40
2	43	C_6	61	64	17	62	18	37	60
3	72	C_9	17	9	41	6	19	11	A

Fresh methyl linoleate had an origin spot. In sample autoxidized at 0.92 a_w 2-alkenal class was absent and sample autoxidized at 0.79 a_w had two spots with R_f0.08 and 0.72 with 35 and 65% concentration respectively.
A=Absent.

TABLE 5. PATTERN OF 2,4-ALKADIENALS ISOLATED FROM OXIDIZING METHYL LINOLEATE SUPPORTED ON CELLULOSE POWDER AT DIFFERENT WATER ACTIVITIES

Spot no.	R_f ×100	Identity carbon no.	Relative concn of individual members at indicated a_w								
			0.02	0.11	0.22	0.32	0.43	0.51	0.71	0.79	0.92
1	20	—	6	A	24	28	82	30	82	A	A
2	52	C_6	57	58	A	A	11	36	A	95	41
3	80	C_7	37	42	A	A	8	A	A	5	59
4	96	—	A	A	76	72	A	35	18	A	A

Fresh methyl linoleate had a spot at R_f 0.20 A=Absent.

TABLE 6. PATTERN OF 2-ALKANONES ISOLATED FROM OXIDIZING METHYL LINOLEATE SUPPORTED ON CELLULOSE POWDER AT DIFFERENT WATER ACTIVITIES

Spot no.	R_f ×100	Identity carbon no.	Relative concn of individual members at indicated a_w						
			0.02	0.22	0.32	0.43	0.51	0.79	0.92
1	12	C_3	3	22	12	25	73	23	31
2	27	C_4	A	A	37	11	A	8	21
3	37	C_5	A	14	1	A	A	17	A
4	51	C_6	71	9	8	A	27	2	5
5	68	C_8	A	16	7	26	A	51	28
6	83	C_{11}	27	40	15	A	A	A	15
7	70	—	A	A	19	9	A	A	A
8	97	—	A	A	A	29	A	A	A

Fresh methyl linoleate and sample autoxidized at 0.71 a_w had a spot at the origin. 2-alkanone class was absent in sample autoxidized at 0.11 a_w. A=Absent.

The variation in the relative concentration of individual members of 2-alkenals, 2, 4-alkadienals and 2-alkanones in Tables 4, 5 and 6 show that these compounds are formed to different extents depending on the water activity of the autoxidizing linoleate. The present study confirms the earlier observation on walnut oil-cellulose system[2] that the type and quantity of monocarbonyl compounds formed in an autoxidizing lipid system depends on the water activity of the system.

The results presented in Tables 1-5 show that though there is no linear correlation between PV and carbonyl value with water activity, the composition of the monocarbonyl compounds formed at one a_w differs from another even at equal PV or carbonyl values. As monocarbonyl compounds are the ones which give off-odours, variation in the composition of the different classes of monocarbonyls and the individual monocarbonyls among a particular class could give an entirely different odour to the food. Hence the influence of water activity becomes important in controlling the secondary reactions of lipid oxidation in foods.

The mechanism (s) of action of a_w on secondary reactions of peroxides of fatty acids is not known. However, this study underlines the importance of controlling the a_w of food systems so as to control delay or direct the formation of secondary products of peroxide scission, which are involved in causing lipid induced off flavours in processed foods.

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RESEARCH NOTES

MELON SEEDS—EVALUATION OF PHYSICAL CHARACTERISTICS

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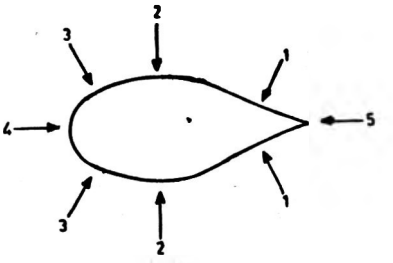
Melon seed kernels are a rich source of protein and fat and are commonly used as adjuncts in sweet/snack foods. The seeds are manually dehulled to get kernels. This operation is very tedious and laborious limiting the capacity to only 100g/person/day. The various physical characteristics of melon seeds, were evaluated in order to find out the possibility of mechanically dehulling them.

Melon seed kernels are rich in protein and fat and have been in use traditionally[1]. However, due to tedious collection and manual dehulling, commercial utilisation is limited to only as an adjunct in some sweet and snack foods. Though lot of work is reported on basic aspects[2], technological work is limited to mechanised seed extraction from water melon fruits and oil production on pilot scale from musk melon and long melon seeds[3,4]. Prior to oil extraction the seeds are reported to be dehulled using a disc huller, but they are not completely dehulled and/or are broken. With a view to explore the development of a mechanised dehulling method, to get pure and whole kernels without breakage, the various physical characteristics of seeds, kernels and husk were evaluated in this study.

Commercial varieties of musk melon (*C. melo*), long melon (*C. melo utilissimus*) and water melon (*C. vulgaris*) seeds, procured from a local shop were used in this study. Dry seeds were soaked for 15 min in tap water at room temperature to get wet seeds, which are manually dehulled. The wet seed was held between thumb and first finger and the edges adjacent to sharp tip pressed by a metal fork to break the hull and separate the kernels. Water melon seeds however, need hammering with a heavy stone/metal piece of around 500 g weight.

Hull breaking load was measured using fruit pressure tester (D. Ballauf Mfg. Co. Inc., Washington, D. C.). The seed was held tight with a plier and the edge was pressed against the tip of the fruit pressure tester. Experiments were conducted around the edge of the seeds at different points as shown below.



Bulk densities were measured using a 50 ml measuring jar. The carrying velocities were measured in a Petkus apparatus. Surface roughness was measured with surtronic surface roughness meter (type 112/1100) and values reported as centre line average (arithmatic mean of excursions from the centre line of the surface profile) at 0.75 cut of length. Changes during soaking were studied. All linear dimensions are measured with vernier calipers and average value of 100 random seeds are reported.

Hull breaking load: It was observed that the hull can be opened only at position 1 (edge near the sharp tip) by applying suitable force; in all other places, the seed gets crushed if pressed beyond a certain force. Hull breaking load at the above position is lowest (0.7-1.1 kg) for long melon and highest (7-8 kg) for water melon seeds. Hull breaking load is reduced appreciably for wet seeds (Table 1).

Carrying velocities: There is a large difference between the carrying velocities of the hull and the kernel as also the seed (Table 2); hulls can therefore, be easily separated in a suitable aspiration system.

TABLE 1. COMPOSITION OF MELON SEEDS AND HULL BREAKING LOAD

Kind of seed	Composition		Hull breaking load (kg)	
	Kernel (%)	Hull (%)	Dry	Wet
Musk melon	75	25	1.6 – 2.3	0.7 – 1.1
Long melon	68	32	0.7 – 1.1	0.5 – 1.0
Water melon	42	58	7.0 – 8.0	4.0 – 5.0

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TABLE 2. CARRYING VELOCITIES OF DRY AND WET MELON SEEDS, KERNEL AND HULL

Kind of seed	Seed part	Dry			Wet		
		Air velocity (m/sec)	Feed rate (g/sec)	Bulk density (kg/L)	Air velocity (m/sec)	Feed rate (g/sec)	Bulk density (kg/L)
Musk melon	Hull	2.44	1.33	0.10	2.78	6.33	0.12
	Kernel	7.00	7.81	0.54	7.78	11.90	0.50
	Seed	7.44	7.81	0.45	8.22	11.36	0.47
Long melon	Hull	2.33	0.69	0.12	2.89	5.43	0.12
	Kernel	7.22	10.00	0.56	7.78	24.80	0.48
	Seed	7.55	14.31	0.46	8.33	12.50	0.46
Water melon	Hull	4.55	6.50	0.20	4.89	3.60	0.20
	Kernel	7.66	15.89	0.50	8.33	10.26	0.60
	Seed	8.33	16.57	0.46	9.11	6.25	0.58

TABLE 3. SURFACE ROUGHNESS OF MELON SEEDS AND KERNELS

Kind of seed	Size of seed	Length range (cm)	Sample		Av. seed wt. (mg)	Roughness in μ			
			No. (%)	Wt. (%)		Dry		Wet	
						Kernel	Seed	Kernel	Seed
Musk melon	Large	>1.15	14	20	47	3.5–5.0	2.5—.0	4.0–6.0	4.0–5.5
	Medium	0.76–1.14	64	63	35	4.0–6.0	3.0–4.5	3.0–4.0	1.5–3.0
	Small	<0.75	22	17	26	6.0–7.2	4.0–5.0	2.5–5.5	3.0–4.0
Long melon	Large	>0.95	7	9	23	3.0–4.5	2.5–3.0	4.0–6.0	3.0–4.5
	Medium	0.71–0.94	79	81	17	3.0–5.3	2.5–3.5	3.5–6.0	4.0–5.0
	Small	<0.70	14	10	13	3.5–5.0	3.0–5.0	3.0–5.0	2.0–4.0
Water melon	Large	>1.35	9	10	130	2.5–3.5	4.0–6.0	2.0–3.0	3.0–4.0
	Medium	1.16–1.34	84	84	120	2.5–3.5	3.5–6.5	1.5–3.0	3.0–4.0
	Small	<1.15	7	6	100	3.5–4.0	4.5–7.0	1.5–2.5	2.5–4.0

TABLE 4. CHANGES IN SIZE DURING SOAKING IN WATER

Kind of seed		Seed size (cm)			% increase by 15 min soaking			Diff. between wet seed and wet kernel (cm)		
		L	B	T	L	B	T	L	B	T
Musk melon	Min.	0.71	0.30	0.09	0.88	2.56	7.14	0.07	0.03	0.01
	Max.	1.17	0.46	0.14	7.77	26.77	33.33	0.19	0.13	0.05
	Av.	0.95	0.38	0.11	3.32	9.18	11.90	0.14	0.08	0.02
Long melon	Min.	0.65	0.25	0.07	1.05	3.33	7.69	0.01	0.01	0.01
	Max.	0.96	0.37	0.10	6.66	20.00	28.57	0.17	0.11	0.07
	Av.	0.79	0.31	0.10	3.63	7.78	10.04	0.11	0.06	0.03
Water melon	Min.	1.09	0.73	0.18	0.92	2.33	3.85	0.21	0.12	0.10
	Max.	1.50	0.87	0.26	7.56	8.22	20.00	0.29	0.25	0.18
	Av.	1.28	0.81	0.22	3.73	4.52	9.31	0.24	0.17	0.14

L=Length B=Breadth T=Thickness

Surface roughness: There is a distinct and appreciable difference in surface roughness of kernel and seed of the respective melon seeds. However size has no significant effect on roughness (Table 3). The kernels of musk melon and long melon are more rough than their seeds whereas seeds are more rough in case of water melon. This distinct difference in roughness would be useful in designing a suitable separating system, similar to a paddy separator.

Changes during soaking: The pick-up of water during soaking by the various constituents are shown in Fig 1. It is observed that maximum amount of water is picked up during the first 15 min. However, 70-80 per cent moisture is picked up by the hull only. The seed kernels become soft during soaking in water. This shows that (*i*) a maximum of 15 min soaking is enough to avoid undue breakages during dehulling, and (*ii*) kernels pick up little water and subsequent drying, if any, will be easy.

