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Protein, Lipid, Mineral and Trypsin Inhibitor Content in Modified Opaque-2 Maize (*Zea mays* L.)

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Protein, lysine, minerals, trypsin inhibitor content and fatty acid composition have been estimated in different grades of modified 'opaque-2' maize kernel. With the increase in kernel vitreosity, protein quality deteriorated, kernel weight increased and the content of various minerals decreased. The decrease was more pronounced for Fe and Mn. Fatty acid composition and trypsin inhibitor activity of modified 'opaque-2' did not differ much from that of chalky 'opaque-2'. In general, protein quality was superior and mineral content was also higher in chalky and modified opaque-2 kernels as compared to normal maize.

The nutritional superiority of 'opaque-2' maize protein over normal maize is well documented[1,2]. More recently breeders are trying to develop varieties with hard endosperm to overcome the agronomic and acceptability problems associated with chalky 'opaque-2' types. A wide range in kernel opacity is recorded even in the presence of 'opaque-2' gene under homozygous recessive condition[3-5]. The present study was undertaken with a view to determine nutritional status of various categories of hard endosperm 'opaque-2' since no such information is available in the literature. The protein, lysine, oil, minerals, trypsin inhibitor content and fatty acid composition in various categories of modified 'opaque-2' kernels have been determined and compared with those of normal and chalky 'opaque-2' maize.

Materials and Methods

The maize varieties, 'SO/SN composite' (modified 'opaque-2' with hard endosperm), 'Shakti opaque-2' composite' (chalky) and 'Vijay' (normal) used for the present investigation were grown under identical agronomy. Sample from each variety consisted of kernels from 15-20 well filled ears, which were controlled pollinated. For separating 'opaque-2' kernels of SO/SN composite into various categories namely 100 per cent opaque, 75 per cent opaque-25 per cent vitreous, 50 per cent opaque-50 per cent vitreous, 25 per cent opaque-75 per cent vitreous and nearly normal (0 per cent opaque-100 per cent vitreous), the kernels were screened against light. The classification was based on the basis of relative kernel opacity. A representative sample of 100 g was drawn from the kernels of each variety/category for chemical analysis and ground to 100 mesh.

For endosperm studies, 25-30 kernels were soaked in distilled water at 4°C for 3 hr. Then the pericarp was removed and endosperm collected after removing embryo. Dried endosperms were ground to 100 mesh.

Protein and lysine: In whole kernel sample, protein (N×6.25) was estimated by micro-Kjeldahl method[6] and lysine by colorimetric method[7].

Minerals: Finely ground samples were digested in tri-acid mixture (10:1:4, HNO_3: H_2SO_4: $HClO_4$ V/V) according to Piper[8]. Iron (Fe), Copper (Cu), Manganese (Mn) and Calcium (Ca) were estimated quantitatively by Atomic Absorption Spectrophotometer. Potassium (K) and Phosphorus (P) were estimated quantitatively by flame photometric[9] and colorimetric[10] methods respectively.

Lipid and fatty acids: Crude fat was extracted by Soxhlet extraction method using hexane as a solvent. Fatty acid analysis was done by gas-liquid chromatography after methylation of fatty acids as described by Sen *et al*[11]. The methyl esters were determined by Shimadzu gas chromatograph having dual column and fitted with flame ionization detector and digital integrator. Coiled stainless steel column (3m×4 mm ID) were packed with 10 per cent (by weight) of stabilized diethylene glycol succinate coated on Chromosorb P 60/80 mesh solid support. The column, detector and injection port temperatures were held at 175°C, 230°C and 230°C. The nitrogen (carrier gas) flow rate was

kept at 40 ml/min, air and hydrogen pressure rate, 0.8 kg/cm^2 and 0.5 kg/cm^2 respectively for flame ionization detector. Retention time was used to identify fatty acids and from peak area obtained by integrator, fatty acid composition was determined.

Trypsin inhibitor activity: Trypsin inhibitor activity was determined according to the method of Kakade *et al*[12]. One trypsin unit is defined as an increase of 0.01 absorbance 3/2 ($A^3/2$) units at 280 nm in 20 min per 10 ml of reaction mixture. Trypsin inhibitor activity is defined as the number of trypsin units inhibited (TUI). Casein was used as substrate.

The data reported are an average of duplicate determinations which agreed closely.

Results and Discussion

Kernel weight, protein and lysine: Results presented in Table 1 indicate increase in kernel weight and protein content with increase in the kernel vitreosity in modified 'opaque-2' maize. Lysine content and chemical scores decreased with the increase in kernel vitreosity. Kernel weight of segregating normal (100 per cent vitreous) was 85.7 per cent of the 'Vijaya' (normal). Kernel weight of chalky opaque-2 kernels (0 per cent vitreous) was 80.5 per cent of 'Shakti opaque-2'.

Protein content of 0, 25, 50, and 75 per cent vitreous kernels was nearly comparable. None of the modified 'opaque-2' kernel categories had a lysine level comparable to that of 'Shakti opaque-2'. The lysine content of normal segregating kernel (100 per cent vitreous) and 'Vijay normal' kernels was the lowest. The chemical score values indicated that with the increase in kernel vitreosity from 0 per cent to 75 per cent vitreous, protein quality deteriorated steadily. Further increase in kernel vitreosity to nearly normal level lowered the protein quality drastically.

Mineral composition: The mineral elements play an important role in metabolic and regulatory processes[13]. Therefore, their concentrations were determined in whole kernel and data are presented in Table 2. The results show that with the increase in kernel vitreosity the content of various minerals studied, decreased. However, the decrease was more pronounced for Fe and Mn. The level of all these minerals was substantially higher in 0, 25 and 50 per cent vitreous kernel types as compared to 'Vijay' normal. Even in 75 per cent vitreous kernels the level of Fe was 95 per cent higher than in 'Vijay' normal. The level of most of the minerals in chalky 'opaque-2' kernels (0 per cent vitreous) was either comparable or slightly lower than that of 'Shakti opaque-2'. 'Shakti opaque-2' had 62, 116, 383, 52, 26 and 123 per cent higher P, K, Fe, Cu, Ca and Mn respectively as compared to 'Vijay' normal. The level of all the minerals except Fe in segregating normal was nearly comparable to that of 'Vijay' normal. Goodshell[14] reported that 'opaque-2' kernels contain more K than normal.

Lipid and fatty acid composition: The data presented in Table 3 indicate that the oil content and fatty acid composition of oil from normal, 'opaque-2' and different categories of modified 'opaque-2' kernels did not show much variation although slight decrease in oil content was observed with increase in kernel vitreosity. The oil content of various modified 'opaque-2' categories ranged from 5.4 to 5.9 per cent in whole kernel and 0.7 to 1.2 per cent in endosperm.

In whole kernel samples linoleic acid content was the highest and was followed by oleic and palmitic acid. Linolenic acid was present only in traces. Similar trend was also observed in the fatty acid composition of endosperm samples (Table 3). Stearic acid accounted

TABLE 1. PROTEIN, LYSINE AND 100 KERNEL WEIGHT OF NORMAL OPAQUE-2, AND VARIOUS CATEGORIES OF MODIFIED OPAQUE-2 KERNELS

Variety/kernel category	100 kernel wt. (g)	Protein %	Lysine (g/16gN)	Chemical score*
Vijay (Nornal)	30.3	8.7	2.49	45
Shakti (opaque-2)	24.4	11.0	4.62	84
SO/SN Composite (*Mod.* 02)				
0% vitr-100% opaque	21.8	11.8	4.02	73
25% vitr- 75% opaque	23.2	11.8	3.90	71
50% vitr- 50% opaque	23.3	12.1	3.77	69
75% vitr- 25% opaque	25.9	12.2	3.50	64
0% opaque-100% vitr	26.0	13.2	2.67	49

*Based on lysine as the first limiting amino acid and 5.5g lysine/16g N in 1973 reference amino acid pattern.

TABLE 2. MINERAL COMPOSITION OF NORMAL, OPAQUE-2 AND VARIOUS CATEGORIES OF MODIFIED OPAQUE-2 KERNELS

Variety/kernel category	P (%)	K^+ (%)	Fe^{+++}	Cu^{++}	Ca^{++}	Mn^{++}
			..... (mg/100 g)			
Vijay (normal)	0.24	0.12	1.61	0.27	2.01	0.21
Shakti (opaque-2)	0.39	0.26	7.78	0.41	2.54	0.47
SO/SN Composite (*Mod.* 02)						
0% vitr-100% opaque	0.36	0.22	6.20	0.43	3.24	0.51
25% vitr-75% opaque	0.35	0.20	6.05	0.41	3.23	0.50
50% vitr-50% opaque	0.32	0.18	4.39	0.41	2.12	0.41
75% vitr-25% opaque	0.27	0.16	3.15	0.25	1.97	0.27
0% opaque-100% vitr	0.26	0.12	1.98	0.25	1.84	0.20

TABLE 3. PER CENT FATTY ACID COMPOSITION OF OIL IN NORMAL, OPAQUE-2 AND VARIOUS CATEGORIES OF MODIFIED OPAQUE-2 WHOLE KERNELS AND ENDOSPERMS

Variety/kernel category	Oil (%)	Fatty acid %					Oleic/ Linoleic
		Palmitic	Stearic	Oleic	Linoleic	Linolenic	
		Whole Kernel					
Vijay (normal)	4.88	16.91	3.33	29.28	50.48	<1	0.58
Shakti (opaque-2)	5.05	15.26	1.92	34.68	48.15	<1	0.72
SO/SN Composite (*Mod.* 02)							
0% vitr-100% opaque	5.88	14.80	2.98	34.95	47.26	<1	0.74
25% vitr- 75% opaque	5.86	14.43	2.58	35.38	47.61	<1	0.74
50% vitr- 50% opaque	5.83	—	2.94	33.09	46.18	<1	0.72
75% vitr- 25% opaque	5.43	14.98	3.14	36.02	45.87	<1	0.79
0% opaque-100% vitr	5.38	14.72	3.11	37.94	44.24	<1	0.86
		Endosperm					
Vijay (normal)	0.86	18.59	4.45	24.02	52.93	<1	0.45
Shakti (02)	0.81	16.71	4.26	23.61	55.42	<1	0.43
SO/SN Composite (*Mod.* 02)							
0% vitr-100% opaque	1.23	16.44	6.03	24.70	52.83	<1	0.46
50% vitr- 50% opaque	0.87	17.15	—	29.52	53.21	<1	0.55
75% vitr- 25% opaque	0.79	16.16	3.97	28.56	51.35	<1	0.56
100% vitr- 0% opaque	0.72	17.33	3.23	33.84	45.60	<1	0.74

TABLE 4. TRYPSIN INHIBITOR ACTIVITY IN NORMAL, OPAQUE-2 AND VARIOUS CATEGORIES OF MODIFIED OPAQUE-2 KERNELS

Variety/kernel category	TUI/g flour
Vijay (normal)	386
Shakti (opaque-2)	426
SO/SN Composite (*Mod.* 02)	
0% vitr-100% opaque	442
25% vitr- 75% opaque	410
50% vitr- 50% opaque	410
75% vitr- 25% opaque	410
100% vitr- 0% opaque	402

for less than 3.4 per cent in whole kernel. The low level of linolenic acid content is indicative of very good keeping quality of maize oil. Jellum[15] has also reported that palmitic, oleic and linoleic acids are the major fatty acids in normal maize.

Trypsin inhibitor activity: The result of trypsin inhibitor activity in 'Vijay' normal, 'Shakti opaque-2' and different categories of modified 'opaque-2' kernels are presented in Table 4. The trypsin inhibitor activity of 'Shakti opaque-2' was 10 per cent higher compared to 'Vijay' normal. It was also higher (10 per cent) in 100 per cent opaque as compared to 100 per cent vitreous kernels. The trypsin inhibitor activity in 25, 50 and 75 per cent vitreous kernels was slightly higher compared to nearly normal kernels.

The results presented here clearly show substantial improvement in nutritional quality of various categories of modified 'opaque-2' maize as a result of increase in lysine; The higher Fe content in chalky and modified 'opaque-2' compared to normal maize is of nutritional significance in view of general Fe deficiency in human diet. Similarity in the fatty acid composition of normal, 'opaque-2' and modified 'opaque-2' maize indicates that opaque-2 gene does not affect the fatty acid composition.

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Effect of Reducing and Oxidizing Agents on the Farinograph Characteristics of Indian Wheats

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The reducing agents had a marked effect on the dough development time, stability and MTI of the varieties 'WG 357' and 'WL 711' even at 20 ppm level of L-cysteine HCl which decreased further with increasing level of reducing agent though not commensurately. Potassium bromate and ascorbic acid countered the effect of reducing agents to a negligible extent even at 20 ppm level of L-cysteine HCl combined with 75 ppm of the oxidants. Salt seems to counteract to some extent the effect of L-cysteine HCl in the dough. The consistency of the dough at 25°C was higher by 30 to 60 B.U. and at 35°C that was lower by 50 to 70 B.U. reckoned from the 500 B.U. line using FWA for mixing the dough with and without the reducing agents. SSL had negligible effect on dough consistency in the presence of the reducing agents and 50 ppm of AA.

Mixing properties of dough are important in breadmaking processes according to Bushuk *et al.*[1] Farinograph has been used extensively in the study of rheological behaviour of wheaten doughs[2]. North American wheats have been found to respond favourably to oxidizing improvers for baking. They have high protein content and tend to produce bucky doughs. Indian wheats on the other hand, have been reported by Maninder and Bains[3] to have medium to low protein with critical mixing and absorption constraints. Bains and Irvine[4] obtained practically no response to potassium bromate in test bakings on some of the improved Indian wheats. Since information on the effect of various reducing and oxidizing agents on the farinograph curve characteristics is not available, this investigation was carried out. The paper describes the interaction of L-cysteine HCl, sodium bisulphite ($NaHSO_3$), potassium metabisulphite (KMS) along with ascorbic acid (AA) and potassium bromate ($KBrO_3$) on the farinograms of 'WG 357' and 'WL 711' varieties of wheats.

Materials and Methods

Bulk samples of commercial varieties of wheat, 'WG 357' and 'WL 711', from 1976–77 crop, were conditioned to 15.5 per cent moisture before being milled into straightgrade flours in the Buhler Pneumatic Laboratory Mill (MLU-202), The flours were stored in air-tight containers.

Analytical methods: The flours were analysed for moisture, protein, damaged starch, ash and diastatic activity, by the AACC methods[5]. The colour grades of the flour were measured in the Kent-Jones and Martin flour colour grader, Series 3 (Henry Simon Ltd., England).

Farinograph curves: Brabender farinograph assembled with stainless steel clad mixer was used. The dough was mixed using the AACC constant flour, (50 g) 14 per cent moisture method. A number of variables were investigated in relation to the dough properties of the varieties.

(*a*) Effect of reductants in conjunction with oxidants on dough properties.

(*i*) Reductants and their levels were: L-cysteine HCl, 0, 20, 40, 60 and 80 ppm; and $NaHSO_3$ or KMS, 0, 10 and 20 ppm.

(*ii*) Oxidants and their levels were: AA or $KBrO_3$, 0, 25, 50 and 75 ppm.

(*b*) Effect of salt (1.5 g/100 g flour) and reductants as in section a (*i*)

(*c*) Effect of temperature (25°, 30° and 35°C) and reductants, as in section a(*i*)

(*d*) Effect of SSL (0.5g/100g flour), AA (50 ppm) and reductants as in section a(*i*)

Farinograph water absorptions (FWA) used in (*a*), (*b*), (*c*) and (*d*) experiments were: 'WG 357', 64.0 and 'WL 711', 61.5 per cent.

The farinograms were interpreted for dough development time (DDT), stability, mixing tolerance index (MTI) and softening according to the AACC methods[5].

Results and Discussion

The yield of 'WG 357' straight grade flour was 71.7 per cent as compared with 73.9 per cent for 'WL 711'. The per cent protein, damaged starch, ash and diastatic activity (mg maltose/10g) for 'WG 357' were 8.7, 10.1, 0.50, and 235; and for 'WL 711' were 8.8, 9.7, 0.49, and 250 respectively.

Farinograph studies

(*a*) Effect of reducing and oxidizing agents on farinograph curve characteristics

(*i*) L-cysteine HCl in conjunction with AA or $KBrO_3$:

The dough development time and stability of 'WG 357' and 'WL 711' doughs decreased considerably even at the level of 20 ppm of L-cysteine HCl which decreased further with the increased levels of 40, 60 and 80 ppm (Table 1). Ascorbic acid or potassium bromate at the level of 25 to 75 ppm when incorporated with varying levels of L-cysteine HCl seemed to have hardly any counter effect on the dough development time and stability of the dough. The dough of 'WG 357' had a better mixing tolerance index (55 B.U.) than that of 'WL 711'. The values increased to 85, 90, 105 and 130; and 95, 105, 115 and 145 B.U., respectively when the amount of L-cysteine HCl was increased from 20 to 80 ppm. Ascorbic acid counteracted the reducing action of L-cysteine HCl on continued mixing whereas $KBrO_3$ further decreased the mixing tolerance of the respective L-cysteine HCl control doughs without any oxidant. Probably, on continued mixing in the presence of air and $KBrO_3$, the dough suffered over oxidation compared with the regulated effect of AA towards extended mixing.

(*ii*) $NaHSO_3$ in conjunction with AA or $KBrO_3$:

From the results in Table 2, it is seen that 10 ppm

TABLE 1. EFFECT OF L-CYSTEINE HCL WTH AND WITHOUT OXIDANTS (75 PPM)* ON THE FARINOGRAMS OF 'WG 357' AND 'WL 711' FLOURS

		'WG 357'				'WL 711'			
L-Cys. HCl (ppm)	Oxidant (ppm)	DDT (min)	Stability (min)	MTI (B.U.)	Softening (B.U.)	DDT (min)	Stability (min)	MTI (B.U.)	Softening (B.U.)
0 (Control)	Nil	5.2	7.7	55	80	4.6	6.8	80	90
L-Cys. HCl									
20	Nil	3.0	4.8	85	85	3.2	4.0	95	95
20	AA	2.6	4.5	75	80	3.5	4.1	85	110
20	$KBrO_3$	3.2	3.8	95	95	3.3	3.6	105	100
40	Nil	2.4	3.3	90	110	2.7	2.9	105	105
40	AA	2.4	3.1	90	100	2.8	2.8	100	100
40	$KBrO_3$	2.8	3.0	110	120	2.8	3.0	110	130
60	Nil	2.2	2.9	105	120	2.4	2.6	115	125
60	AA	2.0	3.0	105	110	2.6	2.7	105	120
60	$KBrO_3$	2.5	2.6	120	145	2.5	2.4	120	150
80	Nil	2.3	2.5	130	150	2.0	2.5	145	155
80	AA	1.8	2.3	120	110	2.4	2.4	110	130
80	$KBrO_3$	1.8	2.1	140	170	2.2	2.3	145	155

*Data for oxidant levels of 25 ppm and 50 ppm not included.

TABLE 2. EFFECT OF $NaHSO_3$ AND KMS WITH AND WITHOUT OXIDANTS (75 PPM)* ON THE FARINOGRAMS OF 'WG 357' AND 'WL 711' FLOURS

Reductant (ppm)	Oxidant (ppm)	'WG 357' DDT (min)	'WG 357' Stability (min)	'WG 357' MTI (B.U.)	'WG 357' Softening (B.U.)	'WL 711' DDT (min)	'WL 711' Stability (min)	'WL 711' MTI (B.U.)	'WL 711' Softening (B.U.)
0 (Control)	Nil	5.2	7.7	55	80	4.6	6.8	80	90
$NaHSO_3$									
10	Nil	4.5	5.5	60	90	4.4	5.0	75	100
10	AA	4.5	6.9	55	90	4.5	6.3	90	90
10	$KBrO_3$	4.5	6.4	75	80	4.5	5.4	95	85
20	Nil	3.6	4.4	90	90	3.8	4.1	95	100
20	AA	4.6	6.4	55	85	4.0	5.5	95	90
20	$KBrO_3$	3.1	4.9	80	85	3.8	5.0	95	85
KMS									
10	Nil	3.7	6.0	80	90	3.3	4.3	100	100
10	AA	3.7	5.3	75	85	4.2	5.0	80	85
10	$KBrO_3$	4.2	5.6	85	82	3.5	4.7	85	80
20	Nil	3.0	4.2	90	90	2.8	2.8	100	105
20	AA	2.4	3.8	90	105	3.2	3.0	90	95
20	$KBrO_3$	3.2	4.0	90	100	2.8	3.0	110	110

*Data for oxidant levels of 25 and 50 ppm not included.

of $NaHSO_3$ had a slight reducing action on the mixing properties of dough, whereas 20 ppm markedly affected those properties. AA seemed to counteract appreciably the reducing action of $NaHSO_3$ whereas bromate had a negligible effect.

(*iii*) KMS in conjunction with AA or $KBrO_3$:

KMS, at the levels of 10 and 20 ppm, was almost equivalent to 20 ppm L-cysteine HCl in its reducing action in the dough. Oxidants had a negligible counteraction on KMS doughs as seen from results given in Table 2.

TABLE 3. EFFECT OF REDUCING AGENTS WITH SALT (1.5 g/100 g) ON THE FARINOGRAMS OF 'WG 357' AND 'WL 711' FLOURS

Reductant (ppm)	'WG 357' Max. consistency (B.U.)	'WG 357' DDT (min)	'WG 357' Stability (min)	'WG 357' MTI (B.U.)	'WG 357' Softening (B.U.)	'WL 711' Max. consistency (B.U.)	'WL 711' DDT (min)	'WL 711' Stability (min)	'WL 711' MTI (B.U.)	'WL 711' Softening (B.U.)
0 (Control)	500	5.2	7.7	55	80	500	4.6	6.8	80	90
0 (Salt)	410	7.5	14.0	20	30	420	6.0	12.1	35	45
L-Cys. HCl										
20	435	4.2	7.5	50	65	430	6.0	10.0	50	60
40	435	4.0	5.5	55	70	430	4.2	6.9	60	70
60	440	3.2	3.8	75	85	430	3.6	5.0	75	80
80	440	2.7	3.2	95	90	430	3.4	3.8	90	95
$NaHSO_3$										
10	420	7.0	12.4	30	40	410	5.9	11.0	40	45
20	410	6.7	11.0	50	45	420	4.9	9.7	50	50
KMS										
10	420	7.0	11.0	35	45	410	5.5	9.3	45	50
20	430	4.8	7.3	55	65	430	4.0	6.3	55	60

TABLE 4. EFFECT OF REDUCING AGENTS AND TEMPERATURE ON THE FARINOGRAMS OF 'WG 357' AND 'WL 711' FLOURS

Reductant (ppm)	Max. consistency (B.U.)		DDT (min)		Stability (min)		MTI (B.U.)		Softening (B.U.)	
	25°C	35°C	25°C	35°C	25°C	35°C	25°C	35°C	25°C	35°C
					'WG 357'					
0 (Control) L-Cys. HCl	530	445	4.7	2.8	6.5	7.0	80	50	110	45
20	535	445	3.0	2.2	4.0	4.0	115	65	125	75
40	530	450	2.6	2.0	3.0	3.8	125	80	130	105
60	530	445	2.2	1.8	2.1	2.7	130	95	140	120
80	530	450	1.8	1.8	1.9	2.3	140	120	150	140
$NaHSO_3$										
10	530	425	4.2	2.5	5.8	5.0	85	55	115	50
20	530	440	3.5	2.4	5.0	4.0	95	60	120	70
KMS										
10	525	435	3.7	2.4	4.3	4.0	100	50	120	70
20	530	430	2.8	2.4	3.4	3.8	140	55	130	55
					'WL 711'					
0 (Control) L-Cys. HCl	560	425	4.5	3.9	5.2	5.5	100	55	130	55
20	555	450	3.5	2.6	4.1	3.1	115	80	135	85
40	560	450	2.8	2.5	2.8	2.8	140	95	142	110
60	560	450	2.8	2.2	2.3	2.7	155	115	155	120
80	560	450	2.4	1.9	2.1	2.2	160	130	165	145
$NaHSO_3$										
10	530	430	4.5	3.2	5.1	4.6	105	70	110	65
20	540	435	3.8	3.0	4.6	3.8	110	75	125	70
KMS										
10	535	430	3.4	2.7	4.0	3.8	110	60	130	75
20	545	440	2.7	2.7	2.5	2.9	130	65	140	90

'WG 357' Max. consistency (30°C), 500 B.U.
'WL 711' Max. consistency (30°C), 500 B.U.

(*b*) Reducing agents and salt:

There was a uniform drop in the consistency of 'WG 357' dough from 500 B.U. to 410 B.U., due to the presence of salt (1.5g/100 g flour) in the dough system (Table 3). The reducing agents had a negligible effect on the peak consistency of the dough in all cases containing salt. These results show that there was no interaction between salt and the reducing agents in the dough system. To mix the dough to the peak consistency (410 B.U.) using the FWA, the dough development time of 'WG 357' was increased by 44.2 per cent and of 'WL 711' by 47.8 per cent, respectively. A distinct stabilizing effect of salt in the doughs of both the varieties without L-cysteine HCl was observed. The stability of 20 ppm of L-cysteine HCl dough was similar to that of control dough without salt. At this level of L-cysteine HCl in the dough, there was a stabilizing effect of salt on the dough consistency. The effects of $NaHSO_3$ and KMS on the stability of dough were distinct at both the levels but were below those of the doughs which contained salt alone. Incorporation of salt increased the mixing tolerance of the dough of both the varieties. At 20 ppm of L-cysteine HCl, the dough has similar tolerance to mixing as the control without the salt. In the case of $NaHSO_3$ and KMS, the doughs were more tolerant to mixing at 10 ppm than those at 20 ppm level of incorporation. There was a perceptible softening of dough without salt, but in the presence of increased amounts of the reducing agents, the values for softening increased proportionately.

TABLE 5. EFFECT OF REDUCING AGENTS WITH SSL (0.5g/100g) AND AA (50 ppm) ON THE FARINOGRAMS OF 'WG 357' AND 'WL 711' FLOURS

Reductant (ppm)	'WG 357'				'WL 711'			
	DDT (min)	Stability (min)	MTI (B.U.)	Softening B.U.)	DDT (min)	Stability (min)	MTI B.U.)	Softening (B.U.)
0 (Control)	5.5	6.9	55	95	5.4	6.8	65	90
L-Cys. HCl								
20	3.1	5.0	70	80	3.2	4.3	80	100
40	2.3	3.8	85	95	2.8	2.9	95	100
60	2.2	3.0	95	105	2.5	2.6	105	115
80	2.0	2.5	110	130	2.4	2.0	110	125
$NaSHO_3$								
10	4.6	6.5	55	100	4.9	5.5	65	90
20	4.0	5.8	55	90	4.1	5.1	75	100
KMS								
10	3.1	5.2	65	85	4.1	4.3	75	95
20	2.6	3.5	95	100	2.7	3.3	90	100

Peak consistency from 485 to 500 U.B.

(*c*) Reducing agents and temperature:

The doughs of 'WL 711' mixed at 25°C (Table 4) were slightly more stiffer than those of 'WG 357'. Overall decrease of consistency when the doughs were mixed at 25° and 35°C was in the range of 15.1 to 16.1 per cent. Doughs containing L-cysteine HCl suffered some reduction in the dough consistency compared to the effect of $NaHSO_3$ and KMS when added at 10 to 20 ppm levels. With the increased temperature of mixing, there was a fall of 44.5 per cent in the dough development time at 20 ppm L-cysteine HCl as compared to 18.2 to 26.7 per cent of the 60 ppm L-cysteine HCl, in the case of 'WG 357' doughs.

The relative stabilities and MTI values decreased as the level of L-cysteine HCl was increased in the dough. Comparably, the decrease was more when the doughs were mixed at 25°C because of their high peak consistencies compared to those of doughs mixed at 35°C. With respect to the peak consistency at 25°C, the degree of softening was more when dough containing L-cysteine HCl was mixed at 25°C. The degree of softening caused by $NaHSO_3$ and KMS likewise was higher at the lower temperature than at the higher temperature of mixing. The trend of interaction of temperature and the reducing agents was similar in the case of 'WL 711' doughs.

(*d*) Reducing agents with SSL and AA:

The dough development times of 'WG 357' and 'WL 711' were decreased by the incorporation of 20 ppm of L-cysteine HCl in the dough having 0.5 g SSL/100g and 50 ppm AA (Table 5) which decreased further when the amount increased from 40 to 80 ppm, though not commensurately. The effect of 10 and 20 ppm of $NaHSO_3$ in the dough was not as marked as that of 20 ppm of L-cysteine HCl whereas KMS was almost similar in its action to that of L-cysteine HCl. There was practically no effect of SSL on the mixing tolerance which decreased with the increased level of L-cysteine HCl in the dough beyond 20 ppm. Softening of dough increased as the level of L-cysteine HCl increased from 40 to 80 ppm in the doughs of both the varieties. In the case of $NaHSO_3$ and KMS, softening was similar to that of the control doughs. It is thus seen, that the effect of SSL and AA on the dough properties was not revealed by the farinograph in the presence of reducing agents in the doughs of both the varieties.