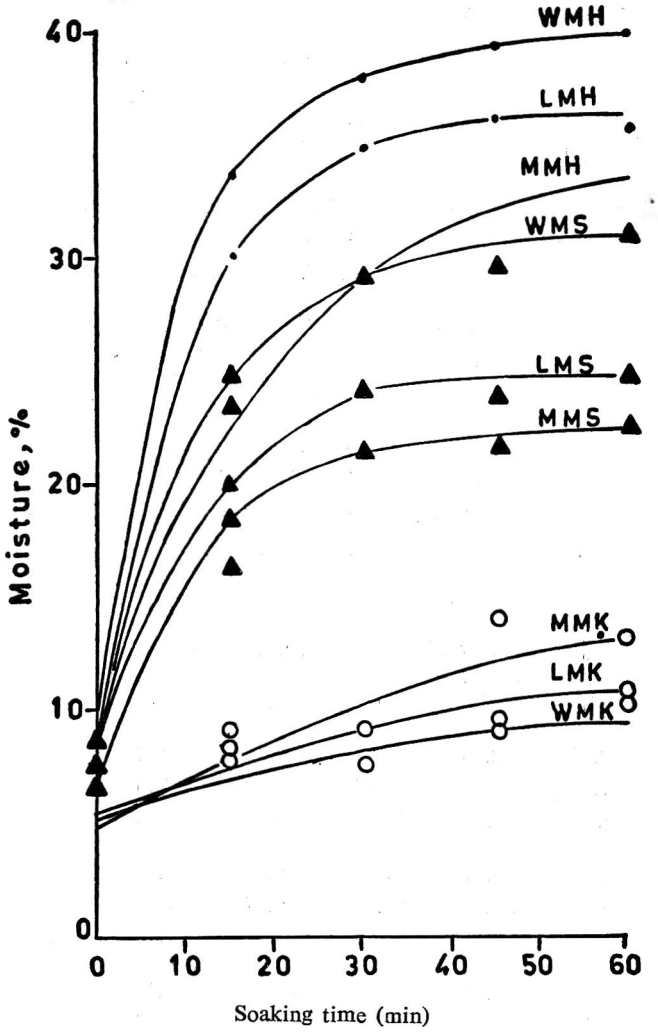

Fig 1. Water pick-up by melon seeds during soaking
MM—Musk melon H—Hull LM—Long melon
S—Seed WM—Water melon K—Kernel

Size of the seeds and changes during soaking were also studied (Table 4). The length, breadth and thickness of the seeds increase from 3-10 per cent and no significant variation in behaviour was observed with any particular type of melon seeds. But the difference between the size of the seed and the kernel of water melon is found very significant as compared to musk melon and long melon seeds. This showed that water melon seed and kernel can be separated with a proper screen more easily than other melon seeds. The results show the possibility of mechanically dehulling the seed, and separating the kernels.

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INFLUENCE OF DIFFERENT SEED-BORNE FUNGI OF RICE (*ORYZA SATIVA* L.) ON THE PRODUCTION OF CITRININ BY *PENICILLIUM CITRINUM*

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Influence of different seed-borne fungi of rice (*Oryza sativa* L.) on the growth and citrinin production by *Penicillium citrinum* was studied. *Aspergillus niger* and *Trichoderma viride* completely suppressed the citrinin production by *P. citrinum*. *Drechslera oryzae* and *P. crotalariae* failed to inhibit the citrinin production. No correlation could be observed between type of microbial interaction and citrinin production by *P. citrinum*.

Citrinin, a lemon yellow toxin, produced by *Penicillium citrinum* is reported to be a natural contaminant

of rice[1]. It has received special attention of toxicologists in view of its nephrotoxicity[2] and phytotoxic nature[3]. Inspite of its varied biological activities no attention has been made to control this toxin production. Though Wicklow *et al.*[4], Agarwal and Thakur[5], Malini *et al.*[6] and Reddy and Reddy[7] have suggested the use of certain fungi to control aflatoxin production, no such studies have been made to control citrinin production. Hence, it was considered worthwhile to investigate the interaction of different fungi with *P. citrinum* and its influence on citrinin production.

The influence of different seed-borne fungi of rice (*Oryza sativa* L.) on growth of *P. citrinum* was evaluated by inoculating the buffered 2 per cent malt extract agar (pH 6.0) with 26 different fungal species pairing with *P. citrinum*. Each pair was inoculated over the agar surface maintaining equidistance to all test pairs. The plates were incubated at 27±2°C and examined on alternate days for 10 days and the type of interaction was graded as suggested by Johnson and Curl[8].

1. Mutual intermingling of two organisms — A
2. Mutual inhibition on contact; the space between the two colonies is small, but clearly marked — B
3. Mutual inhibition at a distance — C
4. *Penicillium citrinum* growth was suppressed by seed-borne fungi — D
5. *Penicillium citrinum* suppressed the growth of seed-borne fungi — E

The fungi with different responses towards *P. citrinum* were inoculated in a synthetic broth (Adye and Mateles)[9] in pairs to determine their individual effect on the production of citrinin. The flasks were incubated at room temperature (27±2°C) for 10 days. At the end of the incubation period, 25 ml of culture filtrate was employed for citrinin extraction. The details of extraction and estimation were essentially similar to those suggested by Damodaran *et al.*[10] The lemon yellow spot of citrinin on chromatoplates was eluted in 3 ml of 0.1M carbonate-bicarbonate buffer (pH 10.0), and centrifuged. One ml of Folin ciocalteau (FC) reagent and 1 ml of 15 per cent sodium carbonate were added to the supernatant of test solution and incubated at 37°C for 15 min. Control was also maintained simultaneously. The intensity of blue colour thus developed was read at 640 nm in spectrocol. Experiment was repeated thrice and the mean of three observations is presented in Table 1.

From Table 1 it is clear that the response of *P. citrinum* towards different seed-borne fungi of rice varied with the species. *A. terreus*, *A. flavus*, *F. moniliforme*, *P. janthenellum* and *P. crotalariae* failed to influence the vegetative growth of *P. citrinum*. On the other hand, *M. echinata* and *S. atra* were inhibited by *P. citrinum* at a distance. *A. alternata*, *C. brachysporum*, *C. tuberculata*, *D. oryzae*, *D. spiciferum* and *Phoma* sp. were comparatively mild in inhibiting the growth of *P. citrinum* from a distance, while *Trichoderma viride*, *P. fumiculosum*, *F. equisetii*, *C. lunata* and *A. niger* not only inhibited the growth of *P. citrinum* but also overgrew it. The reaction of the other fungi was intermediate

TABLE 1. INTERACTION OF DIFFERENT SEED-BORNE FUNGI OF RICE (*ORYZA SATIVA* L.) AND ITS EFFECT ON CITRININ PRODUCTION BY *PENICILLIUM CITRINUM*

Fungi	Citrinin production (ppb)	Type of interaction
Control	230	—
Aspergillus niger	Nil	D
A. terreus	105	A
A. flavipes	115	B
A. flavus	100	A
Alternaria alternata	110	C
Absidia corymbifera	180	B
Acrocylindrum oryzae	180	B
Curvularia lunata	75	D
C. brachysporum	110	C
C. tuberculata	115	C
Chaetomum globosum	100	B
Drechslera rostrata	115	B
D. oryzae	225	C
D. spiciferum	110	C
Fusarium equisetii	75	D
F. moniliforme	110	A
Memnoniella echinata	75	E
Nigrospora oryzae	105	B
Penicillium janthenellum	110	A
P. funiculosum	115	D
Phoma sp.	110	C
Phaeotricocoonis crotalariae	220	A
Stachybotrys atra	180	E
Paeciliomyces varioti	120	B
Trichoderma viride	Nil	D
Thielvia nemicola	110	B

Influence of different seed borne fungi of rice on citrinin production by *P. citrinum* was studied. *A. niger* and *T. viride* were responsible for complete suppression of citrinin production by *P. citrinum*. Similarly, *F. equisetii*, *C. lunata* and *M. echinata* also suppressed the citrinin production to a great extent. On the other hand, *D. oryzae*, *P. crotalariae*, *S. atra*, *A. corymbifera* and *A. oryzae* could not interfere much with the citrinin production by *P. citrinum*. Other species of *Penicillium* like, *P. funiculosum* and *P. janthenellum* were also responsible for the partial inhibition of citrinin synthesis. The remaining fungi inhibited the synthesis of citrinin by *P. citrinum* moderately. There was no correlation between the type of interaction with *P. citrinum* and citrinin production.

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EFFECT OF MOLASSES COMPOSITION ON ETHANOL FERMENTATION

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Fermentation of molasses obtained from different sources with variable chemical composition was tested in media using *Saccharomyces cerevisiae* HAU-1 for ethanol production. The ash content of these molasses varied from 9 to 18% and total sugar from 42.5 to 54.2%. Fermentation efficiency improved with some molasses by supplementation with urea and phosphate.

In India, industrial ethanol is produced mostly from cane molasses. The composition of these molasses varies depending on the cane variety, soil type and the type of sugar produced (mill/khandsari). Further, the quality of the molasses is also determined by the clarification process (sulphitation/carbonation). The quality of the molasses may determine the yeast strains needed for ethanol production. Schiweck[3] has reported that the composition of beet molasses influences the yield of ethanol and suggests the need to develop yeast strains to suit the molasses composition. We have earlier reported the development of a yeast strain HAU-1 that can ferment unclarified molasses efficiently[1]. In this paper we report the performance of this yeast strain in fermentation media prepared using molasses from different sources.

Details of the yeast *S. cerevisiae* HAU-1 used in this investigation have been reported previously[2]. The strain was maintained on yeast extract (0.2 per cent)-peptone (0.2 per cent) -dextrose (2 per cent)-agar (2 per cent) medium by regular subculturing.

Molasses samples were collected from eight different Northern Indian Sugar Mills/Distilleries.

The inoculum medium comprised 12° Brix molasses solution containing 0.2 kg urea and 0.02 kg phosphoric acid per 100 kg fermentable sugars[3]. The pH was adjusted to 5.0 and the medium was sterilized at 10 psi for 15 min. The inoculum medium (50 ml) in 150 ml flask was seeded with a 24 hr old yeast culture. After 12 hr incubation at 30°C, the contents of the flasks were transferred to 250 ml in 500 ml flask and again incubated for 12 hr. This was used to inoculate 1 l of unsterilized production medium in a 2 l flask.

The production medium contained diluted molasses (about 14-16 per cent sugars), phosphoric acid and urea as described above. This was allowed to ferment

TABLE 1. COMPOSITION OF MOLASSES

Sl. No.	Clarification process	Total sugars (%)	Fermentable sugars (%)	pH	Ash* (%)	N_2* (%)	P* (%)
1	DC/DS	51.8	47.2	5.5	13.0	0.52	0.075
2	DS	54.2	49.6	4.8	9.0	0.37	0.067
3	DC/DS	50.6	44.2	5.0	13.6	0.30	0.030
4	DC	48.5	43.9	5.3	11.6	0.55	0.030
5	DS	50.6	45.6	5.0	18.2	0.46	0.075
6	M	49.3	44.2	4.8	13.8	0.44	—
7	M	48.5	44.5	5.1	13.0	0.46	—
8	M	42.4	38.4	4.8	12.0	0.32	—

*On fresh wt basis
ND Not determined
DC Double carbonation
DS Double sulfitation
M Mixed

upto 48 hr at 30°C. Alcohol production was monitored at regular intervals by withdrawing 20 ml samples.