Hlynka[6] studied the effect of bisulphite on the rheological properties of dough using the farinograph and observed considerable reduction in the dough strength. Meredith and Hlynka[7] observed notable decrease in farinograph dough stability from 11.75 min to 1.25 min by 1μ mole of cysteine which was ascribed to its reducing action on the disulfide bonds. Upto 53 to 63 ppm of cysteine, a logrithmic decrease in dough development time was stated by Henika and Rodgers[8]. Reduction to the extent of 10 fold in the mixing time with cysteine has been reported by Henika[9] when the speed of mixer blades of the 'Do Corder' was increased from 65 to 200 rpm. Increased dough development time by salt was reported by Hlynka[10] using the farinograph. The tightening effect of salt on dough was also revealed

by the mixograph test according to Bennet and Coppock[11].

From these results, it is seen that the doughs of the medium protein wheats were considerably susceptible to the action of reducing agents even with 20 ppm L-cysteine HCl. Comparably, $NaHSO_3$ at similar levels was less effective, whereas KMS was almost equal to L-cysteine HCl in its reducing action. Ascorbic acid countered the reducing action of various reductants on continued mixing whereas potassium bromate affected those properties adversely. Salt notably affected the dough development time which decreased with the increased temperature of mixing using the farinograph water absorption in the test.

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Characteristics of Enzymatic Hydrolysate of Faba Bean (*Vicia faba* Minor) Protein

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The rates of hydrolysis of faba bean protein isolate by different proteolytic enzymes and the effect of preheating has been studied. A hydrolysate preparation has been obtained free from bitterness and highly soluble at low pH. A stepwise enzyme reaction was observed with different protein components. Amino acid composition of the hydrolysate shows that it is deficient in sulphur containing amino acids.

Studies with plant proteins have become increasingly important because of concern over population growth and widespread protein malnutrition. Fan and Sosulski[1] determined the solubility characteristics of nine legume species and observed that some legume proteins have high dispersibility of nitrogen at neutral pH. Except methionine, several of these legume proteins had excellent amino acid composition when compared with FAO reference amino acid pattern[2].

Proteins for use in liquid foods and beverages must possess a high solubility in the system. Many soft drinks have a pH in the range of 3-4, at which majority of proteins are only slightly soluble. Furthermore, the stability of native proteins in the acid pH range is often limited, as exemplified by soy protein[3]. Various chemical modifications like succinylation[4] or mild alkaline hydrolysis[5] have been reported to improve the desired functional properties of proteins. The important drawback

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of these chemical treatments is the deterioration of the nutritional quality, due to the blocking or destruction of essential amino acids[6].

Degradation of the proteins using a proteolytic enzyme increases the solubility and the nutritive value. The enzyme hydrolysis has several advantages over acid hydrolysis of proteins. The formation of bitter peptitides is one of the main problems of protein hydrolysates for food use. Higher the content of hydrophobic amino acid in a protein, the more pronounced is its tendency to form bitter tasting hydrolysates[7].

Petritschek[8] concluded that the level of bitterness was a function of enzyme-substrate system in the hydrolysis of proteins. By careful control of hydrolysis process, in particular by terminating the process at a well defined stage, it is possible to produce hydrolyzates of soy protein which are highly soluble at an acid pH, yet free from bitterness[9].

Faba bean which is an important legume crop in many parts of the world is unsuitable for direct use as protein isolate[10]. The controlled hydrolysis of faba bean protein may be more suitable in different food products.

The present study was undertaken to elucidate the basic characteristics of enzymic hydrolysis of faba bean protein isolate and to determine some of the functional and nutritional characteristics of the hydrolysate.

Materials and Methods

The faba bean (*Vicia faba* Minor) seed was bought from local market (Denmark). The seed was cleaned by aspiration and screening. All the proteolytic enzymes used for the study were supplied by Novo Industri, Denmark.

Moisture, ash, crude fibre, protein, and fat were determined by AACC[11] procedure. Protein values were determined by Kjeldahl method. The degree of hydrolysis (DH) is defined as the ratio of the number of peptide bonds cleaved to the total number of peptide bonds[12] monitored by pH-stat.

Protein isolate: The seeds of faba bean were dehulled using Weston Moisture Meter rolls, followed by aspiration and hand picking of hulls where necessary. The dehulled cotyledons were ground into a flour in an Udy grinder and sifted to pass a 100 mesh Tyler screen.

In preparation of protein isolate, 10 per cent dispersion of legume flour (100 mesh) was made in distilled water, and the pH of the slurry adjusted to 8.5 with IN NaOH and stirred for 30 min at 40°C. The vessels were provided with automatic water supply and valve adjustment and the stirrers were identical in form and speed. The slurry consisting of soluble and insoluble material was pumped to the decanter (Alfa-Laval. N×207) with a mohno pump and from this to the solid ejecting centrifuge (Alfa-Laval FUP N×207) where the light particles not removed by decanter were separated. The extraction procedure was repeated to increase the yield of protein. The pH of the combined extracts was adjusted to 4.5 with IN HCl to precipitate the major proteins. The whole suspension was centrifuged and the filtrate was separated from the residue by decantation. The surface of the protein curd was washed with water and the curd redispersed in distilled water (20 per cent DM), adjusted to pH 7.0 and spray dried at an inlet temperature of 210-230°C and an outlet temperature of 90-100°C. The dried product obtained from spray drier contained 90.5 per cent protein (dry basis).

Preparation of protein hydrolysate: Hydrolysate of faba bean protein isolate was prepared by use of ALKALASE (Novo Industri A/S, Denmark). The following hydrolysis parameters were used: Substrate concentration, 10 per cent protein, enzyme concentration 2 per cent, Alkalase, 0,6L, temperature 50°C, and pH, 8.0. The protein suspension was heated to 90°C in a waterbath for 12 min and immediately cooled to 50°C before addition of enzyme. The pH was maintained with 4N NaOH during hydrolysis by means of Radiometer pH-stat equipment as shown in Fig. 1. The hydrolysis was terminated after desired reaction time by heating to 85°C for 5 min and cooling immediately to room temperature. This procedure effectively inactivates

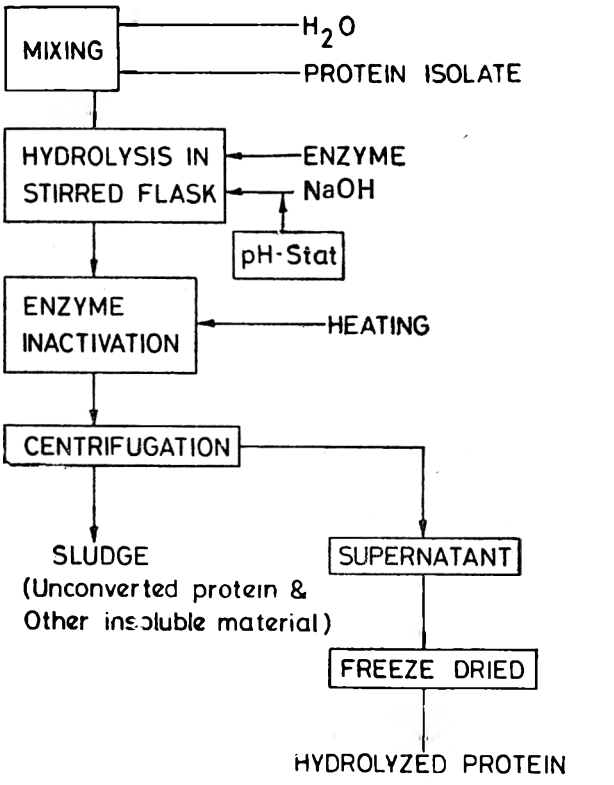


Fig. 1. Flow diagram of the preparation of enzymic hydrolysis of faba bean protein isolate.

the alcalase. The suspension was adjusted to pH 7.0, centrifuged and freeze dried. The protein and peptide contents in all hydrolysates were determined by Kjeldahl method (N×6.25). The protein hydrolysates were also prepared with other enzymes and cooked protein.

Proteolytic activity: The proteolytic activity was determined by the procedure originally developed by Anson[13]. The procedure is based on the measurement of the amount of denatured hemoglobin which is hydrolysed under specific conditions (pH, 7.5, 25°C, 10 min reaction time). The enzyme used was obtained from Novo Industri, Denmark.

Cooking for increased enzyme activity: Cooking of the proteinaceous material is a pre-requisite for enzyme hydrolysis of protein as has been reported by Fukushima[14] and Namba[15]. In order to find optimum cooking time, spray dried fababean protein isolate was dispersed in distilled water in the ratio of 1:10. The suspension was heated to 90°C for different lengths of time at atmospheric pressure and immediately cooled in a deep freeze to 50°C and enzyme reaction was carried out as described earlier in a water bath for 2 hr. The amino nitrogen liberated due to hydrolysis was estimated by Sorenson's formal titration method.

Ultracentrifugation: For ultracentrifugal analysis the flour, protein, and hydrolysed protein were each suspended in a buffered salt solution (0.0325 M K_2HPO_4, 0.0026 M KH_2PO_4 and 0.40 M NaCl) at pH 7.6, $\mu = 0.5$. Sedimentation velocity measurements were made with a Spinco ultracentrifuge model E, operated at 52,000 rpm. Sedimentation coefficients were calculated by the Pickles[16] procedure.

Estimation of amino acid concentration of fababean proteins: Three separate hydrolyses were required to analyze samples for eighteen amino acids. Concentrations of three basic amino acids and twelve acidic and neutral amino acids were determined from hydrolysates obtained by heating 25-35 mg samples with 7 ml of 6N HCl in a sealed evacuated ampule at 110°C for 24 hr. Cysteine and cystine were measured as cysteic acid and methionine as methionine sulphone after performic oxidation and HCl hydrolysis according to the method of Moore[17]. Tryptophan analysis was done on a hydrolyzate obtained by basic hydrolysis of a sample as described by Tkachuk and Irvine[18]. Solvents were removed from the hydrolyzates by rotary evaporation under vacuum and the residue was taken up in sodium citrate buffer (pH, 2.2).

The amino acid analyses of the hydrolysates were carried out by column chromatography on an automated Beckman 120C Analyzer. Amounts of amino acids were determined by comparing the response of the analyser to samples and to amino acid standards.

Results and Discussion

The protein content of the isolate was more than 90 per cent on dry basis (Table 1). The other non-protein material present in the protein isolate was not high. The high yield of protein isolate from flour indicated that the protein was loosely bound with starch. The number of protein components of flour and isolate were same in both cases.

Simultaneous ultracentrifugation patterns were determined for fababean flour and isolate protein using a double sector interference cell and counterbalance as shown in Fig. 2A. In this co-ultracentrifugation process, flour proteins (top sector) and isolate protein (bottom sector) could be compared directly in the photographic frame (Fig. 2A).

Four peaks were observed in flour and isolate proteins of faba bean. From Table 1 and Fig. 5 it is evident that in spite of different amount of salt soluble proteins present in flour and isolate, the nature of peaks and values of sedimentation coefficients were not greatly altered. The protein concentration in the dispersion medium of flour was higher than that of isolate (Table 1) and the sedimentation values of the proteins (flour) was lower due to differential effects of density and viscosity. The data from protein isolates provided the specific sedimentation coefficients of the protein components.

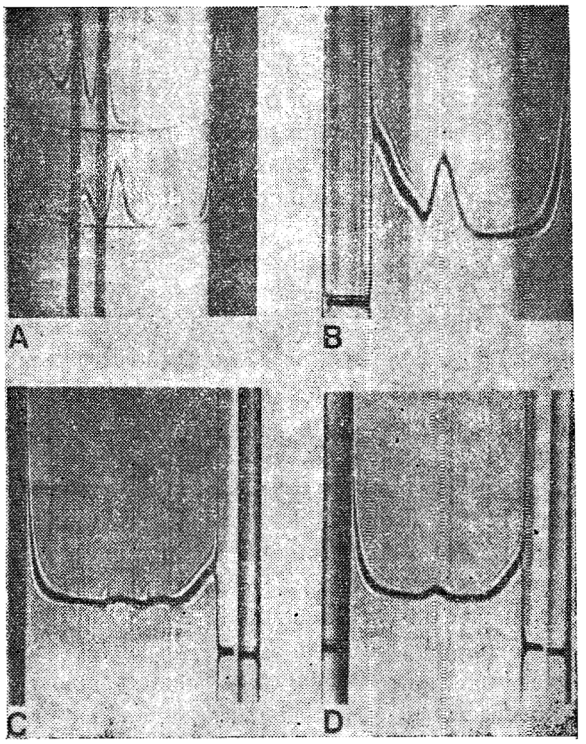
As shown in Fig. 3, about 12 min cooking was necessary for maximum release of amino nitrogen for this substrate enzyme system. The maximum rate of liberation of amino nitrogen happened within 5 min of cooking. It will be shown later that cooked protein had different effect on degree of enzymic hydrolysis due to denaturation of proteins.

TABLE 1. CHEMICAL COMPOSITION* AND PHYSICO-CHEMICAL CHARACTERISTICS OF FABABEAN FLOUR AND PROTEIN ISOLATE

Source	Flour	Protein isolate
Protein (%)	32.5	90.5
Fat (%)	1.4	0.8
Fibre (%)	1.5	0.5
Ash (%)	2.8	1.2
Yield (% of total protein)	—	80.1
Salt soluble protein as (%) of total flour protein ($\mu = 0.5$, pH-7.2)	86.8	70.6
Protein in ultracentrifugation pattern (mg/ml)	12.1	8.2
Sedimentation co-efficients[a]	1.4, 5.8, 8.9, 12.1	2.7, 6.8, 10.5, 15.4

*Dry basis

a In case of flour it is Sobs and for isolate it is $S_{20,w}$.



Fjg. 2. Ultracentrifugation pattern of faba bean proteins (A) and changes during enzymic hydrolysis (B, C, D.)

There is considerable variation in pH values for the optimum activity of the proteolytic enzymes. This depends not only on the enzyme source itself but also on the protein from different sources[19]. On the basis of optimum activity of these enzymes on faba bean protein isolates the hydrolysis were carried out as described earlier. Fig. 4 illustrates the relative activities of proteolytic enzymes of faba bean protein isolate of

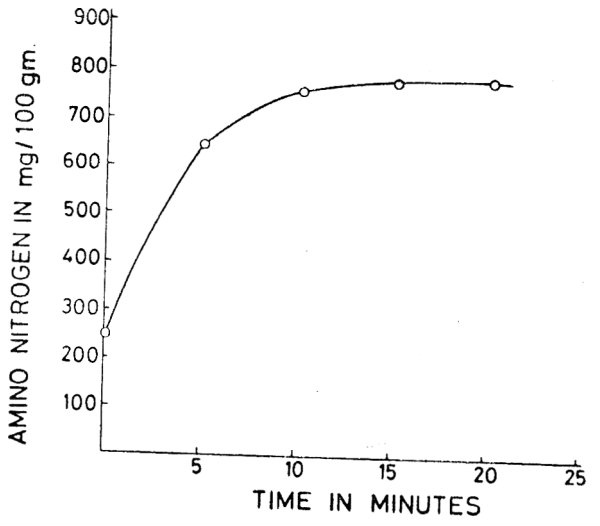


Fig. 3. The effect of cooking time of faba bean protein on enzyme activity.

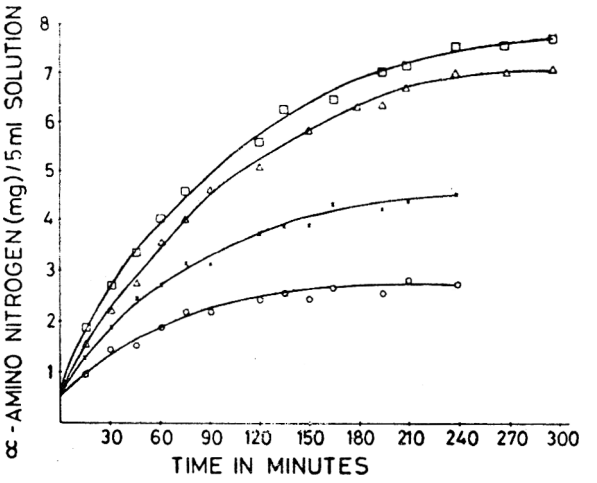


Fig. 4. Relative activities of proteolytic enzyme on faba bean protein isolat .

O—O, Trypsin (8.0 pH, 50°C); ×—×, Bact. proteinase (7.0 pH, 60°C); △—△, Pep in (1.9 pH, 38°C); □—□, Alkalase (8.0 pH, 50°C).

4 per cent dispersion. Trypsin and alkalase showed minimum and maximum proteolytic activities respectively.

Enzymic hydrolysis rate by pepsin is much higher than by bacterial proteinase and trypsin. The ratio of substrate and enzyme remained same in all the cases and none of the product was found bitter.

On the basis of predetermined optimum activity values, the enzyme hydrolysis reaction was carried out. It was found for a given value of E/S the hydrolysis time to reach to a prese degree of hydrolysis (DH) value, was fairly independent on substrate within the

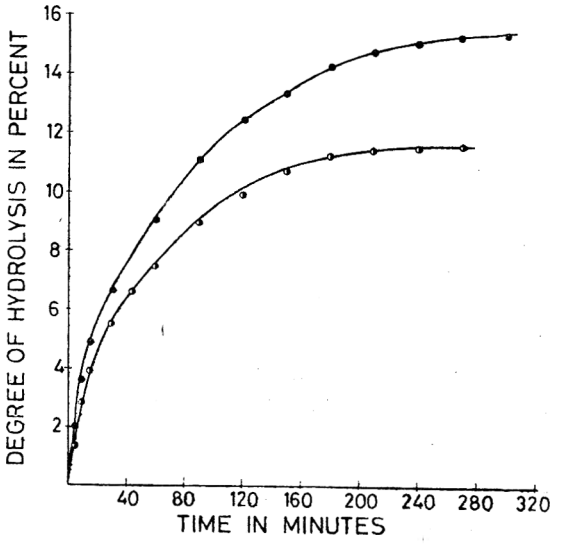


Fig. 5. The effect of heating on degree of hydrolysis of faba bean protein.

●—●, Cooked; ◒—◒, uncooke '.

range of 6-12 per cent protein. The DH was plotted as a function of time for both cooked and uncooked faba bean protein isolates as shown in Fig. 5. The cooked protein showed relatively higher degree of hydrolysis. The high rate of hydrolysis was mainly due to unfolding of protein molecule during heating which caused more number of active sites available to the enzyme. The extent of hydrolysis was also large in case of cooked protein due to the same reason. The reaction rate decreases with time in both cases and the reaction will eventually come to a halt at a given DH value. It is not possible to hydrolyse a protein to 100 per cent degree of hydrolysis with a single enzyme.

The figure 2 B, C and D indicate sedimentation pattern of the hydrolysed (non-cooking) faba bean protein isolate after 5 min, 30 min and 5 hr duration respectively. From the figure it is clear as to how the enzyme alkalase reacted with the different protein components in this protein enzyme system. It was evident from Fig. 2B that 10.5S fraction was still unaffected by the enzyme. The initial resistive nature of 10.5S protein component may be due to less number of active sites present in this protein component.

All the protein components of isolate were reacted by the enzyme alkalase within 30 min of the reaction. Two small peaks were observed initially and one (10.5S) existed even after 5 hr of hydrolysis (Fig. 2C, D).

There were specific differences in the solubility profiles when fababean protein isolate was hydrolysed by the enzyme alkalase as shown in Fig. 7. The change of solubility around pH 3-6 was remarkable due to hydrolysis of proteins. Cooking of protein isolate before hydrolysis increased the solubility of hydrolysed products due to higher DH than that of uncooked hydrolysate (Fig. 6). It is to be noted that 15 per cent of fababean protein was not precipitable when unhydrolysed but after hydrolysis the protein in the whey was 75 per cent

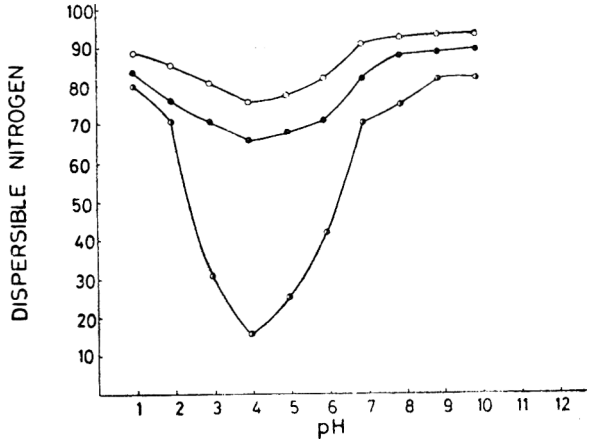


Fig. 6. The solubility profiles of faba bean protein isolate (◒—◒); hydrolysed uncooked protein (●—●); and hydrolysed cooked protein (O—O).

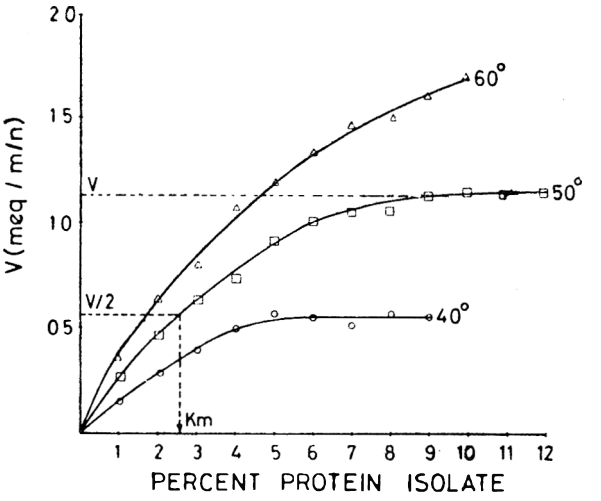


Fig. 7. The effect of substrate concentration on reaction rate at same concentration of alkalase (2%, 0.6 / litre).

at the isoelectric point. Fig. 7 shows the initial reaction rate as a function of substrate concentration (S) for a constant enzyme concentration (E). The curves for enzyme catalyzed reactions have been determined by the pH-stat method for a series of temperatures. The reaction rate is fairly constant, approaching the maximum V, at high substrate concentration. At 50°C the maximum reaction rate for fabaprotein—alkalase system was found at 10 per cent protein concentration. The constant reaction rate at high substrate concentration can be economically exploited for industrial production. Initially the reaction rate doubles, for every 15°C increase in hydrolysis temperature (Fig. 7). The hydrolysis time to reach a preset DH value decreases exponentially with increasing temperature, unless the heat inactiva-

TABLE 2. ESSENTIAL AMINO ACID CONTENTS AND INDICES OF FABA BEAN PROTEINS AND HYDROLYZED PRODUCTS

Essential amino acid	Amino acid (g/100 g protein)			
	FAO provisional pattern	Flour	Protein isolate	Hydrolyzed product
Isoleucine	4.0	4.0	4.9	4.8
Leucine	7.0	7.2	8.5	9.2
Lysine	5.5	6.1	6.4	6.8
Methiomine + cystine	3.5	2.3*	1.7*	1.6*
Phenylalanine + tyrosine	6.0	7.0	8.0	8.6
Threonine	4.0	3.5	3.2	3.5
Tryptophan	1.0	0.9	1.1	1.1
Valine	5.0	4.7	5.7	5.6
Chemical score		65	48	44

*First limiting amino acid as determined by chemical analysis.

tion becomes significant. The hydrolysis time is inversely proportional to the activity of the enzyme. From Fig. 7, Km was calculated as 0.2 mol/l. The value of Km is inversely proportional to the chemical affinity of the enzyme for the substrate. The smaller the value of Km, the greater the affinity of the enzyme for the substrate.

The essential amino acid distribution of fababean flour, protein isolate and hydrolyzed protein isolate as compared to FAO provisional pattern is shown in Table 2. Amino acid analysis was used to predict the protein quality of the isolate and hydrolyzed product and the chemical scores were based on 1973 FAO Provisional Pattern[20].

The first limiting amino acid of flour, protein isolate and hydrolyzed product was sulphur-containing amino acids. Comparing the values (Table 2) for sulphur containing amino acids in fababean flour, protein isolate and hydrolyzed product, it was apparant that the water soluble fraction which was discarded in isolate preparation was richer in these amino acids than the precipitated isolate.

Acknowledgement

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Yeast Flora from Must, Fermenting Must and Wines With and Without Spices

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Sixty isolates of naturally occurring yeasts were isolated from must, fermenting must and wines from two grape varieties namely, 'Beauty Seedless' and 'Perlette' grown in Haryana region and were identified. A total of eleven different species belonging to five different genera namely *Kloeckera*, *Candida*, *Rhodotorula*, *Schizosaccharomyces*, and *Saccharomyces* were present. Except for the *Kloeckera* in 'Beauty Seedless' all the others were present in both the grape varieties. The asporogenous yeasts were predominant during the early phase of fermentation whereas sporogenous yeasts were predominant during the active and late phase of fermentation. Only *Sacch. cerevisiae* was found in wines. Asporogenous yeast i.e. *Kloeckera*, *Candida* and *Rhodotorula* produced less alcohol than sporogenous yeast i.e. *Schizosaccharomyces* and *Saccharomyces*.

Quality of wine depends on the quality of grapes used. Grapes produced in some parts of India do not have much flavour[1]. Therefore, attempts were made to improve the flavour of wines produced from these grape varieties by addition of flavoured spices to the fermenting must and wines.

The study of yeast flora of grapes, fermenting must and wines is important from enological and microbiological point of view. A number of reviews on the yeast flora of grape, fermenting must and wine have been published[2-10]. This paper reports the nature and occurrence of yeast flora in must, fermenting must and wines with and without spices.

Materials and Methods

Two varieties of grapes namely 'Beauty Seedless' and 'Perlette' obtained from Haryana Agricultural University experimental farm were used in this study. Grapes were crushed by Garolla type crusher stemmer (Amos Maschines Fabrik K. C. Hill Bonn W. Germany) after 2 hr of harvest. Sugar content of the must was determined with a Brix hydrometer. Since the sugar content of these varieties was low (16° Brix), sterile cane sugar syrup was added before fermentation to raise the °Brix to about 23.

Six different treatments were given to the must. In each treatment 15 kg of crushed grapes were used. Treatment No. 1 contained no spices (control). In treatment No. 2 spice mixture was added at the rate of 1.77 g/l. In remaining four treatments, cardamom, cinnamon, clove and mace were added separately at the rate of 0.5 g/l. Composition of spice mixtures is as follows: cardamom, 0.07 per cent, cinnamon, clove, longpepper, black and white pepper, ginger, poppyseeds, fenugreek and mace, 0.01 per cent each, nutmeg, coriander, fennel, cumin, mustard and liquorice, 0.005 per cent each; spices were autoclaved at 120°C for 20 min before addition. In case of 'Perlette' the skin was removed after 20 hr while in case of 'Beauty Seedless', it was removed after 48 hr in order to extract more colour from the skin. Fermentation was carried out at room temperature which varied between 35° and 38°C. Yeasts from must, fermenting must and wines were isolated by enrichment culture technique[11] and identified according to methods described by Lodder[12]. Alcohol production capacity of individual yeast was tested in a synthetic medium[13].

Results

Results presented in Table 1 show that predominant yeasts isolated from must, fermenting must and wines from 'Beauty Seedless' belong to genera *Rhodotorula*, *Kloeckera*, *Candida* and *Saccharomyces*. After 24 hr, *R. glutinis* (var. *glutinis*) was found in must where no

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TABLE 1. PREDOMINANT YEASTS IN MUST, FERMENTING MUST AND WINES DURING FERMENTATION OF 'BEAUTY SEEDLESS' GRAPE VARIETY

Treatment	Must	Must at different times of fermentation			Wines
		24 hr	48 hr	72 hr	
Control	*R. glutinis* var. *glutinis* *Kleockera* sp. *Sacch. uvarum*, *C. utilis*, *Kl. javanica* var. *lafarii* *Candida* sp.	*R. glutinis* var *glutinis*	*Sacch. italicus*	*Sacch. cerevisiae*	*Sacch. cerevisiae*
Spice mix	–do–	*Kl. corticis*	*Sacch. cerevisiae*	–do–	–do–
Cardamom	–do–	*Sacch. uvarum*	–do–	–do–	–do–
Cinnamon	–do–	*Schizosacch. pombe*	–do–	–do–	–do–
Clove	–do–	*Kl. corticis*	*Sacch. bisporus*	–do–	–do–
Mace	–do–	*C. utilis*	*Sacch. cerevisiae*	–do–	–do–

spices were added whereas the must with spice mixture and clove contained *Kl. corticis* (synon *Kl. magna*) with cardamom *Sacch. uvarum* (synon *Sacch. carlsbergensis*), with cinnamon *Schizosaccharomyces pombe* and with mace *C. utilis*. On the second day of fermentation except in control and the clove treated one, all the other treatments contained *Sacch. cerevisiae*, *Sacch. italicus* (synon *Sacch. steineri*) was found in must without spice, and *Sacch. bisporus* (synon *Sacch. mellis*) in must with clove. After 72 hr of fermentation only *Sacch. cerevisiae* was found in all the treatments. The wines made with various treatments also contained *Sacch. cerevisiae*.

Table 2 shows the predominant yeasts isolated from must and wines from 'Perlette'. Yeasts belonging to genera *Rhodotorula*, *Candida* and *Saccharomyces* were found in the must of 'Perlette'. After 24 hr of fermentation except in treatment with cinnamon and mace, all other treatments contained *Saccharomyces*. Treatment with cardamom contained *Schizosaccharomyces pombe;* while treatment with mace contained *C. utilis*. After 48 and 72 hr of fermentation, yeasts belonging to genera *Saccharomyces* were found to be dominant flora in all the treatments. After completion of fermentation only *Sacch. cerevisiae* was present in all the wines.