Total nitrogen, ash, phosphorus, total reducing sugars and unfermentable sugars in different batches of molasses were estimated by standard AOAC methods[4]. Ethanol was determined by the method of Caputi *et al.*[5] pH was determined using a Elico Model LI-10 pH meter.

Molasses from different sources differed in composition (Table 1). The ash content varied from 9 to 18 per cent, pH from 4.8-5.5 and total sugars from 42.5 to 54.2 per cent.

To determine the effect of molasses composition on alcohol production, various molasses were diluted to 14-15 per cent sugar concentration, inoculated with the yeast and allowed to ferment. As seen in Table 2, the rate and quantity of alcohol production varied with the source of molasses.

To find out whether the fermentation rate and the final amount of ethanol produced from these molasses can be improved by nutrient supplementation, the medium was supplemented with N and P as described. It was found (Table 3) that supplementation did not improve the final level of ethanol in molasses No. 6 and 8 while there was significant improvement both in the rate of fermentation and in the final amount of alcohol produced from other molasses. Molasses No. 2, 3 and 4 yielded higher amounts of alcohol when supplemented with urea. There was, however, no signincant improvement in the final amount or

TABLE 2. ETHANOL PRODUCTION BY HAU-1 FROM DIFFERENT MOLASSES

Sl. No.	% initial sugar concn.	% ethanol (v/v) produced at indicated time (hr)		
		24	36	48
1	15.1	6.1	8.0	8.6
2	14.7	3.5	5.0	6.1
3	15.8	2.4	3.5	4.6
4	15.3	6.6	8.4	8.4
5	14.5	7.6	7.9	8.6
6	15.7	9.0	9.1	9.1
7	15.3	7.5	9.1	9.1
8	14.3	7.5	8.3	8.4

TABLE 3. EFFECT OF SUPPLEMENTATION ON FERMENTATION RATE AND PER CENT ALCOHOL (V/V) PRODUCED

Sl. No.	Control		Urea		Phosphoric acid		Urea + Phosphoric acid	
	24hr	48hr	24hr	48hr	24hr	48hr	24hr	48hr
1	6.1	8.6	6.7	8.6	6.1	8.6	7.1	9.1
2	3.5	6.1	6.2	8.6	3.5	6.2	6.2	8.6
3	2.4	4.6	5.6	8.5	2.5	4.6	6.5	8.6
4	6.6	8.4	6.7	8.3	6.7	8.8	7.2	9.0
5	7.6	8.6	7.9	8.7	7.8	8.6	7.9	8.8
6	9.0	9.1	9.2	9.2	9.2	9.2	9.2	9.4
7	7.5	9.1	7.8	9.2	7.4	9.1	7.9	9.2
8	7.5	8.4	7.9	8.5	7.7	8.6	8.2	8.5

rate of ethanol production by the addition of only phosphate. Thus, performance of the yeast can be improved only in some molasses by supplementing with a N source. Generally, in most distilleries, molasses medium is supplemented with either a nitrogen or a phosphate source or sometimes with both. This appears unnecessary with all molasses and the distilleries could save considerably by testing the molasses before use.

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THERMAL STABILITY AND CHANGES IN TRYPSIN INHIBITOR DURING GERMINATION AND COOKING OF HORSE GRAM

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Application of dry heat at 80°C for 60 min did not inactivate trypsin inhibitor in horse gram meal, only 20% activity was lost when subjected to 100°C heat for 60 min. However, autoclaving of meal at 120°C at 15 lbs pressure destroyed the inhibitor activity completely within 10 min. The extracted inhibitor lost 100% activity within 20 min. Soaking (8 hr) of horse gram seeds decreased the inhibitor activity to the extent of 30%. Germination had no effect whereas germination followed by cooking resulted in 90% decrease in the inhibitor activity.

biflorus

Horse gram (*Dolichos ~~tab tab~~*) is extensively cultivated in Australia, Burma, India and Sri Lanka[1]. The use of dry seeds as human food is limited due to its poor cooking quality; they are however, consumed as sprouts in many parts of India. Horse gram seeds are known to contain trypsin and chymotrypsin inhibitors, hemagglutinins[2], flatulence causing factor and polyphenols[3]. The trypsin inhibitory activity in dry beans is appreciably destroyed by heating[4], although resistance of trypsin inhibitors to heat is also documented[5]. Horse gram seeds are sprouted and cooked before they are consumed as human food. Changes in protein content, *in vitro* protein digestibility and polyphenols due to germination in horse gram seeds have been reported.[6] In the present investigation, we have studied thermal stability of trypsin inhibitor in horse gram and changes in trypsin inhibitor activity during germination and cooking.

Horse gram seeds were obtained from Dry Land Research Station, Solapur, cleaned manually and preserved at 4°C until use. The seeds were powdered in Wiley Mill to pass through 60 mesh sieve. The meal was defatted using petroleum ether. The defatted meal was extracted with distilled water (1:20 w/v) for 2 hr at room temperature by using a mechanical shaker. The extract was filtered and filtrate was used for the assay after appropriate dilution[7]. Trypsin activity was determined by the method of Erlanger *et al.*[8] using Na-benzoyl-DL-arginine-p-nitroanilide (BAPNA) as the substrate. The quantity of p-nitroanilide liberated by enzyme was then estimated at 410 nm using a spectrophotometer. One unit of trypsin activity was defined as 1 μ mole of p-nitroanilide released per min. The inhibitor extract was added to the enzyme after adding buffer and allowed to stand at room temperature (28±2°C) for 3 min for reaction. Enzyme inactivated by acetic acid was used as the blank along with other reagents as followed for trypsin assay. Trypsin inhibitor was estimated from the residual trypsin activity of the mixture of inhibitor extract and trypsin, and was expressed in terms of trypsin inhibitor unit per gram of sample. One trypsin inhibitor unit was defined as the amount of inhibitor that inhibited one unit of trypsin activity.

Ten gram seeds sterilized with 0.1 per cent mercury chloride were soaked in distilled water at 4°C for 8 hr, placed on double layer of filter paper in petri dishes and incubated at 30°C. The germinated seeds were dried to a constant weight, powdered in a Wiley Mill and passed through 60 mesh sieve. The seeds of different stages of germination were cooked in boiling distilled water until they were softened to a uniform mass when pressed between thumb and fore finger as described by Sharma *et al.*[9]

The trypsin inhibitor from horse gram meal was extracted in different extractants. Both distilled water and 0.005 NaOH were equally effective in extracting the inhibitor and better than 5 and 10 per cent NaCl solutions. Hence, in all subsequent experiments the inhibitor was extracted with distilled water (1:20 w/v) and diluted to obtain 40-60 per cent inhibition of the commercial preparation of enzyme used for assay.

Heat treatment has been shown to reduce the activity of trypsin inhibitor in many food legumes[4]. The application of dry heat at 80°C for 60 min did not inactivate the inhibitor in horse gram meal. The inhibitor lost only 20 per cent activity when subjected to 100°C for 60 min. Autoclaving of meal at 120°C at 15 lbs pressure destroyed the activity of the inhibitor completely within 10 min (Fig 1). The extracted inhibitor lost 100 per cent activity at 100°C within 20 min (Fig 2).

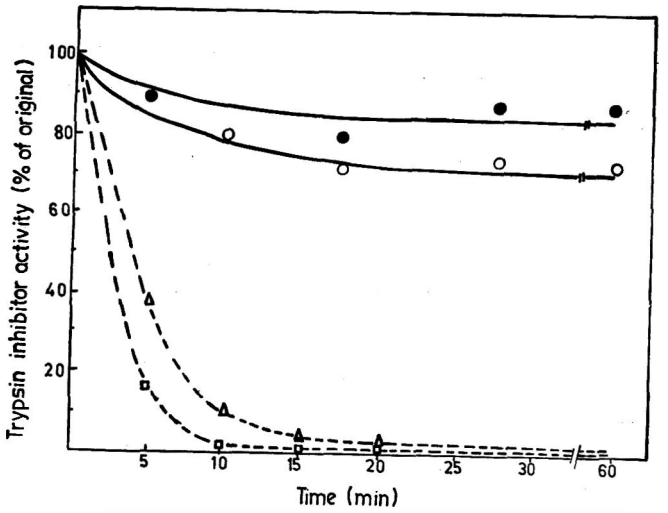

Fig 1. Effects of dry and moist heat on horse gram trypsin inhibitor. Flour incubated at: 80°C (●——●) and 100°C (O——O). Whole seeds (△——△) and flour (□——□) autoclaved at 120°C at 15 lb pressure.

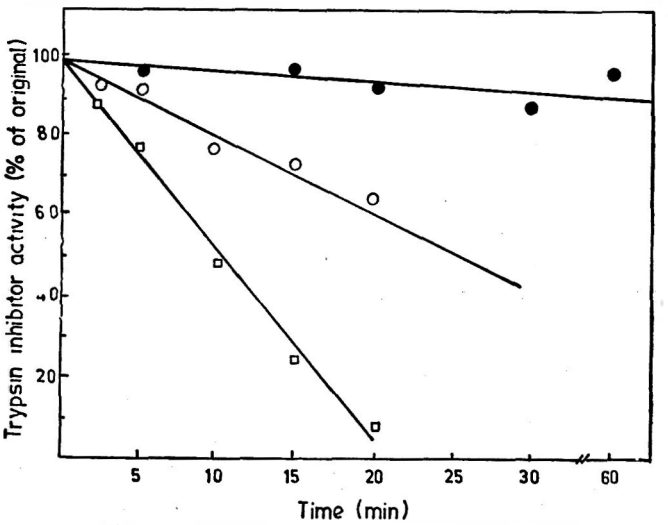

Fig 2. Heat inactivation of extracted trypsin inhibitor from horse gram meal. Incubated at: 50°C (●——●), 80°C (O——O) and 100°C (□——□).

Data on changes in trypsin inhibitor activity during seed germination are presented in Table 1. The soaking of horse gram seeds decreased trypsin inhibitor activity by 35 per cent. However, germination had no effect on the activity of the inhibitor. When germinated seeds were cooked, there was a drastic reduction (90 per cent) in the inhibitor activity. Heat resistant trypsin inhibitor activity has been shown in case of winged bean meal[7]. This was attributed to the presence of polyphenols. The occurrence of polyphenols in the seed coat of horse gram seed is reported[6]. It is not known whether polyphenols present in horse gram exert similar influence.

TABLE 1. EFFECT OF GERMINATION AND COOKING ON TRYPSIN INHIBITOR ACTIVITY OF HORSE GRAM

Treatment	Treatment period (hr)	Trypsin inhibitor activity (units/g sample)	
		Uncooked	Cooked
Raw (dry seeds)	—	94.00	—
Water soaked at 4°C	8	55.49	21.23
Germinated	12	57.79	6.06
	24	57.79	6.06
	48	56.59	6.06

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DETECTION OF ADULTERANT *KARANJA* (*PONGAMIA GLABRA*) SEED CAKE IN OTHER OILSEED CAKES

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Two colour tests have been developed for the detection of *karanja* (*Pongamia glabra*) seed cake in other oilseed cakes. Alcoholic extracts of *karanja* cake or mixtures containing 1% or more of *karanja* cake gave red colour ring with sulphuric acid while alcoholic extracts of seed cake mixtures containing 5% or more *karanja* cake gave yellow colour with phosphoric acid.