Yeast isolated at different stages of fermentation were tested for their capacities to produce alcohol. Table 3 shows the alcohol produced by various yeasts. Alcohol produced by asporogenous yeast like *Kloeckera*, *Candida* and *Rhodotorula* ranged from 3 to 4 per cent, while alcohol produced by sporogenous yeast like *Saccharomyces* and *Schizosaccharomyces* ranged from 4 to 9 per cent.

Discussion

To develop the economically feasible process, fermenation was carried out at room temperature and the high cost involved and process of temperature regulation was avoided. Due to the high room temperature, fermentation was over within 3 days. During the present investigation 11 different species of predominant fermenting yeasts belonging to 5 different genera i.e. *Kloeckera*, *Candida*, *Rhodotorula*, *Saccharomyces* and *Schizosaccharomyces* were isolated from two grape varieties namely, 'Beauty Seedless' and 'Perlette'. The yeasts found in almost all conditions in grapes and

TABLE 2. PREDOMINANT YEASTS IN MUST, FERMENTING MUST AND WINES DURING FERMENTATION OF 'PERLETTE' GRAPE VARIETY

Treatment	Must	Must at different times of fermentation			Wines
		24 hr	48 hr	72 hr	
Control	*R. glutinis* var. *glutinis*, *Sacch. bisporus*, *Candida* sp.	*Sacch. uvarum*	*Sacch. cerevisiae* *Sacch. bisporus*	*Sacch. cerevisiae*	*Sacch. cerevisiae*
Spice mix	–do–	*Sacch. bisporus*	*Sacch. cerevisiae*	–do–	–do–
Cardamom	–do–	*Sacch. pombe*	*Sacch. italicus*	–do–	–do–
Cinnamon	–do–	*Sacch. uvarum*	*Sacch. bisporus*	–do–	–do–
Clove	–do–	–do–	–do–	–do–	–do–
Mace	–do–	*C. utilis*	*Sacch. cerevisiae*	–do–	–do–

TABLE 3. AMOUNT OF ALCOHOL PRODUCED BY VARIOUS YEAST ISOLATES

Yeast	Alcohol produced (%)
Kloeckera javanica var. *lafarii*	3.1
Kloeckera corticis	3.9
Kloeckera sp.	4.0
Rhodotorula glutinis var. *glutinis*	3.2
Candida sp.	3.7
Candida utilis	4.0
Schizosaccharomyces pombe	4.1
Saccharomyces uvarum	5.6
Saccharomyces bisporus	6.7
Saccharomyces italicus	7.5
Saccharomyces cerevisiae	9.0

wines throughout the world are *Sacch. cerevisiae* and *Kl. apiculata*. But during the present investigation we were able to isolate *Kloeckera* yeast only from 'Beauty Seedless'. Three spices of *Kloeckera*, *Kl. javanica* var. *lafarii*, *Kl. corticis* and *Kl.* sp. (unidentified) were found by us from must and early phase of fermentation of 'Beauty Seedless'. The presence of *Kloeckera* in must and fermenting must of grapes grown in Haryana region has not been reported so far.

Rhodotorula glutinis (var. *glutinis*) was isolated from must and fermenting must of 'Beauty Seedless' and must of 'Perlette'. Mavlani and Gulyamova[14] isolated asporogenic yeasts from grapes and vinous substrates from different wine making regions of Uzbekistan and found that *Rhodotorula* was one of the most wide spread forms. Relan and Vyas[11] also reported presence of this yeast in 'Beauty Seedless'.

C. utilis was isolated from must and fermenting must of both the grape varieties. This yeast was found to be associated with fermenting must containing mace. Relan and Vyas[11] reported the presence of *Metschnikowia pulcherrima* (formerly classified as *C. pulcherrima*) and *C. guilliermondii* in grapes grown in Haryana region. Domercq[3] reported the presence of *Candida* yeast in French musts. Minarik and Nagyova[15] reported presence of *Metschnikowia pulcherrima* in must and found that this yeast was typically associated with *Kl. corticis* in freshly crushed must.

During the fermentation sporogenous yeasts were found to be dominant. Among the sporogenous yeasts, *Saccharomyces* was the most frequently encountered during the fermentation of must and in wines. Species of *Saccharomyces* were identified as *Sacch. cerevisiae* *Sacch. italicus*, *Sacch. uvarum* and *Sacch. bisporus*. They outnumbered all others in their frequency of occurrence in fermenting must. Only *Sacch. cerevisiae* was found to be predominant flora of all wines. Domercq[3] reported presence of *Sacch. bisporus* in French vineyards. Toledo *et al*[4]. also found *Sacch. uvarum* in fermenting must. *Sacch. italicus* was isolated by us from fermenting must of both the grape varieties. Occurrence of this yeast is not usual and not isolated frequently by others except by Domercq[4] from French vineyard and Relan and Vyas[11] from 'Casba' variety of grape grown in Haryana. *Sacch. bisporus* was also isolated by us from both the grape varieties. *Schizosacch. pombe* was isolated from initial phase of fermentation. Occurrence of this yeast in grapes grown in Haryana is very common[11,16]. This yeast helps in improving the quality of wine since it has the ability to ferment and convert malic acid to alcohol[17,18].

It was observed that the asporogenous yeasts were the predominant flora of must found in the early phase of fermentation. During the active phase of fermentation yeasts belonging to genera *Saccharomyces* and *Schizosaccharomyces* take over to complete the fermentation, because of their ability to produce high concentrations of alcohol (Table 3). Disappearance of asporogenous yeast during the late phase of fermentation suggest their inability to tolerate and to produce the high concentration of alcohol.

In general addition of spices for production of spiced wine before the fermentation of grapes didnot affect much the sequence of yeast in wine fermentation. During active period of fermentation and in later phase of fermentation, yeast flora remained more or less same in the treatments containing different spices and control.

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Adjuncts in Brewing. II. Tapioca Starch

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The possibility of the use of tapioca (*Manihot esculenta*) starch was examined for its suitability as malt adjunct in brewing. The use of adjunct varied from 15 to 50%. Analysis of worts showed that reducing sugars conent was, in general, comparable to control wort. The alcohol content of the adjunct beers was slightly less than the control. The adjunct beers were organoleptically comparable to control samples.

Tapioca (*Manihot esculenta*) is widely grown in south India. Beer is mainly produced from barley malt which is an expensive raw material. In recent years there is a trend in the industries to replace this expensive raw material partially or completely by use of cheaper starchy materials. The demand of barley malt for other purposes is also on the increase. Therefore, in this paper, with the aim of reducing the cost of beer production potentiality of tapioca starch as a malt adjunct in brewing has been investigated.

Materials and Methods

Barley variety 'C-164' used in the present investigation is a commercially grown variety and was obtained from the Department of Plant Breeding, Haryana Agricultural University, Hissar. Tapioca starch was supplied by Sago Research Laboratory, Salem, Tamil Nadu (India). Barley and barley malt were analysed for various ingredients by AOAC[1] method. The starch content was estimated by the method of Hassid and Nuefeld[2] and diastatic activity of malt was determined as described by AOAC[1].

Preparation of wort: Different combinations of malt and tapioca starch (Table 1) were made to prepare the wort, keeping the raw materials concentration at 15 per cent for a final brew of 2.5 l. Tapioca starch was used on an equivalent starch basis of 50 per cent in malt and was gelatinized in 2 l. of water at 15 lb pressure for 10 min (Ethiraj, S. personal communication, Haryana Agricultural University, Hissar). After the gelatinization the coarsely ground malt was mixed with the gelatinized, tapioca starch and diluted to 3.8 to 4.1 l with distilled water depending upon the level of adjunct combined with the malt (Table 1). A decoction process of mashing

TABLE 1. COMPOSITION OF RAW MATERIALS AND INITIAL TOTAL VOLUME FOR WORT PREPARATION

Malt+Tapioca starch (%) (%)	Malt+ adjunct (g)	Initial total vol (l)
Malt (Control)	375+0	3.2
85+15	320+28	3.8
75+25	280+47	3.8
65+35	245+75	4.0
50+50	188+94	4.2

TABLE 2. CHEMICAL COMPOSITION OF RAW MATERIALS

Raw materials	Moisture (%)	Starch (%)	Protein (N×6.25) (%)	Ether extract (%)	Red. sugars (as % maltose by wt)
Barley (C-164)	10.9	56.3	8.6	2.25	0.18
Barley malt	8.1	48.5	9.0	2.33	2.08
Tapioca starch	—	100.0	—	—	—

Values are on dry wt basis

modified by Dhamija and Singh[3] was followed. After mashing, the wort was filtered through an ordinary two fold filter paper covered with cheese cloth. This filtrate called 'Sweet wort' was mixed with hops and was autoclaved at 5 lb pressure for 30 min to get a clear wort. The hot wort was filtered into a sterile container, cooled and analysed.

Preparation of malt, yeast culture and inoculum preparation, hops, beer fermentation and analysis of wort and beer were same as previously reported[3].

Results and Discussion

Chemical composition: The grains with a low protein and fat contents but higher starch content are considered suitable for brewing. The chemical composition of the raw materials is given in Table 2. Tapioca starch which is free of protein and fat was tried in combination with malt rich in enzymes (diastatic activity 120) for the production of wort.

Wort analysis: The wort is a complex solution containing a variety of solutes such as carbohydrates (dextrins, maltose, glucose), organic nitrogenous materials and inorganic salts. The chemical make up as well as the physical properties of the wort finally determine the quality of the beer produced. Table 3 shows the analysis of worts prepared from different levels of tapioca starch as an adjunct.

The specific gravity of wort represents the total dissolved solids in wort and is referred also as the original extract of wort. The extract of adjunct wort was comparable to that of control when tapioca starch was used to the extent of 15 per cent. However, further increase in the levels of adjunct resulted in a corresponding decrease in extract values. These variations can be attributed to the type of adjunct used and the variation in the concentration of various enzymes in different malt-adjunct combinations. The colour of the worts prepared using tapioca starch as adjunct were lighter than the control, indicating the effect of adjunct used. Reducing sugars play an important role in establishing the quality and identity of beer as the alcohol content of beer is largely dependent upon the concentration of sugars (fermentable) in wort. The reducing sugars content goes on decreasing with the increase in the concentration of the adjunct which is probably due to the corresponding decrease in the concentration of various enzymes in different malt-adjunct combinations. The reducing sugar values are nearer to the limits prescribed for average American wort.

The protein contents of adjunct worts are lowered

TABLE 3. ANALYSIS OF WORTS PREPARED USING TAPIOCA STARCH AS MALT ADJUNCT

Treatments (%)	Sp gr	Extract (°plato)	Colour (SRC*)	Red. sugars (% maltose by wt.)	Protein (% by wt)	pH	Total acidity (% by wt as lactic acid)
85M+15T	1.03338	8.93	9.83	7.51	0.38	5.35	0.13
75M+25T	1.03296	8.28	9.67	7.28	0.38	5.35	0.13
65M+35T	1.02874	7.25	8.15	6.66	0.31	5.30	0.13
50M+50T	1.02862	7.21	7.90	6.59	0.25	5.30	0.13
All malt (control)	1.03581	8.98	9.85	7.72	0.50	5.75	0.10
Av. American wort	1.04755–1.04965	11.80–12.30	3.00–5.00	7.00–8.50	0.38–0.50	5.20–5.80	0.11–0.12

*Standard reference colour

M = Barley malt

T = Tapioca starch

TABLE 4. ANALYSIS OF BEERS PREPARED USING TAPIOCA STARCH AS MALT ADJUNCT

Treatment (%)	Sp gr	Apparent extract (oplato)	Real extract (% by wt)	Colour (SRC*)	Red. sugars (% maltose by wt)	Protein (% by wt)	pH	Total acidity (% by wt as lactic acid)	Alcohol (% by wt)
85M+15T	1.00434	1.12	2.03	7.29	0.89	0.31	5.15	0.13	2.27
75M+25T	1.00456	1.17	2.08	7.29	0.91	0.25	5.15	0.13	2.08
65M+35T	1.00484	1.24	2.19	5.63	0.72	0.19	4.90	0.14	2.02
50M+50T	1.00498	1.28	2.26	5.41	0.62	0.19	4.95	0.14	2.02
All malt (control)	1.00432	1.10	2.05	7.84	0.76	0.31	5.20	0.10	2.75
Av. American beer	1.01071–1.01410	2.73–3.60	4.08–5.45	2.50–3.50	0.90–1.55	0.24–0.38	4.10–4.50	0.13–0.17	3.10–3.90

*Standard Reference Colour. The alcohol content (% by wt) according to Indian Standards Institution specification is 2–10.
M = Barley malt
T = Tapioca starch

with the subsequent additions of adjunct suggesting that a major portion of protein in wort is contributed by the malt only. The pH and total acidity of all the adjunct worts are comparable to that of control and lie within the limits of average American wort[4]. The variations in pH and total acidity values among the various beers prepared from different levels of tapioca starch were practically nil, indicating the effect of type of adjunct only.

Analysis of beer: Table 4 shows the analysis of beers prepared using tapioca starch as an adjunct. The apparent and real extract for various beers, so prepared were higher than that of control but lower than average American standard. However, the variations among the various beers prepared from different malt adjunct combinations were very small. It may, perhaps, be a reflection of the type of adjunct used and the presence of higher amounts of dextrins in adjunct beers. The adjunct beers were lighter in colour than the control. All the beers including control were darker than the average American standard. This difference is due to the difference in the colour of malt, adjuncts used and the conditions employed for the production of these worts or beers.

The reducing sugars in the adjunct beers are comparable to that of control and are nearer to the average American standard. However, slightly higher content of sugars in adjunct beers than control may be due to the presence of dextrins with reducing activity in these beers. The values for protein content of the beers were comparable to control and average American standard when tapioca starch was used to the extent of 25 per cent. However, a decrease in protein content was observed with further addition of the adjunct. Thus, it suggests that most of proteins of wort or beer are contributed by the malt and slight differences are a reflection of the adjunct used. The pH values of adjunct beers are slightly less, or more or less comparable to that of control but higher than average American standard. Total acids in the beers derived from tapioca starch were slightly more or comparable to control. This suggests that adjuncts do not show much influence on pH and total acidity. The low alcohol content in beers prepared using tapioca starch may be the result of relatively lower amount of fermentable sugars present in the corresponding worts. The alcohol content of all the beers lie within the limits (2.0 to 10.0 per cent) prescribed by Indian Standards Institution[5].

The beers, after a storage of 15 days were tested organoleptically. It was found that the adjunct beers were comparable to control. The beer produced from 50 per cent tapioca was adjudged to be the best and comparable to the commercial beer (Rozy Pelican of Haryana Breweries, Murthal, Haryana).

From the above studies, it is evident that the beers prepared by the use of adjuncts are comparable, both, organoleptically and to some extent analytically to the beer produced from all malt.

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Malting Quality of New Varieties of Ragi *(Eleusine coracana)*

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Nine recently released high yielding strains of ragi have been evaluated for their malting quality. 'Indaf 3', 'Indaf 9', 'Indaf 1' and 'Annapurna' were found to be good malting varieties, while 'Indaf 7' and 'WB 1' were not suitable because of their poor germinating power and low amylase activity.

Malting of ragi (*Eleusine coracana*) has been practised both in the household as well as in commerce in India and some African countries[1] as the malted ragi flour or the extract from it can find use in the preparation of weaning food, infant food, beverage or other pharmaceutical preparations[2,3]. Recently many high yielding varieties of ragi have been developed and released and the evaluation of their relative malting quality with special reference to their use as a malted flour free from bran for preparation of weaning foods is reported here.

Materials and Methods

Cleaned seeds (200 g) in 9 ragi strains procured from V. C. Farm, Mandya, were washed with 0.1 per cent aqueous dispersion of lime and were steeped in running water at about 25°C for 24 hr. The excess water was drained out and the germination allowed for 3 days in thin layers spread on wet cloth. Nongerminated seeds were removed by sieving and the germinated grains dried in an air oven at 50°C for 20 hr, brushed gently to remove the rootlets and weighed to determine the malting loss. It was ground in a Raymond mini mill to pass through 80 mesh (B.S.S.) and used for amylase activity and viscosity determinations.

One g of meal was homogenised with 100 ml acetate buffer of pH 4.8 for 10 min in a waring blender, and one ml. of the filtrate was used for amylase assay at 37°C for 30 min by method of Bernfeld using dinitrosalicylic acid for colour development[4]. A 20 per cent cold water slurry of the meal was heated at 70°C on a water bath with occasional stirring for 20 min, cooled to room temperature (27°C) and the viscosity of the slurry was measured with a Brookfield Viscometer (L.V.T. model).

For the preparation of malted ragi flour with minimum bran, 100 g of dry malted ragi was mixed with 10 per cent extra water, tempered for 15 min, pulverised in a McGill hammer mill and sieved through 200 mesh (B.S.S.) sieve. The plus fraction was reground twice, sieving between grinding passes. The combined flour samples were used for colour measurements with a reflectance meter (Photo-volt) with tristimulus green filter. For determination of bran content, 25 g of seeds was soaked in water for 24 hr, and macerated in a waring blender for 30 min. The mash was filtered through a 200 mesh (B.S.S.) sieve and residue washed with water until free from starch, (as tested through starch-iodine test) collected on filter paper, dried at 105°C for 16 hr and weighed. Protein was determined by microkjeldahl method using 6.25 as the conversion factor from nitrogen to protein.

Results and Discussion

There were variations in the content of bran and protein among the varieties studied (Table 1). The protein varied from 6.3 to 8.3 per cent while the bran content ranged from 9.7 to 14.9 per cent. 'WB 1' had the lowest bran content (9.7 per cent) among the 9 varieties. Most of the samples had good germinating ability except 'Indaf 7' and 'WB 1' where the germination percentage was low (67 and 80 per cent respectively). The reasons for this low germination in these two varieties are not known.

The moisture content in the varieties after steeping for 24 hr at 25°C varied from 34.5 to 39.9 per cent. The weight loss due to removal of rootlets ranged from 6.8 to 12.1 per cent, the loss being low in 'Indaf 7' and 'WB 1' which had low germinating power. The loss was about 11-12 per cent in the other varieties.

The yield of refined malt flour varied from 62.8 to 70.8 per cent. Whiteness in the malt flour also varied widely from 54-70 P. V. units. Here again varieties 'Indaf 7' and 'WB 1' which had poor germination power gave malt flours with low reflectance readings. The protein content in the refined malt flour varied from 3.8 to 5.0 per cent. Generally, varieties with high initial protein content gave refined malt flour with higher protein than those with low values. The protein recovery

TABLE 1. YIELD AND CHARACTERISTICS OF MALT FLOUR FROM 9 RAGI VARIETIES

Variety	Protein* (N×6.25) %	Bran* content %	Moisture after 24 hr soaking in water at 25°C %	Germination %	Rootlets %	Malt flour*		Refined malt flour		
						Amylase activity+	Viscosity (Centipoise units)	Yield %	Protein* % (N×6.25)	(Colour tristimulus)
Indaf 1	6.3	14.6	35.4	96	11.5	176	20.4	70.8	3.8	69
Indaf 3	8.3	11.3	36.5	93	10.8	136	23.5	68.1	4.6	70
Indaf 5	7.0	13.3	36.3	95	11.1	170	22.0	62.8	4.3	64
Indaf 6	7.3	14.6	35.8	90	12.1	199	19.6	70.3	3.9	66
Indaf 7	8.0	12.9	39.9	67	8.3	75	46.4	66.7	5.0	54
Indaf 8	7.9	14.9	34.5	95	11.0	149	27.8	69.5	4.8	65
Indaf 9	6.6	12.5	36.6	97	11.4	181	20.1	67.0	4.0	68
Annapurna	7.1	12.5	37.3	98	12.0	185	18.1	69.5	4.1	67
WB 1**	8.2	9.7	38.4	80	6.8	94	29.9	67.1	4.5	57

+Amylase activity expressed as mg of maltose produced by 1 g of malt at 37°C for 30 min when acted on 1 ml of 1 per cent starch substrate.

**White ragi seeds. The colour of most of the seeds of this variety change to dark brown during germination.

*Values expressed on 12 per cent moisture basis.

in the refined malt flour was about 60 per cent of the initial value.

The amylase activity in the malted flours ranged from 75 to 199 units. The viscosity values also varied from 46.6 to 18.1 C.P. units. Varieties with high amylase activity gave slurries with low viscosity which was to be expected.

From the point of view of suitability for producing a malt flour that could be used in weaning foods where high amylase activity, low slurry viscosity and a moderate yield of white flour free from bran is desired, 'WB 1' and 'Indaf 7' have performed very poorly. 'Indaf 1', 'Indaf 9', 'Annapurna' and 'Indaf 3' are the more suitable varieties. The others are intermediate in their behaviour. While germinating studies at differing moisture or temperature conditions may alter the amylase values in the varieties, the common germination procedure employed in the present study (24 hr soaking and 72 hr germination at 25-26°C) are practical for household or village level use which is aimed at in the present study.

Acknowledgement

We wish to thank Mr. C. H. Laxmaniah of V. C. Farm, Mandya for kind supply of pure bred varieties for this study.

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Oxidative Rancidity in the Skin and Muscle Lipids of Oil Sardine *(Sardinella longiceps)*

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Development of oxidative rancidity in the skin and muscle lipids of oil sardine during frozen storage (at-18°C) was investigated by measuring the peroxide value, thiobarbituric acid number and polyene indices and determining fatty acid compositions. Skin lipids contained slightly higher proportion of monounsaturated acids and lower levels of polyunsaturated acids than muscle lipids. Increase in the peroxide value and thiobarbituric acid value, and fall in the polyene indices, are faster in skin lipids indicating more rapid autoxidation in the latter. Peroxide value reached a maximum after four weeks of storage. A marked increase in the rate of formation of TBA-reacting substances was observed as peroxide value started to decrease. The increased susceptibility of skin lipids towards autoxidation could be due either to the accumulation of prooxidant substances or depletion or dilution of naturally occurring antioxidants.

The most important problem in quality preservation of fatty fish is the prevention of oxidative rancidity. Various factors affect lipid oxidation in fish muscle. Apart from the usual ones such as concentration of the reactants and effect of temperature, prooxidant activity of trace metals and other biochemical catalysts in the system probably accelerate the initiation of oxidation[1,2] through direct interaction with oxygen or free radical mechanism. In heavily catalysed systems containing highly unsaturated fatty acids the oxidation sets in without any appreciable induction period, and further reaction proceeds very rapidly[3]. There is a general lack of technological data about all these factors to enable optimum conditions to be chosen for processing and preservation of fatty fish.

The highly unsaturated nature of fatty acids in the lipids of oil sardine makes it very susceptible to autoxidation. This is perhaps the most important problem encountered in the processing and subsequent storage of this fish. The lipid content of this fish varies with season from as low as 2-3 per cent in April-May to 16-20 per cent in November-December[4]. This wide fluctuation also contribute its share in complicating the problem. Earlier studies on the frozen storage of oil sardine from two different seasons showed that the rate of development of oxidative rancidity was higher in the fish with the higher lipid content eventhough the percentages of polyunsaturated fatty acids in both samples were almost similar[5]. Autoxidation in the skin lipids of Atlantic mackerel was 8 times faster than in muscle lipids[3]. The presence of a fat-soluble prooxidant in skin lipids has been postulated[6] and the rapid disappearance of natural antioxidants like tocopherols from frozen sole at a certain season, observed by Ackman[7,8], was attributed to these prooxidants[9]. In oil sardine the subcutaneous layer is a major site of fat storage and the higher rate of oxidation observed in the fish with a higher fat content might have been due to these skin lipids. Accordingly, the progress of autoxidation in the skin and muscle lipids of oil sardine during storage at -18°C under commercial conditions was examined.

Materials and Methods

Fresh oil sardines (*Sardinella longiceps*) were collected from landing sites in the Cochin area and brought to the laboratory packed in ice. Twenty fish were frozen in blocks (-40°C) adding water as glaze. Frozen blocks were wrapped well in polyethylene sheets and stored at -18°C. Samples were drawn for analysis over 37 weeks. The skin with its subcutaneous layer of fat was removed from each fish; the lipids from this portion are designated as 'skin lipids' and that from the remaining muscle, as 'muscle lipids'. Lipids were extracted with chloroform methanol (2:1, v/v) following Bligh and Dyer[10]. Peroxide value, free fatty acids and iodine value (Wijs) were determined by A.O.A.C. methods[11]. Thiobarbituric acid (TBA) values were determined by the distillation colourimetric method[12]. Lipid phosphorus was determined by the method of King[13]. Fatty acid methyl esters were prepared by saponifying the lipid and esterifying the isolated fatty acids using boron-trifluoride methanol complex[11] and the methyl esters were analysed on a gas chromatograph (Toshniwal) fitted with a flame ionisation detector using a stainless

TABLE 1. LIPID CONTENT OF MUSCLE AND SKIN DURING STORAGE

Storage period (weeks)	% lipid content	
	Muscle	Skin
0	6.0	27.4
2	6.3	27.2
4	6.0	27.6
8	6.7	30.8
11	7.0	31.6
14	6.3	33.4
22	5.9	33.2
26	6.6	31.5
37	6.6	32.3

steel column (6′ × $\frac{1}{4}$″ o.d.) packed with 10 per cent Silar 10C on Anachrom ABS 110/120 mesh (Analabs). Analytical parameters, identification of peaks and quantification were as described earlier[14].

Results and Discussion

The total lipid content was about 6.5 per cent of the fresh muscle and storage did not seem to affect the lipid content. Skin lipids showed an increase of about 10 per cent in the total lipid content after 4 weeks, but thereafter, remained at about this level till the end of 37 weeks (Table 1). The increase in the lipid content can be partly due to loss of moisture through drip during thawing and partly to reabsorption of fat from drip fluid during thawing operation.

There was a marked difference in the pattern of free fatty acid production in the two lipids (Fig. 1). The muscle lipids showed a pattern similar to that observed for mackerel[15] and sardine[5]. Skin lipids had very low concentration of free fatty acids. It is known that the accumulation of free fatty acids in lipids during frozen

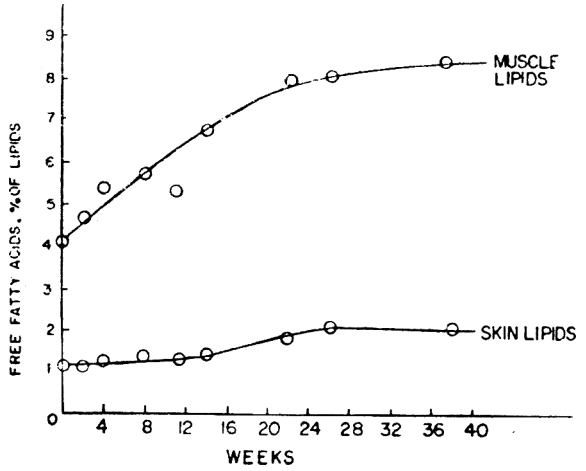

Fig. 1. Accumulation of free fatty acids in the skin and muscle lipids of oil sardine during storage at -18°C.

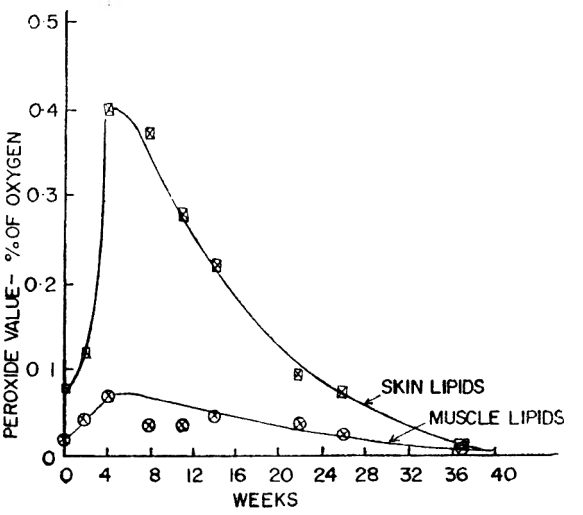

Fig. 2. Changes in the peroxide values of skin and muscle lipids of oil sardine during storage.

storage of fish is mainly due to the breakdown of phospholipids[16,17]. The phospholipid content of skin lipids is very low—0.75 per cent of the lipids, whereas it is 1.38 per cent in muscle lipids—and the low concentration of free fatty acid in skin lipids could be due to this factor.