Karanja (*Pongamia glabra*, Syn. *Pongamia pinnata*), a member of Leguminosae family is known as one of the main commercial non-edible minor oilseed plant in India[1] with a potential to produce over one lakh tonnes of the seed and about 30,000 tonnes of this seed oil annually[2]. *Karanja* oil is non-edible due to the presence of toxic flavonoid compounds particularly karanjin and pongamol[3]. Recently, techniques have been evolved for the removal of these toxic constituents[3-5] and at present purified oil is extensively used in soap and other industries. *Karanja* oil is usually cheaper than many other vegetable oils and its use is likely to increase gradually. Therefore, a large amount of this defatted seed cake is now obtained as by-product. This seed cake is white in colour and has a bitter taste and unsuitable for feeding due to presence of saponins (3 per cent), a toxic and complex amino acid named glabrin and some flavonoid compounds. The toxicity of raw *karanja* cake was studied in our laboratory[6] and it was found that raw seed cake is highly toxic to rats[7,8]. Being the cheapest oilseed cake, *karanja* cake, can perhaps be used for adulterating many other edible or non-edible oilseed cakes. The present communication reports two sensitive and quick colour tests applicable for *karanja* seed cake exclusively or when such cake is admixed with other edible and non-edible oilseed cakes.

Extraction: Twenty five grams of the defatted *karanja* seed cake was refluxed with 100 ml of 95 per cent ethanol over hot water bath for 5 hr. After cooling, the extract was filtered and the filtrate was concentrated under reduced pressure. Then the filtrate was bleached with active carbon (0.5 g) and the carbon was filtered off from the extract.

Detection test A: To 4-5 ml of sulphuric acid in a test tube 4-5 drops (0.25 ml) of the alcoholic extract were added along the sides of the test tube so as to form a ring on the top of the acid layer. The colour of the ring and time taken for development of colour was noted; (*i*) a bright red-coloured ring appearing immediately indicates the presence of *karanja* cake or mixtures containing 4 per cent or more of *karanja* cake, (*ii*) a red-coloured ring appearing within 1 min indicates that the mixture contains 2-4 per cent of *karanja* cake, and (*iii*) a light red-coloured ring appearing in about 2-5 min indicates a mixture containing 1-2 per cent of *karanja* cake. This red coloured ring is due to the presence of saponins in the *karanja* seed cake. It is reported that saponins give striking colours on coming in contact with sulphuric acid[9,10]. When other saponins isolated from the seed cakes of *akashmoni* (*Acacia auriculaeformis*), *babul* (*Acacia arabica*) or *mahua* (*Bassia latifolia*) were treated with sulphuric acid, they gave the pink or red colour indicating that the red-coured ring in the present investigation was due to the presence of saponins in the *karanja* cake. This test was performed with several edible and non-edible oilseed cakes such as groundnut (*Arachis hypogaea*), mustard (*Brassica nigra*), rape (*Brassica napus*), soybean (*Glycine max*), linseed (*Linum usitatissimum*), niger (*Guizotia abyssinica*), neem (*Azadirachta indica*), *undi* (*Calophyllum inophyllum*) and *khakan* (*Salvadore oleoides*). The alcoholic extracts of these oilseed cakes gave colourless, pale yellow, brown or sometimes darkish coloured ring with sulphuric acid (Table 1). The light red to bright red-coloured ring obtained as a result of the presence of *karanja* cake is striking and cannot be mistaken for a false positive test.

Detection test B: To 1 ml of the alcoholic extract in a test tube 2-3 drops of phosphoric acid (88 per cent) was added. An immediate characteristic canary yellow to orange yellow colour indicates the presence of *karanja* cake to the extent of 5 per cent or more in the mixture. This yellow colour is probably due to flavonoid compounds. It has been shown that when pure flavonoid compounds such as karanjin, pongamol and pongaglabrone isolated from the seed cake were treated with phosphoric acid (88 per cent), yellow to orange yellow colour is produced. Thus, this colour test with phosphoric acid may also be specific for seed

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TABLE 1. COLOUR REACTION OF ALCOHOLIC EXTRACTS OF *KARANJA* SEED CAKE WITH OTHER OILSEED CAKES

Oilseed cakes	Ring colour with H_2SO_4 (A)	Colour with phosphoric acid (B)
Karanja	Bright red	Orange yellow
Groundnut	Colourless	Colourless
Mustard	Yellowish green	Light green
Rape	Yellowish green	light brown
Soybean	Light yellow	Colourless
Linseed	Yellow	Light pink
Niger	Dull light brown	White
Undi	Dark brown	Light grey
Neem	Greyish brown	Brown
Khakan	Brown	Steel grey
Karanja (5% or more) +other cake	Bright red	Canary yellow
Karanja (2-4%) +other cake	Red	—
Karanja (1-2%) +other cake	Light red	—

cake like *karanja* which contains a number of flavonoid compounds. This test was also carried out with a number of edible and non-edible oilseed cakes but none of them gave yellow or orange yellow colour (Table 1).

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PREPARATION OF DEHYDRATED *MUKHI* (*COLOCASIA ESCULENTA*) CHIPS

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Chips free of acridity and stickiness were prepared from tubers of 'Mukhi kachu', a highland cultivar of *Colocasia esculenta* Schott. Slices were subjected to nine different processing treatments and dried in cabinet solar dryer. Acid treatment of the tuber chips improved their appearance and acceptability. Chips made by treating with tartaric, citric and hydrochloric acid followed by washing and immersion in metabisulphite were found to be highly acceptable. On reconstitution, the chips were found to be of better eating quality, almost free of acridity and stickiness. The chips retained colour and texture when stored for six months in polyethylene bags.

'*Mukhi kachu*', a cultivar of *Colocasia esculenta*, which is widely grown and used as vegetable, promises to offer a potential supplementary source of calories. But its tuber failed to gain attention for processing into food products owing to its acrid and mucilagenous property. Saha and Hussain[1] reported that the irritating substance in aroids which include 'Mukhi-kachu' was a glycoside. The elimination of acridity and mucilagenous property is necessary to enhance the acceptability of *mukhi* as a supplementary starchy food. According to Abdel *et al.*[2] the mucilagenous

substance present in *mukhi* (*Colocasia esculenta*) is polysaccharide which could be extracted by water[3].

To reduce the acrid feel in the mouth, tamarind and lemon are generally used by housewives. According to the method of Suzuki[4], the acrid principle which is a glycoside, could be extracted by 95 per cent ethanol. So different processing techniques aimed at elimination of acridity, involved the use of heat treatment in slightly acidic media and ethanol extraction, etc. among others. Blanching and sulphuring are other common steps in the preparation of processed foods.

The present study was undertaken to investigate the steps necessary to prepare acceptable dehydrated *mukhi* chips free of irritation and mucilage.

A widely cultivated variety '*mukhi kachu*' locally named, 'Guavir', originally collected from Habiganj, Sylhet, Bangladesh, was chosen for the preparation of dehydrated chips, as it was moderately irritating on one hand and was produced on commercial scale on the other. After sorting and peeling, the '*mukhi*' were sliced, each slice being about 3-4 mm thick. The slices were subjected to nine different treatments as shown in Table 1. The acids used in some treatments were tartaric acid, citric acid and hydrochloric acid. The variously treated slices were then dried for 14 hr in a cabinet solar dryer (1.8m×0.96m) fabricated according to the design proposed by Bangladesh Council of Scientific and Industrial Research[5]. The dehydrated *mukhi* chips were subjected to chemical analysis for their moisture and protein contents according to AOAC[6]. Colour and physical characteristics of each type of chips were also noted.

For reconstitution, the dehydrated *mukhi* chips were soaked in cold water for an hr and then boiled in water (400g/l) for 40 min. The rehydrated chips were served to a panel of 10 semi-trained volunteers of an age groups of 20-25 years, for organoleptic test. The criteria chosen for evaluation of quality were degree of acridity, softness, texture, taste and stickiness. For acridity, softness, texture, and taste, a 'hedonic' scale of 7 was used for ranking which included like very much (7), neither like nor dislike (4), and dislike very much (1). For degree of acridity, the criteria used were not irritating (7) and very much irritating (1). A freshly boiled non-acrid sample was served as control. The average score for degree of acridity,

TABLE 1. PROCESSING STEPS IN THE PREPARATION OF DEHYDRATED *MUKHI*++ CHIPS

Processing treat.	A	B	C	D	E	F	G	H	I
1. Sorting, peeling, slicing	+	+	+	+	+	+	+	+	+
2. Blanching at 65–70°C for *5 min.	+	—	—	—	—	—	—	—	—
3. Dipping in 95% ethanol for 18 hr.	—	—	—	—	—	—	—	+	—
4. Dipping in water for 15 hr and draining	+	+	+	+	+	+	+	+	—
5. Boiling in water for 10 min	+	+	+	+	+	—	—	—	—
6. Boiling in acid sol. for 10 min.	—	—	—	—	0.5*	0.25*	0.25*	—	—
7. Blanching at 70-75°C for 5 min.	—	—	—	—	—	—	—	+	—
8. Dipping in water for 2 hr and washing	—	—	—	—	+	+	+	—	—
9. Immersing in KMS sol.	0.3*	0.3*	0.5	0.5	0.3	0.3	0.75	0.3	—

—Chips were not subjected to this step

+Chips were subjected to this step

++*Mukhi* slices were dried in solar drier cabinet for 14 hr.

Potassium metabisulphite (KMS) treatment was given for 10 min for all treatments except in D where it was 2 hr.

Figures indicate the % concn. of tartaric, HCl (v/v) and citric acid for E, F and G respectively in sl. no. 6. In sl. no. 9 figures indicate % concn. of metabisulphite solution.

TABLE 2. PHYSICAL AND CHEMICAL QUALITY OF DEHYDRATED *MUKHI* CHIPS

Dehydrated chips	Texture	Colour	Moisture (%)	Crude protein (% on dry wt. basis)	Protein loss in processing (%)
A	Hard, non-uniform	Blackish white	10.75	8.90	28.11
B	Hard, non-uniform	Blackish white	10.64	8.96	27.62
C	Comparatively soft	Blackish creamy and non-uniform	7.44	9.45	23.66
D	Comparatively soft	Blackish creamy and non-uniform	7.84	9.71	21.56
E	Comparatively soft	Creamy and uniform	10.38	9.60	22.43
F	Comparatively soft	Creamy and uniform	10.40	5.34	56.86
G	Comparatively soft	Creamy and uniform	5.83	7.79	37.07
H	Comparatively soft	Brownish white	11.24	8.55	30.93
I	Soft	Brownish white and uniform	9.14	12.34	0.0

softness, texture and taste was calculated separately and standard error was calculated for each. The extent of mucilagenous property retained after reconstitution was also noted by organoleptic test by the same set of 10 panelists by the degree of stickiness.

Physical characteristics and the results of chemical analyses of different dehydrated *mukhi* chips were shown in Table 2. Among different types of chips prepared, the physical appearance of chips-E, F and G were better having a shiny creamy uniform colour with crispness. Chips-H was brownish white while chips-A, B, C and D were blackish white in colour. In the present study, chips either blanched or unblanched, were not found to have developed any undesirable colour during dipping in water for 15 hr. Blanching is known to prevent the enzymatic activity which may cause undersirable colour development during processing and storage.