Fig. 2 and 3 illustrate the changes taking place in the peroxide value and TBA number of the muscle and skin lipids during storage. Peroxide value reached maximum in both lipids after four weeks of storage and then decreased. However, the peroxide value of the skin lipids is five times more than that of the muscle lipids at this stage. There is a five-fold increase in the peroxide value of the skin lipids whereas the muscle lipids showed only a three-fold increase. TBA values reached a maximum in 22 weeks in both samples at which point skin lipids recorded a value 9 times higher than that of the muscle lipids. Similar higher rate of oxidation of skin lipids has been observed in Atlantic mackerel[3]. The rate of development of TBA-reacting substances is faster in the skin almost from the beginning. Subsequent decrease in the TBA values is rapid and within four

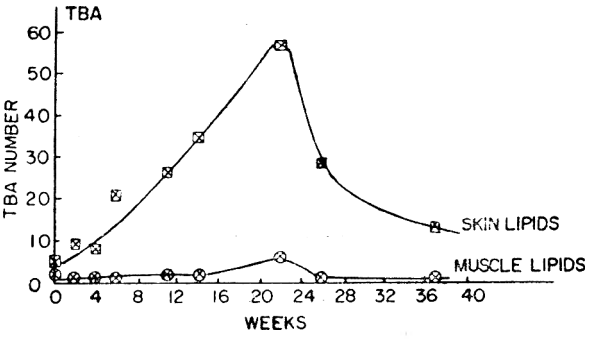

Fig. 3. Changes in the TBA values of skin and muscle lipids of oil sardine during storage.

weeks of reaching the maximum the value came down to almost the original level and continued thereafter for the rest of the period. This decrease could be due to reaction of TBA-type compounds, with amino acids, peptides, etc. released from the decomposition of proteins[18]. One reason for the lower TBA values in muscle lipids is the higher free fatty acid concentration, which is known to suppress formation of aldehydes[19]. The sharp increase in TBA values parallels the fall in the peroxide value, since malonaldehyde and other carbonyls are the decomposition products of hydroperoxides, the primary products of lipid oxidation. Although a direct correlation between TBA number and other indices of oxidative rancidity has been observed in many instances, this could not be established in complex systems containing proteins[20]. Deng *et al.*[21] have reported a similar increase in TBA values with decrease in peroxide value in frozen *chamos*. This decrease in peroxide value and the accompanying increase in TBA number corresponds to the bimolecular period of lipid oxidation in a model system[22].

Muscle and skin lipids only vary slightly in their fatty acid compositions (Table 2). Total saturated acids are almost the same (36.7 per cent in muscle lipids and 36.5 per cent in skin lipids), palmitic acid being the major constituent (about 18 per cent). Skin lipids have a higher proportion of monoenoic acids and a lower proportion of polyunsaturated acids. Ke *et al.*[23] have noted similar pattern in the skin and muscle lipids of the Atlantic mackerel.

TABLE 2. FATTY ACID COMPOSITION OF MUSCLE AND SKIN LIPIDS OF OIL SARDINE

Fatty acids	Mole percent in	
	Muscle	Skin
14:0	9.4	10.2
15:0	1.6	0.9
16:0	18.6	18.2
18:0	6.1	6.1
19:0	1.0	1.1
Total saturated	36.7	36.5
16:1	9.7	13.6
18:1	9.3	10.0
20:1	1.1	1.0
22:1	1.2	0.6
Total monoenoic	21.3	25.2
18:2	2.5	3.8
18:3	1.4	1.5
18:4	2.3	2.1
20:2	0.7	1.3
20:4	2.7	2.1
20:5	15.2	14.5
22:4	3.0	3.4
22:5	2.8	2.7
22:6	11.5	7.1
Total polyenoic	42.1	38.5

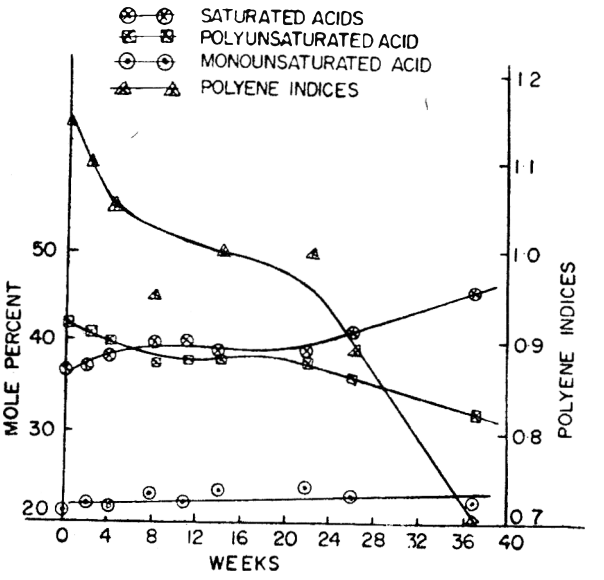

Fig. 4. Changes in the levels of fatty acids and polyene indices in the muscle lipids of oil sardine during storage.

Fig. 4 and 5 show the effect of frozen storage on the fatty acid compositions of the muscle and skin lipids. In both cases there is an increase in the proportion of saturated acids and a decrease in the levels of polyunsaturated acids. In muscle, the rate of these changes slows down after eight weeks, remains steady for

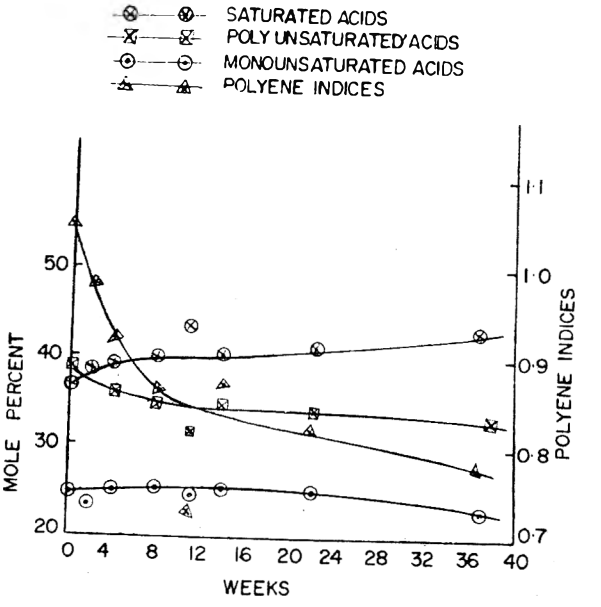

Fig. 5. Changes in the levels of fatty acids and polyene indices in the skin lipids of oil sardine during storage.

another 14 weeks and there after a further rapid phase sets in. The pattern is slightly different in skin lipids. After the initial stage the changes proceed at a slower rate, without a marked break in the middle. No appreciable difference was observed in the extent to which the individual polyunsaturated fatty acids in skin and muscle lipids were affected. The end of the initial rapid phase coincides roughly with the peak concentration of peroxides. Polyene indices for the two samples are also shown in Fig. 4 and 5. The fall in polyene indices is more rapid in the skin lipids in the initial stages indicating a higher rate of development of rancidity. After 22 weeks, the rate of decrease in polyene indices is faster in the muscle lipids.

The parameters of oxidative rancidity that have been considered all indicate that the skin lipid of oil sardine is less prone to hydrolytic attack but more prone to autoxidation than the muscle lipid. This may explain why fish with higher seasonal fat content was earlier found to be more susceptible to autoxidation[5]. The susceptibility to autoxidation of skin lipids cannot be explained by fatty acid composition, but must be sought in other parameters such as sluggishness to hydrolytic attack, the presence of prooxidant substances in the subcutaneous layer as the lipid content increases, or the depletion or dilution of naturally-occurring antioxidants.

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Sorption Isotherms and Monolayer Moisture Content of Raw Freeze Dried Mutton

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Desorption and adsorption isotherms were obtained at different initial moisture levels of raw freeze dried mutton to obtain the monomolecular moisture content by the hysteresis curve and the modified BET equation. The effect of equilibrium relative humidity of the product on the oxidation of fat, brown discolouration and enzymic activity was also studied. The monolayer value for raw freeze dried mutton was approximately 5% with corresponding ERH value of about 21%.

The moisture content of a food product affects its storage stability in a number of ways. Microbial growth occurs above 60 per cent ERH, browning reactions, both enzymic and non-enzymic, are maximum between 30 and 60 per cent ERH and same level are maintained even above 60 per cent also. Lipid oxidation causing rancid smell increases both at higher and lower level of ERH[20]. It is well known that a water content slightly more than the monomolecular moisture content is desirable in a food for its optimum stability[2,3]. The determination of monomolecular content of moisture is, therefore, of considerable importance in determining the storage stability of a food product. The moisture sorption data provide useful guidance for processing and packaging the dehydrated foods. In most cases the monolayer moisture content is a good index when specific stability data are not available.

Monomolecular values can be arrived at from the study of desorption and adsorption isotherms. In the study, reported here, the hysteresis curve was drawn from various desorption and adsorption isotherms and the monomolecular moisture content of raw freeze dried mutton was established from modified BET equation[4] and from the hysteresis curve.

Materials and Methods

Equilibrium moisture content (EMC) at different RH values was determined by equilibrating[5] powdered raw freeze dried mutton chunks at the different RH values and at a constant temperature of $25 \pm 1.5°C$[5,6]. The moisture levels in the powder were adjusted for the equilibration studies by vacuum drying or spraying known quantity of water on to the powdered chunks in the case of adsorption and removing them from the freeze drier at different stages in case of desorption isotherms. The EMC was plotted against the RH to obtain the isotherms. The lower point of intersection of the adsorption and desorption isotherm gives the monolayer moisture content[7,8]. The monomolecular layer adsorption value for water vapour (Vm) was calculated from the equation described by Salwin[4]. The hysteresis curve was drawn from adsorption and desorption isotherms to confirm the values derived from Salwin's equation.

Times of estimation: Various analytical values of ERH up to 75 per cent ERA were obtained after equilibriation of the product with different saturated salt solutions for a period of 15 days. At Higher ERH values of 86,93 and 97 per cent values were, determined as soon as mould growth appeared (5 to 9 days), and further storage experiments could not be done.

Thiobarbituric acid (TBA) values: TBA values were determined according to the method of Taraldgis[9].

Browning: The brown discolouration of the product was measured according to the method described by Iyengar *et al.*[10] by extraction with 80 per cent alcohol for 18 hr to determine optical density at 420 nm.

Adenosine triphosphatase activity: ATPase activity was measured according to the method of Hay *et al*[11].

Peroxide value and free fatty acid content: The peroxide value was determined according to Martinez and Labuza[12] while FFA was determined according to the AOCS method[13].

Visual colour was judged as per 9-point hedonic scale, with value 9 as excellent to value 1 as extremely poor.

Results and Discussion

Monolayer moisture content of raw freeze dried mutton: The monolayer moisture content from the equation of Salwin[4] for raw freeze dried mutton was 5.0 g/100 g

of dry solids, corresponding to an ERH of 21 per cent while the monolayer desorption value was found to be between 6.2 and 6.4 g/100 g of dry solids with corresponding ERH between 19 and 21 per cent.

Karel[14] and Salwin[15] have reported the molonayer values for raw freeze dried beef. The former found 4 per cent moisture while latter reported 3.5 per cent moisture (8 per cent ERH) on fat free dry solid basis as the monolayer value. Iglesias and Chirife[16] found the monolayer value of air dried beef at 30°, 55° and 70°C as 5.4, 5.1 and 4.5 per cent respectively on non-fat dry basis.

A hysteresis curve was drawn with the desorption and adsorption data (Fig. 1). This curve also indicates the monolayer value as about 5 g/100 g of dry solids with corresponding ERH of about 19 per cent which corresponds well with the calculated values. The phenomenon of hystersis is also clearly evident in this curve.

Effect of ERH on the growth of microbes on mutton: Leistner and Rodel[17] provided tables for showing relationship of water activity with the multiplication and growth of microorganisms. They stated that no microbial growth is possible below 65 per cent ERH and bacterial growth occurred only above 85 per cent ERH.

In the present investigation, microbial growth was observed above 67 per cent ERH level. The higher the moisture content, the earlier the growth appeared. While it took about 5 days for microbes to grow below 40 per cent moisture, the growth was observed within 24 hr above this moisture. At 93 per cent ERH the microbial growth appeared very early and the growth was noticed next at 86 per cent ERH level (in about 8 days). At 97 per cent ERH, however, growth was not noticed even after 15 days in very low (upto 6 per cent), moisture product though a slight bad smell and brown discolouration (indicative of putrefaction) was observed.

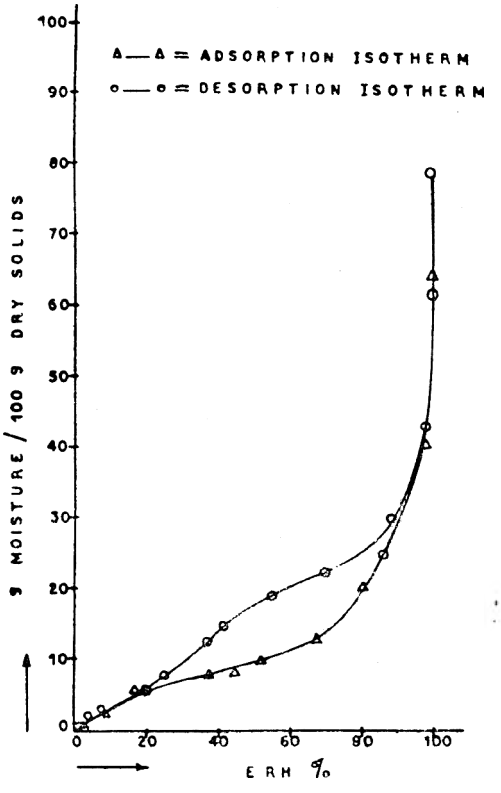

Fig. 1. Hysteresis curve showing the monomolecular region and the effect of hysteresis.

Effect of ERH on deterioration of colour, fat and enzymes: Sharp[18] showed that major deteriorative changes in dehydrated meats, which usually contain 30-40 per cent fat, was the oxidative rancidity occurring in the presence of oxygen and yellow or orange discolouration which occurred even in the absence of oxygen which was termed as non-enzymatic browning. He further found that while lipid oxidation increased both below and above the monolayer value, the non-enzymic browning occurred only above the monolayer moisture content of the product.

In the present investigation initial bleaching was noticed even within 24 hr at higher relative humidities (above 33 per cent ERH). Bleaching became more evident with longer storage period. The browning was however, noticed only above 43 per cent ERH (Table 1). The extinction at 420 nm decreased from initial levels which can be attributed to the bleaching of the muscle pigments with the increase in ERH from 0 to about 43 per cent. Visual colour scores agreed well with the extinction values at 420 nm. The increase in extinction values above 43 per cent ERH level was due to perhaps the browning reactions (enzymic as well as non enzymic) since the brown colour was quite evident visually and was much in contrast with the colour of the same product below 43 per cent ERH. Maximum browning of product was observed in petridishes kept at 67 per cent ERH, the product was spoiled microbiologically and the colour could not be judged and compared for more than 5 and 8 days at 93 and 86 per cent ERH respectively.

The TBA values were found to increase both ways from the 33 per cent ERH level. No rancid or bad smell was observed till *equilibirum* in the product at any ERH inspite of high TBA values. This gives a clue to the spoilage of the product at a particular ERH level (Table 1). TBA values were found to be highest at about 67 per cent ERH and then declined slightly. At 97 per cent ERH, the TBA values were found to decrease abruptly perhaps due to decrease in the TBA values, after an increase upto accestain level, like peroxide values, as has been confirmed by Arya *et al*[19].