Except chips-I, all the others were treated with different concentration of potassium metabisulphite solutions for various periods of time. Sulphuring prevents undesirable browning and blackening of the product during drying and storage. The presence of sulphur dioxide also helps retention of upto 50 per cent of vitamin C and more than 60 per cent beta-carotene in the dried product[5]. The acid-treated slices had more acceptable colour and appearance. The moisture contents in the chips varied from 5.83 to 11.24 per cent, with the highest content in chips-H and the lowest value in chips–G. Chips-G prepared by treating slices with 0.75 per cent potassium metabisulphite solution for 10 min had the lowest moisture content. Slices treated with 0.5 per cent potassium metabisulphite solution for 2 hr and 10 mins produced chips-D and chips-C. respectively. The moisture per cent in chips-D and chips-C, were 7.84 and 7.44 respectively. Processing techniques that included 0.75 per cent metabisulphite treatment appeared to decrease protein content, though insignificantly. For better storage quality, lower moisture contents is important. Treatment of chips by dilute acids like tartaric acid (a major acid in tamarind), hydrochloric acid and citric acid (a major acid in lemon) improved the physical appearance of the chips conferring attractive creamy colour and comparatively soft texture and as such enhanced acceptability. Higher concentration of tartaric acid was needed to eliminate acridity compared with citric acid and hydrochloric acid. Hydrochloric acid treatment caused higher reduction of protein in chips. Hydrochloric acid treatment appears to cause hydrolysis of protein as well as slime polysaccharide quite considerably. HCl treatment may not be practised since tartaric acid and citric acid produced equally good result without causing much loss of protein. Assuming no loss of protein from Chips-I (as the processing method involved peeling, slicing and drying only) the of loss of protein in other chips varied from 21.56 to 56.86 per cent, maximum loss occurring in chips-F. The losses may be attributed to washing out of the water soluble protein. The maximum loss of 56.86 per cent protein in chips-F might have resulted from the hydrolysis of protein by HCl followed by removal of free amino acids and smaller peptides.

TABLE 3. ORGANOLEPTIC SCORE OF RECONSTITUTED *MUKHI* CHIPS

Reconstituted chips	Acridity Mean±S.E.	Softness Mean±S.E.	Texture Mean±S.E.	Taste Mean±S.E.
A	6.0±0.21	4.5±0.31	4.5±0.22	4.0±0.15
B	6.2±0.25	5.0±0.26	4.5±0.27	4.0±0.21
C	7.0±0.00	4.0±0.26	4.5±0.31	5.0±0.21
D	7.0±0.00	4.0±0.26	4.0±0.15	5.0±0.26
E	7.0±0.00	6.0±0.26	5.0±0.26	4.0±0.15
F	7.0±0.00	6.0±0.21	5.0±0.26	5.0±0.26
G	7.0±0.00	4.0±0.21	4.5±0.22	4.0±0.21
H	7.0±0.00	4.5±0.27	4.5±0.22	5.0±0.26
I	5.5±0.17	4.0±0.15	5.0±0.33	4.0±0.26

Mucilagenous property was negligible in A, B, C, D, E and F, and not present in F and H. It was present only in I.

Organoleptic test of reconstituted chips (as shown in Table 3) indicated that rehydrated *mukhi* chips were moderately acceptable to the panelists who rated them between 'neither like nor dislike' and like very much'. From the view point of non-acrid nature, except A and B other samples were highly acceptable. The mucilagenous substance in all chips were either removed totally or reduced to a great extent in comparison with that in chips-I. The chips-F and chips-H were completely free of mucilagenous property. Considering all the criteria of eating quality of reconstituted chips, chips E, chips-F, and chips-G seemed to be highly acceptable. The chips appeared to retain colour, texture and softness when preserved in polyethylene bags over a period of six months.

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MICROBIOLOGICAL QUALITY OF MILK AND SPRAY DRIED SKIM MILK POWDER USED AS ICE CREAM INGREDIENTS IN KARNAL

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Ten parameters were chosen for the microbiological analysis of raw milk and skim milk powder. Milk from experimental dairy of NDRI contained Gram negative organisms and coliforms even after pasteurization, indicating post-pasteurization contamination. Application of the paired 't' test for milk was found to be significant at 1% level in case of standard plate count and acid producers. Skim milk powder was found to be the second main source of contamination to the ice cream mix which contributed spore formers to the finished product.

The hygienic quality of ice cream mainly depends on the ingredients used for manufacture[1]. Microorganisms in ice cream are very important from the public health point of view as well as to avoid legal complications under PFA[2]. Many reports are available on the food poisoning occurring through ice cream[3]. Very few reports[4] are available on the extent of contamination through the ingredients and their impact on the quality of the finished product. Keeping these points in view the present investigation was taken up to study the extent of contamination coming from the ingredients, raw milk and spray dried skim milk powder used in the manufacture of ice cream in Karnal city and the type and number of microorganisms present.

The samples of milk and spray dried skim milk powder from the experimental dairy of this Institute (source A) and also from the market (source B) were asceptically collected in sterile bottles and examined for total bacterial count, acid formers, proteolytic, chromogenic, Gram negative, coliforms, yeast and molds, psychrotrophs, *staphylococci* counts and also spore counts in the case of skim milk powder. Total bacterial count was determined by the ISI method[5]. The plates used for total counts were also used for counting acid producing colonies (light or deep blue in colour); proteolytic colonies showed zone formation around the colonies, whereas chromogenic colonies showed pigment production by the bacteria. The Gram negative count was determined as suggested by Meiklejohn[1]. Coliform count was determined by the most probable number (MPN) method by using Eosine methylene blue agar (EMB) as suggested by APHA[6]. Yeast and mold counts were determined as suggested by APHA[7]. Psychrotrophic count was determined

TABLE 1. TYPES OF ORGANISMS (COUNT PER G OR ML) IN MILK

Type of organism	Source A (8)			Source B (8)		
	Minimum	Maximum	Average	Minimum	Maximum	Average
SPC	6.2×10^3	25.0×10^3	16.75×10^3*	0.8×10^3	4.0×10^3	2.212×10^3*
Acid producers	5.2×10^3	9.6×10^3	7.42×10^3*	0.3×10^3	2.0×10^3	1.075×10^3*
Proteolytic	0.1×10^3	2.0×10^3	0.562×10^3	—	—	—
Chromogenic	17.0	100.0	18.875	—	—	—
Gram negative	24.0	400.0	132.125	—	—	—
Coliforms						
MPN	2.0	92.0	27.1625	—	—	—
Total	7.0	40.0	22.875	—	—	—
Yeast and molds	2.0	156×10^2	32.5×10^2	—	—	—
Psychrotrophs	3.0	720.0	143.75	—	—	—
Staphylococci	—	3.0	0.375	—	—	—

*A and B significantly different at 1% level.

Figures in parentheses indicate number of samples used for testing.

as suggested by Witter[8]. Staphylococcal counts were determined as per Sharpe *et al.*[9] Spore count was estimated according to Ingram[10].

The results of the microbiological analysis of milk are given in Table 1. None of the samples from Source A and Source B were found to exceed the standard plate count (SPC) limit of 30,000/ml for pasteurized milk as prescribed in ISI specifications. Four samples (50 per cent) from Source A and all samples (100 per cent) from Source B were within limits of ISI specifications (10 coliforms/ml) for pasteurized milk[5].

Milk from Source B was of better quality than that from Source A, standard plate count and acid producers[11] significantly differed between them. The difference between the two sources can be attributed to the fact that experimental dairy of this Institute (Source A) were pasteurizing the milk before addition while in market (Source B) it was boiled before making the mix. Presence of Gram negative organisms, coliforms, yeast and molds in milk from Source A even after pasteurization indicates post-pasteurization contamination of milk[12]. Singh[13] reported the heat resistance of coliform organisms. Presence of psychrotrophs in a few samples may be from dirty water and utensils[12]. Verma[14] has observed higher average standard plate count, coliform count and yeast and mold count. This can be attributed to the fact that the samples analysed were of raw milk and has not been subjected to any heat treatment. *Staphylococci* were present (3/ml) in sample A. Sharpe *et al.*[9] reported the occurrence of *Staphylococci* in heat treated milk.

The results of the microbiological quality of the skim milk powder are given in Table 2. Only source A used skim milk powder for raising the solids-not-fat (SNF) content of the ice cream mix. Two samples (25 per cent) according to MPN for coliforms were found to be within the limit of ISI specifications of not more than 90 coliforms per gram for dried milk powders[16].

Extent of contamination coming from the skim milk powder as shown in Table 2 was due to the survival of microorganisms during the high heat-treatment of production of skim milk powder. Verma[14] observed total plate count per gram in dried milk to be 4,800. This was lower than the present investigation (21.262/g av). Higher standard plate count observed during

TABLE 2. MICROBIOLOGICAL QUALITY OF SPRAY DRIED SKIM MILK POWDER PREPARED FROM MILK FROM EXPERIMENTEAL DAIRY N.D.R.I., KARNAL

Type of organism	Minimum	Maximum	Average
SPC	3.2×10^3	39×10^3	21.262×10^3
Acid producers	2.2×10^3	16×10^3	6.437×10^3
Proteolytic	—	3×10^3	1.187×10^3
Chromogenic	—	6×10^3	1.45×10^3
Gram negative	230.0	950.0	601.25
Coliforms:			
MPN	13.0	430.0	153.00
Total	18.0	460.0	182.25
Yeast and molds	2.2×10^3	10.6×10^3	5.2×10^3
Staphylococci	40.0	5.600	952.5
Spores	160.0	6.300	2420.0

Average of 8 samples.

the present investigation in skim milk powder can be considered as the contamination occurring during grinding and filling of the powder[11]. Lower coliform count (62/g) than the present investigation (153/g) was observed by Verma[14]. Average yeast and mold were found to be 52,000/g while Verma[14] reported lower counts (yeast 570 and molds 200/g). High yeast and mould indicates unsanitary conditions of handling and contamination from air. Higher staphylococcal count (5600/g) was found in one sample, indicating contamination from nose and hands of the employees. Spore count ranged from 160 to 6,300/g showing that they might have survived the high heat treatment[15]. Chances of contamination of the product by air which has high spore load cannot be ruled out. Acid producers were found to be mostly Gram positive cocci in chains and the average count was 6,437/g. Average proteolytic count per gram was 1.187×10^3 and they were found to be mostly Gram positive spore formers in chains. Gram negative bacterial count ranged from 230 to 950/g. High Gram negative bacterial count can be considered to be heat resistant and due to post preparation contamination of the product. Heat resistance of coliforms has been observed by Singh[13]. Lightbody[12] reported post-pasteurization contamination of milk by Gram negative rods.

It was concluded that milk from Source A contained Gram negative organisms and coliforms even after pasteurization, indicating post pasteurization contamination. If the milk was pasteurized or boiled before making ice cream mix, the contamination could be avoided. Similarly skim milk powder was found to be the second chief source of contamination in the ice cream mix and contributed spore formers. This could be avoided if the milk powder was stored in an air tight container and dust free room.

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ENHANCEMENT OF COLOUR AND TEXTURE OF MUTTON BY THE USE OF LACTIC ACID BACTERIA

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Studies were carried out with mutton and three lactic cultures, namely *Lactobacillus plantarum*, *Lactobacillus bulgaricus* and *Streptococcus lactis*. Retention of colour after 6 days at 5°C under 90-98% RH was better in mutton samples treated with *L. bulgaricus* and *S. lactis* as also mixture of the two. Percentage myoglobin content in untreated samples was lower than in treated samples. An improvement in texture was also seen in treated samples as indicated by the texturometer readings. There was detectable drop in pH in treated samples during the first three days of storage which got stabilized and remained nearly constant during the subsequent period of storage.

Colour and texture constitute two major quality attributes that decide the consumer acceptability of fresh meat. Colour is the most important factor deter-

mining retail selection since the consumer equates it to freshness and relies on it as the criterion of quality[1,2]. Although tenderness improves on storage under chilled temperatures, the colour deteriorates, predominantly due to bacterial growth and also due to oxidation. Treatment with chemicals has been shown to be useful in retaining the colour, but they have adverse effect on biological systems. The present trend in preservation is towards an ecological approach. Many of the micro-organisms which form part of the natural flora of carcass may be able to bring about improvement in meat quality. Tenderness is improved in the presence of organic acids. As lactic acid is produced by lactic *Streptococci* and *Lactobacilli*, a combination of these could be more effective. In addition to being harmless, these organisms also check the saprophytic, pathogenic and toxigenic bacterial growth[3-8].

In the present study an attempt was made to extend the shelf-life of meat by changing the relative preponderance of the natural microflora of meat in such a way as to improve its quality.

The *longissimus dorsi* (LD) muscles from hind legs of young 'Bannur' sheep were collected from the local market. LD muscles of the same animal were used for treatment as well as for control. Muscles used were from the same region in all the three trials.

Lactobacillus plantarum, *Lactobacillus bulgaricus* and *Streptococcus lactis* were grown overnight in Rogosa broth, MRS broth and lactic broth respectively in sufficient quantity. A thick inoculum of the test culture was prepared in 10 ml sterile peptone water as per the method of Bartholomew *et al.*[9] The inoculum was diluted with sterile peptone water to get a cell concentration of 10^{10}/ml; 5 ml of this inoculum was smeared on the surface of 100 g muscle with the help of a pipette[9]. In the case of mixtures of cultures the two inocula were mixed in 1:1 proportion of which 5 ml was used for the treatment. *L. bulgaricus* and *S. lactis* were used individually and also in combination, whereas *L. plantarum* was used only in combination with *S. lactis*. The initial microbiological analysis of the treated samples showed a count of 10^8 cells/g of meat. After treatment the muscles were hung on hooks at 5°C under 90-98 per cent RH for 6 days along with the control (100 g muscle without any treatment).

The meat pigments were extracted and estimated by the method of Broumand *et al*[10]. Texturometric readings were taken on a texturometer (Zenken Co. Ltd). pH values were determined by the method of Reddy *et al.*[4] The subjective evaluation of cooked samples was carried out after 6 day storage by a taste-panel of 10 members. The results were recorded at regular intervals upto 6 days.

Table 1 shows that the per cent myoglobin content in untreated sample was brought down from about 94 per cent to about 62 per cent whereas in the experimental sample the decrease was much lower. Better colour retention in treated samples may be attributed to the suppressive action of lactic acid bacteria on spoilage organisms, which are predominantly Gram negative aerobes that cause surface discolouration of meat at low temperatures. The role of bacterial growth in colour alterations of tissues and tissue extracts have been studied by a number of workers during storage in air at low temperatures[11-15,17]. All the aerobic bacteria were active at 4±1°C and would give the same results causing the pigment to change from red to brown and finally to purple at post-spoilage level[12]. On the other hand, *Lactobacilli* and *Streptococci*, which do not utilize oxygen to any appreciable extent, may not bring about the discolou-

TABLE 1. PER CENT MYOGLOBIN AND PH OF MUTTON TREATED WITH MICROBIAL CULTURES AND STORED AT 5°C AND 90-98% RH

Storage period	*L. bulgaricus*		*S. lactis*		*L. plantarum* + *S. lactis* (1:1)		*L. bulgaricus* + *S. lactis* (1:1)	
(days)	Control	Treated	Control	Treated	Control	Treated	Control	Treated
0	95.0	95.0	96.8	96.8	94.7	94.7	93.0	93.0
	(5.59)	(5.59)	(5.70)	(5.53)	(5.53)	(5.52)	(5.60)	(5.60)
3	71.3	81.0	71.3	82.0	68.8	80.5	71.3	78.0
	(5.80)	(5.30)	(5.81)	(5.36)	(5.64)	(5.12)	(5.70)	(5.31)
6	64.2	72.8	64.2	74.5	60.3	72.0	62.6	71.3
	(6.08)	(5.30)	(5.95)	(5.34)	(5.80)	(5.14)	(6.00)	(5.30)

Figures in the parentheses are pH values

ration of meat. Robach and Costilow[12] did not observe any colour alteration either at room temperature or at 4°C in meat treated with *L. plantarum*. Similar results have also been reported by Ledward[11] for *Lactobacilli* spp.

There was a definite fall in the pH of treated samples during the first three days of storage (Table 1). Afterwards it got stabilized and remained more or less constant during the subsequent period of storage. These results are in agreement with those of Reddy *et al.*[4,5] who studied the effect of resting cells of lactic cultures on beef quality under similar conditions of storage. The fall in pH in treated samples was mainly due to the production of lactic acid by test cultures. The higher pH in control samples may have resulted from production of ammonia and amines due to decarboxylation and deamination of amino acids by the natural flora of meat.

The texturometric readings are given in Fig 1. Samples treated with *L. bulgaricus*, *S. lactis* and a mixture of the two showed definite improvement in tenderness. In samples treated with *L. bulgaricus* and *S. lactis* the texturometer reading was 5.93 and 7.40 units/volt respectively. The values for the samples treated with the mixture was 7.11 while the values for controls were 4.47, 6.83 and 6.47 units/volt respectively. Tezcan and Yucel[16] observed a slight increase in tenderness of beef treated with butter milk rich in *Lactobacilli*, and Ockerman *et al.*[17] observed a uniform increase in tenderness of bovine tissue treated with *L. mesenteroides* for 7 days under low temperatures. The mechanical strength of raw meat resides chiefly in its connective tissue components[20]. Any change in structure of collagen molecule may therefore alter the tenderness of meat. According to Ali and Yeates[18] lactic acid may break the acid-labile electrovalent cross-linkages in the collagen molecule thereby unfolding the triple helical structure of collagen. This would make the collagen more pliable and soft. They have also reported greater degree of hydration and better swelling at lower pH values. A low pH may also weaken the sarcolemma. The improved tenderness in treated samples may also be due to the controlled proteolytic activity of natural flora of meat in the presence of the test cultures. The lower values of tenderness in controls may be attributed to low hydration of connective tissues and to precipitation of certain meat proteins at higher pH of 5.8 to 6.0. Bouton *et al.*[19] observed maximum toughness in *Longissimus dorsi* muscles from sides of bovine at the same pH range.

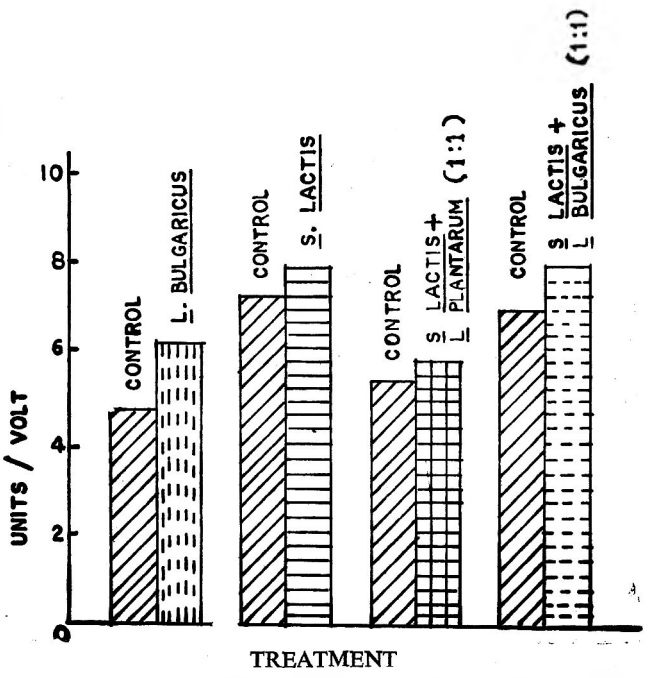

Fig 1. Texturometric readings of mutton samples treated with microbial cultures after six days of storage at 5°C and 90-98% R.H.

Organoleptically all the samples were acceptable. The added cultures did not impart any off-taste or off-odour to the meat. Thus the present study indicates that there is scope for bringing about better quality attributes like colour, texture, etc. to raw meat by treating with lactic acid bacteria.

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A RAPID MADEIRIZATION PROCESS TO IMPROVE MANGO DESSERT WINES

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Mango dessert wines were added ascorbic acid (0.1% w/v) which helped in ageing rapidly upon madeirization at 50°C for 7 days. This process reduced titratable acidity, volatile acidity, increased the pH, volatile esters, aldehydes, colour brightness and organoleptic scores of dessert wines. Madeira prepared by this process from 'Totapuri' variety of mango was more acceptable than those from 'Raspuri', 'Mulgoa', 'Dashehari' and 'Langra' varieties. Madeirized wines were more acceptable than the corresponding dry and dessert wines.

A recent study on mango wines indicated that madeirization at 50°C for 24 days improved the organoleptic quality of mango dessert wines with the exception of those having strong varietal aroma[1]. In the present paper the effect of adding an antioxidant namely ascorbic acid and shorter baking period of one week on the composition and quality of dessert wines made from five commercial mango varieties is reported. Dry, dessert and madeirized wines were prepared from 'Raspuri', 'Totapuri; 'Mulgoa', 'Dashehari' and Langra' varieties of mangoes. Venification, dessert wine preparation and methods of analysis were same as those reported earlier[1]. For rapid madeirization, ascorbic acid was added at 0.1 per cent (w/v) to the dessert wines, mixed and bottled in 650 ml bottles leaving 25 ml head space in each and heated in B.O.D. incubator at 50±2°C for 7 days. After baking, the bottles were cooled to 18°C. The rapidly madeirized wines were not aged further before chemical analysis and sensory evaluation. Organoleptic evaluation of wine samples was done by a panel of 5 judges on a standard 20-point score card described by Ough and Baker[2]

Fortification and madeirization led to a reduction in pH of wines (Table 1). Upon madeirization ester content increased by 9 to 46 mg/l over the corresponding dessert wines. Increase in esters in madeirized wines upon prolonged baking of dessert wines from 23 varieties varied from 2 to 45 mg/l[1] indicating the possible role of ascorbic acid in formation and retention of esters in madeirized wines. The slight increase in aldehyde level in madeirized wines over the corresponding dessert wines indicates limited oxidation in spite of the presence of ascorbic acid. The dessert and madeira wines from 'Raspuri' and 'Langra' varieties had more esters compared to wines from other varieties. This is probably due to high ester content in dry wines and their brandies used for their preparation. All the three types of wines from 'Totapuri' were low in esters as compared to the wines of the other varieties. Similar changes in chemical composition on madeirization of apple wines have been reported recently[3].

The colour and brightness of dry wines was better than that of dessert wines as mango brandy and sugar syrup added to dry wine during preparation of dessert wine diluted these characteristics (Table 2). Wines from 'Raspuri' variety showed maximum colour intensity and brightness, while those of 'Totapuri' the least. Madeirization increased the colour and brightness of all dessert wines, the increase varying from 6 to 44 per cent for colour and from 4 to 35 per cent for brightness over the corresponding dry wines. Such variations in colour and brightness seem to be due to the inherent

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TABLE 1. INFLUENCE OF RAPID MADEIRIZATION ON COMPOSITION OF MANGO WINES

Variety	pH			Titratable acidity (as g tartaric acid/100 ml)			Volatile acidity (as g acetic acid/100 ml)			Total volatile esters (as ethyl acetate mg/l)			Total aldehydes (as acetaldehyde mg/l)		
	Dry	Dessert	Madeira	Dry	Dessert	Madeira	Dry	Dessert	Madeira	Dry	Dessert	Madeira	Dry	Dessert	Madeira
'Raspuri'	3.90	4.00	4.00	0.85	0.82	0.82	0.073	0.067	0.056	170	294	340	157	93	97
'Totapuri'	3.70	3.85	3.95	1.07	0.92	0.86	0.089	0.083	0.067	80	106	146	61	58	73
'Mulgoa'	3.85	3.90	3.94	0.89	0.76	0.70	0.078	0.067	0.056	86	147	156	72	63	65
'Dashehari'	3.80	3.76	3.85	0.87	0.89	0.85	0.100	0.089	0.073	116	135	156	61	67	81
'Langra'	4.12	4.00	4.10	0.65	0.75	0.73	0.112	0.100	0.078	162	215	251	95	81	92

TABLE 2. EFFECT OF RAPID MADEIRIZATION ON COLOUR, BRIGHTNESS AND ORGANOLEPTIC QUALITY OF MANGO WINES

Variety	Colour A 420 nm			Brightness A 420 nm + A 520 nm			Organoleptic score (out of 20)		
	Dry	Dessert	Madeira	Dry	Dessert	Madeira	Dry	Dessert	Madeira
'Raspuri'	0.495	0.420	0.523	0.609	0.522	0.631	13.5	14.75	15.79
'Totapuri'	0.215	0.194	0.310	0.275	0.252	0.381	13.2	14.85	15.86
'Mulgoa'	0.310	0.260	0.409	0.366	0.306	0.480	10.0	12.83	13.81
'Dashehari'	0.208	0.187	0.276	0.264	0.238	0.342	11.0	13.04	14.31
'Langra'	0.420	0.367	0.523	0.569	0.423	0.684	13.0	14.32	15.41

differences in pigment composition of these varieties. The scores for colour and appearance for all madeira wines were better than their corresponding dry and dessert wines. Sensory evaluation of wines indicated that dry wines from 'Mulgoa' and 'Dashehari' are not acceptable as table wines due to high astringency. Fortification of dry wines with sugar and brandy mellowed this character slightly. Dessert wines from 'Totapuri', 'Raspuri' and 'Langra' possessed acceptable taste. Madeirization improved organoleptic characters of all the five dessert wines. Improvement was mainly observed in colour and appearance, bouquet, freedom from acetic odour and development of a characteristic madeira flavour. Madeiras of all varieties possessed organoleptic characteristics of standard wines. Since 'Raspuri', 'Mulgoa', 'Dashehari' and 'Langra' are good table purpose varieties and are fairly expensive as raw material for the wine making. 'Totapuri' which is less acceptable for table purpose is cheap and can be used for wine making. Mild colour and flavour of this variety seem to be ideal as the resulting wine had a pleasant light golden yellow colour and a distinct mild fruity flavour. Ester content of wines from this variety was also modest. Rapid madeirization process which involved baking of dessert wines in the presence of air and added ascorbic acid for a short duration helped to improve the quality of dessert wines.

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STUDIES ON PERSISTENCE OF FENITROTHION AND PHOSALONE RESIDUES ON BRINJAL

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Dissipation of fenitrothion and phosalone residues were studied on brinjal fruits over a period of 14 days after spraying of the insecticides. Based on the half life and total values a waiting period of 3.31 days for fenitrothion and 1.29 days for phosalone is recommended.

Two organophosphorus insecticides fenitrothion (0, 0-dimethyl-0-(3-methyl-4-nitrophenyl)-thionophosphate) and phosalone (0, 0-diethyl S-(6-chloro-2--oxobenzoxolin-3-yl) methyl phosphorodithioate) were reported to be effective against spotted leaf beetle[1] and mites[2] which cause serious damage to brinjal. The present studies were undertaken to ascertain the rate of dissipation of residues of the two insecticides on brinjal fruits and determine the safe period before the produce is consumed.

About 500 g fruit samples were drawn at random from each plot first within one hr and subsequently after 1, 3, 5, 7, 10 and 14 days of spraying. The samples were cut into small pieces and finally a representative sample of 50 g was taken for analyses after thorough mixing and quartering. The samples were extracted thrice, first with 100 ml and subsequently with 50 ml of redistilled n-hexane and acetone for fenitrothion and phosalone, respectively. The extracts were filtered under vacuum through a Buchner funnel fitted with Whatman No.1 filter paper. The extracts were concentrated, passed through a glass column containing a mixture of activated charcoal-celite-magnesia (2:1:1) and then eluted with redistilled n-hexane and acetone until about 500 ml eluate was collected. The cleaned-up extracts were then dried over anhydrous Na_2SO_4, filtered, concentrated and made up to 10 ml from which aliquots were drawn for analysis.

The samples were analysed for the presence of fenitrothion and phosalone by the procedure recommended by Getz and Watts[3] as modified by Jain *et al.*[4]

Recovery of fenitrothion and phosalone from the fortified fruit samples was 78 to 82 per cent and 79 to 81 per cent respectively.

The residue data were then subjected to statistical analysis to compute RL_{50}, an index of the rate of dissipation and t_{tol}, the time gap to be followed before the produce is consumed, by the procedure suggested by Hoskins[5].

TABLE 1. RESIDUES OF FENITROTHION AND PHOSALONE ON BRINJAL FRUITS

Days after treatment	Fenitrothion		Phosalone	
	Residue (ppm)	% dissipation	Residue (ppm)	% dissipation
0	2.77	—	4.80	—
1	1.04	62.46	1.78	62.92
3	0.36	87.01	0.60	87.50
5	0.10	96.39	0.14	97.09
7	n.d.	100.00	n.d.	100.00
Regression	Y=1.3791–0.2779X		Y=1.6290–0.2956X	
RL_{50} (days)	1.083		1.018	
t_{tol} (days)	3.470		1.290	

Residue is not detectable (n.d.) after 7 days in both the insecticides.

The data pertaining to the residues of fenitrothion and phosalone on brinjal fruits are presented in Table 1. It is seen that an initial deposit of fenitrothion of 2.77 ppm declined to 1.04 ppm resulting in over 62 per cent dissipation of the initial deposit just after one day. After 7 days, the level of fenitrothion fell below the detectable limit. RL_{50} and t_{tol} values were found to be 1.083 and 3.470 days respectively. t_{tol} value obtained in the present study is in close agreement of 3.30 days reported by Rajukkannu *et al.*[5]

The initial deposit of phosalone of 4.80 ppm dropped to 0.14 ppm after 5 days with a 97 per cent decline of the initial deposit. RL_{50} and t_{tol} values were found to be 1.018 and 1.290 days respectively; the t_{tol} value obtained by us is slightly less than that of 1.55 days reported by Rajukkannu *et al.*[5]

From the results of the present investigation, a waiting period of 1.290 and 3.310 days for phosalone and fenitrothion, respectively is suggested to avoid any residue hazard to consumers of brinjal.

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BOOK REVIEWS

Commercial Chicken Production Manual: by Mack O. North, Published by the AVI Publishing Company, Inc., Westport, Connecticut, 3rd ed., 1984; pp: 710; Price: $42.50.

The third edition of the book entitled "Commercial Chicken Production Manual" is aimed at helping the poultry producers for more profit from the growing chickens. The book consists of 41 chapters covering subjects like modern breeds of chicken, chick hatcheries, management, feeding, disease aspects, their control and prevention etc. Extensive revised information is available in this edition. Product standards and cost data are updated. Genetics, nutrition, economy and production of chicken have been discussed thoroughly, with more cross references. Although the bibliography is lacking in this book, the scientific results and data are presented in both Metric and English systems. Chapter on the various diseases caused by bacteria, virus, protozoa and fungi is very useful for controlling and prevention of many poultry diseases and to maintain the flocks disease-free and economical.

This book providing such a wealth of information on commercial chicken production, is very useful not only as a reference guide but serves as a text as well.

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Food Analysis—Principles and Techniques: Vol I. Physical Characterization: by Dieter W. Gruenwedel and John R. Whitaker (Ed.), Marcel Dekker Inc., New York, 10016; 1984; pp: 352; Price: $ 59.75

"Food Analysis—Physical Characterization" is the first volume of the proposed eight volume treatise on "Food Analysis—Principles and Techniques", conceived to meet the need for an up-to-date detailed treatment of methods in food analysis. The present volume has seven chapters written by seven well known experts in the area.

Foods are chosen primarily for enjoyment, nutrition being a secondary criterion. Colour, texture and flavour are the important criteria which determine the level of acceptability of the product. Four chapters are devoted to these aspects. Colour is used by consumers to judge the overall quality of the product. Colour measurement systems, scales, and differences; instrumentation and methodology are discussed in Chapter 3. Chapter 4 on reheological techniques emphasizes the need for fundamental test methods as compared to the emprirical methods adopted, and with this in view, brings out principles of fundamental methods, techniques and examples of applications of rheological techniques to foods. Mechanical or textural properties of foods as an aspect of quality; the principles, instrumentation, methods of measurement, and future needs of research form the subject matter of fifth chapter. Sensory analysis to set standards of quality, control of quality, development of product; selected sensory analytical methods, and correlation of sensory analysis with chemical and physical methods are discussed in the second chapter.

Statistical evaluation of experimental data; automated analysis of proteins, water-soluble vitamins, additives, contaminants, carbohydrates, inorganic compounds, enzymes, fatty acids and amino acid using continuous-flow analytical systems like the Technicon Autoanalyzer II which can analyse gas, liquid or solid samples or the Analytical Cartridge suitable for solvent extraction, dialysis, dilution, reagent addition, hydrolysis or many combinations of these operations; and application of micro-and mini-laboratory computers in a modern food analytical laboratory are discussed in three separate chapters.

The treatise, as mentioned by the editors, is not a handbook of methods of analysis; rather, the emphasis is on the principles to enable those not familiar to develop sufficient knowledge. Uniformity has been maintained in the presentation with respect to introductory remarks, historical development of the subject, principles of techniques which are illustrated with examples, summary and conclusions, future needs, and references.

Students and research workers will find the treatise as an authoritative source book of knowledge in the area.

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Pineapple—An Industrial Profile: by Central Food Technological Research Institute, Mysore, 1986; pp: 61; Price: Rs. 15.

Comprises 9 chapters, which include raw materials, area and yield; post-harvest handling; technology

of processing; specifications of raw materials and finished products; processing industry; utilization of pineapple processing waste; waste and effluent disposal and sanitation; scheme for a pineapple processing unit; equipment and their availability. Statistical information are given in the seven appendices. The book is useful to the entrepreneurs in setting up of pineapple processing units.

Pepper—A Profile: by Central Food Technological Research Institute, Mysore, 1986; pp: 43, Price: Rs. 15.

The 8 chapters of the book include: Cultivation; Production; Processing, Handling and Grading; Microbiological Aspects; Foreign Trade; Standards and Specifications. It is a comprehensive source of information on pepper covering all aspects from farm level to the end use.

AFST(I) News

Jammu Chapter

The Annual General Body Meeting was held on Thursday, 27th March, 1986. The following office bearers were elected unanimously for the year 1986.

President:	Sh. A. K. Bhatia
Vice President:	Sh. S. S. Langer
Hony. Secretary:	Dr. G. N. Qazi
Hony. Treasurer:	Dr. (Mrs). Geeta Handa
Councillors:	Sh. Nazir Ahmed Wani and Sh. Shashikant Goswami

Annual General Body Meeting of the Association

The twenty first Annual General Body Meeting was held on Saturday, 15th March 1986 at Hotel Oberoi Towers, Nariman Point, Bombay. The Meeting was attended by 139 members. Dr. A. G. Naik Kurade, President of the Association presided over the meeting.

The President highlighted the activities of the Association during the year. Dr. D. Narasimha Rao, Hony. Exec. Secretary, presented the minutes of the previous General Body Meeting and explained the follow up action taken on the suggestions made during the last AGBM. Members suggested that the minutes of AGBM should be circulated to Chapters within three months after AGBM.

Hony. Exec. Secretary then presented his report for the year 1985. The Secretary said that the membership of the Association at present was 2269. This includes 1791 regular members including affiliate and foreign members, 266 life members, 23 corporate members and 189 student members.

The Association has at present 19 Chapters; a new Chapter was formed at Kanpur. Chapters organised various Symposia, Conferences and Workshops in food science and technology and other allied subjects.

The Central Executive Committee met 6 times during the year at Headquarters. One emergency meeting was also held.

The Secretary informed the members that the financial position of the Association continued to be satisfactory but needed improvements in view of the increase in the cost of publication of two Journals, postage and secretarial expenses. JFST was being given freely to the members. As the cost of publication was increasing every year, he requested the Chapters to contribute liberally to support the publication of the Journal.

The Journal of Food Science and Technology is in its 23rd year of publication. A total of 131 papers had been published during the year (Volume 22). The new Editor Dr. N. Chandrasekhara had taken charge from January 1986.

The Indian Food Industry Journal had published 10 reviews, 5 technology papers and 441 abstracts. The Proceedings of 3rd and 4th ICFOST had been published during the year.

Fellowships: The Association had taken a decision to award Fellowships entitled "Fellow of Association of Food Scientists and Technologists" (FAFST) to honour those who had made outstanding contributions in the field of Food Science and Technology. Fellowship Award would begin from 1985. The subcommittee constituted by the CEC has prepared the guidelines for the award and had been circulated to Chapters.

Sixth ICFOST(I): The Sixth ICFOST(I) had been organised by the Bombay Chapter with the theme 'Perspectives of Food Industries in nineteen ninetees'.

News Letter: A monthly News letter was started to build up better communication and coordination between the Headquarters and the Chapters. It covered the activities of Headquarters and Chapters. Any information of interest or innovations in the field of food science and technology or other allied subjects would also be published in the 'News Letter'.

National Directory of Food Scientists and Technologists: The Secretary informed that it had been decided to publish a National Directory of Food Scientists and Technologists working in India. Proformas were circulated to the members requesting them to send their biodata.

Professional Placement Cell: A Professional Placement Cell had been created at the AFST(I) office at the headquarters during the year to facilitate the graduates of food science/technology/other allied subjects to get suitable employment as also to help the employers of food industries in getting employees of their choice.

The Association in collaboration with CFTRI, Mysore organised a National Symposium on 'Production and Processing of Meat and Poultry Products' during 17-18th January 1986 at Mysore.

International Food Conference: It had been decided to organise an International Conference on Food Science and Technology during the year 1987-88 at Mysore. A sub-committee has been constituted to identify the areas of interest and prepare a preliminary draft. The

Committee had suggested the following theme for the conference: 'Food Science and Technology for Better Quality of Life—A perspective for the 21st Century'.

The following are the persons selected for the various awards:

Prof. V. Subrahmanyan Industrial Achievement Award was given to Dr. B. L. Amla, Director, CFTRI, Mysore.

Best Student Award was given to Mr. N. M. Sachindra, College of Fisheries, University of Agricultural Sciences, Mangalore.

Suman Food Constultant Travel Award was awarded to Mr. N. M. Sachindra, College of Fisheries, University of Agricultural Science, Mangalore.

Gardner's Award was given to the paper entitled 'Manufacture of Butter Powder from Buffalo Milk' by Prasad and S. K. Gupta of the National Dairy Research Institute, Karnal, India, published in *this Journal* of 1984, Vol. 21, No. (4), page 211.

No nominations had been received for the Laljee Godhoo Smarak Nidhi Award.

None was selected for Dr. P. B. Rama Rao Memorial Award and Young Scientist Award.

The Hon. Exec. Secretary thanked all those who have helped him to discharge his duties during his tenure of office as the Secretary of the Association.

The Secretary's report was approved by the General Body after considerable discussion. This was followed by the presentation of reports by the Chapters. Dr. M. G. Sathe, Dr. Sreenivasan, Mr. Kankan, Dr. K. L. Nagarsekhar, Mr. Dravid and Mr. Sood presented the activities of Poona, Madras, Delhi, Bombay, Bangalore and Hyderabad Chapters in that order.

Sri R. Y. Vasudeva, Treasurer of the Association presented his report. There was considerable discussion regarding improvement of the financial position of the Association. It was pointed out that the cost of printing the journals was increasing continuously. Members agreed in principle that it is necessary to increase the membership fee and the subscription rates of IFI and JFST. It was suggested that CEC could take a decision to increase the membership fee after discussing it in the joint CEC meeting. Treasurer's report was then approved by the GB.

The appointment of Sri A. K. Krishnamurthy as auditor for the year 1986, was approved by the GB. The request of Sri A. K. Krishnamurthy to increase the audit fee from Rs. 400/- to Rs. 500/- was also agreed upon by the GB.

The guidelines of AFST(I) Fellowship Award was presented by the Chairman of the Fellowship Committee. It was mentioned that Fellowship of the AFST(I) should be conferred on those who have made outstanding contributions in the field of Food Science and Technology through (*a*) Research and Development (*b*) Education and Training (*c*) Organisation and Management of institutions individually or preferably in a combination thereof. It was suggested that Fellowship Award shall begin from 1985. Considerable discussions took place on the suggested guidelines. The proposal of the President of AFST(I) to coopt a few experts from the Chapters and to form a committee for identifying the outstanding persons in the field of Food Science and Technology for the award of AFST(I) Fellowships for the year 1985 was unanimously agreed. There was a long discussion on the guidelines for holding National and Regional Symposia and the sharing of funds sanctioned by DST/CSIR/ICAR/DRDO between Chapter and headquarters. Majority of the members were in favour of the suggestion to share the funds on mutual consent between HQ and Chapters.

The amendments approved by the CEC was presented to the General Body. Some minor amendments were suggested to the exising memorandum of the constitution. It would be necessary to consider them in a special general body meeting convened for the purpose, these clauses were not taken up for discussion.

The president requested Hony. Exec. Secretary to announce the election results for the year 1986. The Secretary informed that all the office bearers were elected unanimoulsy. They were as follows:

1.	*President designate*	..	Dr. V. H. Potty
2.	*Vice-Presidents*		
	Headquarters	..	Sri M. M. Krishnaiah CFTRI, Mysore.
	Chapters	..	Dr. P. J. Dubash Bombay, Dr. P. Tauro Hissar. Prof. B. P. N. Singh Pantnagar.
3.	*Hony. Jt. Secretary*	..	Dr. T. S. S. Rao DFRL, Mysore.
4.	*Hony. Treasurer*	..	Sri K. C. Chikkappaji CFTRI, Mysore.

The President announced the induction of the new office bearers in absentia since the President elect Dr. T. R. Sharma was not present.

On behalf of the members, Mr. M. K. Panduranga Setty thanked the outgoing CEC for initiating many activities during the year.

The meeting ended with a hearty vote of thanks by Dr. D. Narasimha Rao, Hony. Exec. Secretary.

PUBLICATIONS

OF

THE ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (I)

(CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE CAMPUS, MYSORE-570013)

1. **PROCEEDINGS OF THE SYMPOSIUM ON FATS AND OILS IN RELATION TO FOOD PRODUCTS AND THEIR PREPARATIONS**
   Price: India **Rs. 25/-**
   Abroad: **$10**

2. **PROCEEDINGS OF THE FIRST INDIAN CONVENTION OF FOOD SCIENTISTS AND TECHNOLOGISTS**
   Price: India **Rs. 25/-**
   Abroad: **$10**

3. **TECHNICAL DIRECTORY OF THE CONFECTIONERY INDUSTRY IN INDIA**
   Price: India **Rs. 25/-**
   Abroad: **$10**

4. **PROCEEDINGS OF THE SYMPOSIUM ON STATUS AND PROSPECTS OF CONFECTIONERY INDUSTRY IN INDIA**
   Price: India **Rs. 30/-**
   Abroad: **$10**

5. **PROF. V. SUBRAHMANYAN COMMEMORATION ISSUE**
   Price: India **Rs 30/-**
   Abroad: **$10**

6. **PROCEEDINGS OF THE SYMPOSIUM ON BY-PRODUCTS FROM FOOD INDUSTRIES: UTILIZATION AND DISPOSAL**
   Price: India **Rs. 30/-**
   Abroad: **$10**

7. **PROCEEDINGS OF THE SECOND INDIAN CONVENTION OF FOOD SCIENTISTS AND TECHNOLOGISTS**
   Price: Paper back **Rs. 30/-**
   Calico bound: **Rs. 40/-**
   Abroad: **$ 10**

8. **PROCEEDINGS OF THIRD INDIAN CONVENTION OF FOOD SCIENTISTS AND TECHNOLOGISTS (FOOD LAWS IN RELATION TO DEVELOPMENT OF FOOD INDUSTRIES)**
   Price: India **Rs. 25/-**
   Abroad: **$10**

9. **PROCEEDINGS OF FOURTH INDIAN CONVENTION OF FOOD SCIENTISTS AND TECHNOLOGISTS (ADDITIVES IN PROCESSED FOODS)**
   Price: India **Rs. 35/-**
   Abroad: **$12**

10. **PROCEEDINGS OF THE SYMPOSIUM ON RECENT DEVELOPMENTS IN FOOD PACKAGING**

INSTRUCTIONS TO AUTHORS

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

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. Methods of sampling, number of replications and relevant statistical analyses should be indicated. 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 and 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 sheets. 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 not bigger than 20 cm (oy axis) $\times$16 cm (ox axis). The lettering should be such that they are legible after reduction to column width. 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 issue of the *Journal* for guidance.

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Research Papers

Research Notes

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