The peroxide values also showed similar change (Table 1) and the only difference observed was that these values were found to be minimum at about 23

TABLE 1. EFFECT OF EQUILIBRIUM RELATIVE HUMIDITY ON THE TBA, PEROXIDE AND FFA VALUES AND ON BROWN DISCOLOURATION AND ATPASE ACTIVITY OF RAW FREEZE DRIED MUTTON

Saturated solution	ERH	No. of days equilibrated Mean ±SD	TBA No. (mg/malonaldehyde/kg dry solids) Mean±SD	Peroxide value (m eq. of O_2/kg fat) Mean±SD	FFA (as % oleic acid) Mean±SD	OD at 420 nm Mean±SD	Visual colour score Mean±SD	ATpase activity moles of Pi/mg protein/min	
								Calcium activated Mean±SD	Magnesium activated Mean±SD
Sulphuric acid concentrated	0	15	11.6±1.7	13.9±1.46	2.6±0.2	0.073±0.0063	7±0.7	—	—
Lithium chloride	11	15	7.2±1.6	11.3±1.45	2.7±0.1	0.068±0.0043	7±0.7	—	—
Potassium acetate	23	15	4.4±1.3	8.5±1.66	2.7±0.2	0.068±0.0046	6.6±0.65	—	—
Magnesium chloride	33	15	3.0±1.3	14.8±1.5	3.1±0.2	0.052±0.005	6.3±0.27	0.0154±0.0023	0.0126± 0.002
Potassium carbonate	43	15	6.6±1.0	17.1±1.4	3.3±0.1	0.046±0.009	4.9±0.65	0.023 ±0.0016	0.0199±0.0038
Magnesium nitrate	52	15	7.4±1.7	14.9±1.9	7.3±0.2	0.057±0.0075	4.8±0.57	0.0104±0.0025	0.009 ±0.0027
Cupric chloride	67	15	13.8±1.3	43.1±2.2	4.0±0.2	0.085±0.003	2±0.79	0.0094±0.0027	0.007 ±0.0016
Sodium chloride	75	15	8.8±1.8	20.5±1.5	2.9±0.3	0.072±0.005	3.3±0.44	—	—
Potassium chlroide	86	8	15.3±1.8	25.2±2.0	1.8±0.2	0.061±0.008	3.9±0.41	—	—
Potassium nitrate	93	5	16.1±1.6	9.1±1.2	5.7±0.5	0.051±0.004	3.6±0.65	—	—
Potassium sulphate	97	15	6.7±1.6	8.1±1.4	10.7±1.1	0.064±0.006	3.2±0.57	—	—

per cent ERH level and then increased both below as well as above this level, reaching a maximum at 67 per cent ERH.

The per cent FFA (Table 1) was found to increase gradually with the increase in ERH upto 52 per cent after which it declined upto 93 per cenr ERH. It increased again above 93 per cent ERH.

Potthast *et al.*[20] have found that there was a very slow FFA increase at 3.3 per cent moisture or 10 per cent ERH, but increase was noticeable at 5.6 per cent moisture or 25 per cent ERH in case of raw freeze dried beef. The precooked freeze dried beef, however, did not show any change in FFA even at 70 per cent RH over one month storage. Mathesan[21] stated that the FFA content of raw freeze dried meat increased during storage depending upon the moisture content and he attributed this increase to enzymic action and it was observed even at 2.2 g of water per 100 g non-fat dry solids (7 per cent ERH). In the present work the change in FFA values was also observed even at 11 per cent ERH in comparison to the zero per cent ERH value.

It can be seen that the oxidation of fat in freeze dried mutton was minimum between 23 and 33 per cent ERH level. The maximum hydrolysis of fat at about 52 per cent ERH indicated the presence of lipolytic enzymes, capable of causing hydrolytic rancidity of fat in raw freeze dried mutton. This also gave an indication that the lipolytic enzymes were not destroyed during processing and storage, but were only temporarily inhibited.

The ATPase activity was another indication of the presence of enzymes in this product. Though it was raw aged freeze dried mutton (aged for 72 hr at 5°C and not hot deboned freeze dried mutton) yet there was some activity (though very less when compared to fresh) which was obtained between 33 and 67 per cent ERH levels. Below and above these ERH levels no activity could be obtained. Both calcium and magnesium activated ATPase activity was observed in which the former was slightly higher (Table 1). Matheson[21] found that 40 to 80 per cent of original ATpase activity remained in dried meat after dehydration.

Potthast *et al.*[22] also observed no breakdown of ATP below 25 per cent ERH in hot boned raw freeze dried

beef. They found that ATPase activity increased above 40 per cent ERH with increasing water activity. All the ATP is hydrolyzed after a few days at 70 per cent ERH. At 40 to 55 per cent ERH, there is a residual amount of ATP which stays constant over a long time after a more or less slow decrease of ATP, depending upon the water activity.

Conclusion: From the data obtained in this investigation, the mono-molecular layer adsorption value for water vapour (Vm) was found to be about 5 g per 100 g dry solids (or approximately 20 per cent ERH) in case of raw freeze dried mutton. The oxidation of fat will be more both above and below this value. This moisture content in raw freeze dried mutton will be the most suitable moisture level (contrary to the general belief of about 2 per cent) for longer shelf life and storage stability, since all adverse reactions are likely to be minimum at this level.

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Antioxidative Role of Curry *(Murraya koenigi)* and Betel *(Piper betel)* Leaves in Ghee

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Ghee samples treated with 1.0% betel or curry leaves during clarification showed higher resistance to oxidation and higher sensory scores than those treated with a mixture of BHA + BHT treated samples. The antioxidative effects of betel and curry leaves were thought to be due to the presence of naturally occuring antioxidants.

Ghee (clarified butter fat) is an important dairy product and about 33 per cent of the total milk produced in India is converted into ghee. This product undergoes oxidative deterioration at ambient temperature. The Food Adulteration Rules[1] allow the addition of 0.02 per cent by weight of butylated hydroxy toluene (BHT) or butylated hydroxy anisole (BHA) either singly or in combination as antioxidants. Since continued use of chemical antioxidants such as BHA and BHT has been reported to cause teratogenic and carcinogenic effects in small animals and primates[2,3], commonly used vegetable leaves which are rich in antioxidants would probably prove safe to the health of the consumers. This study deals with the antioxidative effects of betel (*Piper betel*) and curry (*Murraya koenigi*) leaves when added to butter during clarification.

Materials and Methods

Cow's butter obtained by churning the cream in a stainless steel churn, was melted in a stainless steel double jacketed vessel. Fresh curry leaves and betel leaves were cut into small pieces separately and added to different lots of melted butter and then heated to 120°C till characteristic ghee flavour developed. The amount of curry leaves added to ghee were 0.5, 0.8 and 1.0 per cent (W/V) and of betel leaves 0.2, 0.5 and 1 per cent (W/V). The leaves were filtered off before the storage of ghee. A mixture of BHA and BHT (1:1) at concentration of 0.02 per cent by weight was also added to a separate lot of ghee.

Ghee samples were packed and sealed in lacquered tins and stored at 30°C, and were examined for Peroxide value (ml of 0.002 N $Na_2S_2O_3$/g fat), iodine value, free fatty acidity, butyrorefractometer reading and flavour. Peroxide value, iodine value and free fatty acidity were determined according to ISI method[4]. Butyrorefractometer readings were taken on a Bausch and Lomb butyrorefractometer at 40°C. Flavour was evaluated using standard 9 point hedonic scale.

Results and Discussion

Peroxide value: The peroxide values of ghee samples treated with curry leaves, betel leaves and antioxidants changed very little upto 30 days of storage. The control (without antioxidants) showed a steep rise in peroxide with value after 60 days of storage (Fig. 1). Ghee samples added 1.0 per cent curry leaves or betel leaves showed least increase in peroxide value upto 135 days of storage. However, ghee samples treated with betel leaves at 1 per cent concentration proved to be most acceptable and stable even upto 147 days of storage at 30°C.

The antioxidative effect of various treatments in increasing order was exhibited by 0.2 per cent betel leaves, 0.5 per cent curry leaves, 0.8 per cent curry leaves, 0.5 per cent betel leaves, 0.02 per cent BHA+ BHT, 1 per cent curry leaves and 1 per cent betel leaves. Soundarajan[5] is of the opinion that curry leaves contain phenolic compounds like hydroxy-chavicol which could act as a potent antioxidant. The curry leaves and betel leaves contain aspargine, glycine, proline and tryptophan[6]. It is quite likely that these amino acids might also act as potent antioxidants[7,8].

Free fatty acid content in ghee: The extent of hydrolysis of ghee during storage was measured by free fatty acidity (FFA). After 30 days of storage, a progressive increase in free fatty acid content was observed in all ghee samples (Fig. 2). The increase in free fatty acidity was parallel to the development of peroxide value. The control samples of ghee after 147 days of storage at 30°C showed an increase in FFA by more than 100 per cent. Betel leaves at 1.0 per cent concentration provided maximum protection against the hydrolysis were of ghee. The effectiveness against hydrolysis decreases in the order: curry leaves at 1 per cent, betel leaves 1 per cent, BHA+BHT at 0.02 per cent, betel leaves at 0.5 per cent, curry leaves at 0.8 per cent, curry leaves at 0.5 per cent and betel leaves at 0.2 per cent concentration. The maximum permitted level of BHA and BHT, (0.02 per cent) was less effective than curry leaves and betel leaves by a margin of about 10 per cent. It can be inferred that betel leaves and curry leaves at 1.0 per cent proved to be most effective preservatives.

Iodine Value: Although all the samples of ghee during storage showed varying degrees of peroxide formation and increase in free fatty acidity, only slight reduction in iodine value of treated ghee was observed. The initial iodine value of 35.9 in control samples of ghee got reduced to 35.6 after 147 days storage. The changes were non significant in ghee samples treated with higher dosage of betel and curry leaves. Ghee samples showed similar changes in iodine value when treated with BHA+ BHT.

Butyro refractometer reading (BR): Ghee samples prepared with betel and curry leaves exhibited slightly lower BR values (44.5 to 44.8) than the control (45.0).

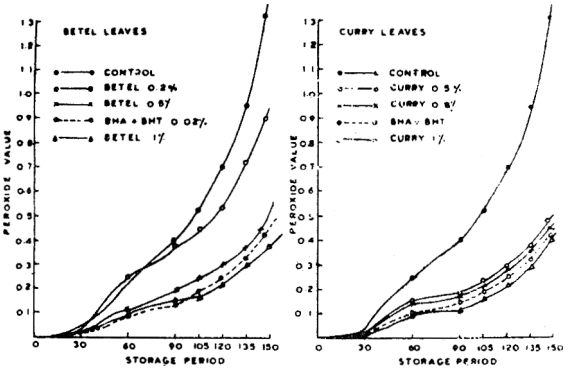

Fig. 1. Change in peroxide value (meq. O_2/kg fat) during storage at 30°C.

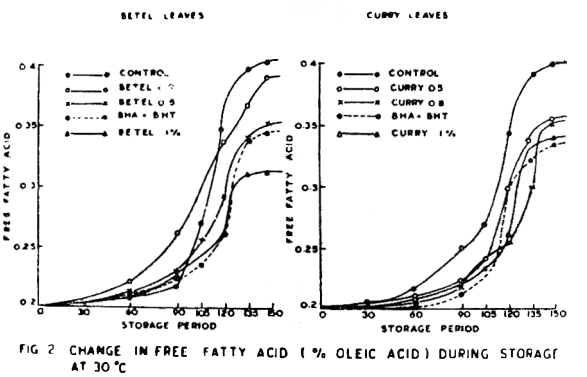

FIG 2 CHANGE IN FREE FATTY ACID (% OLEIC ACID) DURING STORAGE AT 30 °C

Fig. 2. Change in free fatty acid (% oleic acid) during storage at 30°C.

TABLE 1. ORGANOLEPTIC SCORE OF GHEE

	Sensory score	
	Initial	After storage for 147 days
Control	8.33	2.16
BHA+BHT 0.02%	8.30	6.15
Curry leaves 0.5%	8.50	5.50
„ 0.8%	8.40	6.10
„ 1.0%	8.40	6.20
Betel leaves 0.2%	8.30	2.20
„ 0.5%	8.20	5.85
„ 1.0%	8.40	6.50

8.0 Excellent, 7.9 to 6.0 Good, 5.9 to 4.0; Fair; 3.9 Poor

It is likely that the natural compounds which got dissolved in ghee during clarification might have lowered the BR values. On storage the butyro refractometer values of treated samples were lowered by 0.1 to 0.3 units, whereas in control samples the reduction was about 0.5 units.

Ghee samples were also evaluated for appearance, texture and flavour. All the samples of ghee were rated excellent at the beginning of the experiment. The judges preferred ghee samples treated with betel and curry leaves as indicated by their highest scores for flavour and colour. The treated samples were also appreciated for slightly higher intensity of colour. Ghee samples treated with BHA and BHT were rated as ordinary (Table 1).

It can be concluded that betel or curry leaves at 1 per cent concentration may be used instead of BHA and BHT for extending the shelf life of ghee.

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Microorganisms in Ice Creams and their Public Health Significance

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One hundred samples of ice creams of ten different manufacturers, marketed in Calcutta were analysed over a period of two years for standard plate count, psychrophilic and lipolytic bacteria, yeast, mould, enterococci, coliform and fecal coliform and also for the presence of pathogenic microorganisms. 22% of the samples were within the numerical limit suggested by I.S.I., only 12% can be classed as of Grade A quality as per U.S. Public Health Service code. Presence of *E. Coli* was confirmed in 37% of the samples. Most of the strains of *E. Coli* (95.1%) were resistant to penicillin but sensitive to chloramphenicol (91.9%). Multiple resistance was observed in coliforms as well as in the isolates of *E. Coli*. Out of eight serotypes of enteropathogenic variety, all were resistant to more than one antibiotic used in the study. Public Health significance of coliform and *E. Coli* in ice cream was discussed. *Salmonella*, coagulase positive *Staphylococcus aureus*, *Vibrio cholerae* and *Vibrio parahaemolyticus* were not detected.

The hygienic quality of ice cream is assessed chiefly by total count of microorganisms and the coliform count per gram of the food. Some workers[1-8] have reported the microbiological quality of ice creams sold in different cities of India but none of the studies give detailed information in this respect. The present investigation reports the different types of microorganisms, coliforms and fecal coliforms with their serotypes and resistotypes encountered in ice creams marketed in Calcutta.

Materials and Methods

Samples: One hundred samples of ice creams in card board cups from different manufacturers in Calcutta were collected in sterile covered containers from the vendors selling the product. These samples were immediately brought to the laboratory and allowed to thaw. As soon as the thawing was complete the bacteriological analysis of the sample was carried out immediately. Dilution of the sample was made with buffered distilled water.

Standard plate count and psychrophilic plate count: Standard method was used for determining plate counts[9]. Plates were incubated for 48 hr at 32°C for mesophilic plate count and at 7°C for 10 days for psychrophilic plate count.

Yeast and mould: Counting of these organisms was done on acidified potato dextrose agar medium[9] and the inoculated plates were incubated at 23°C for 5 days.

Coliform and fecal coliform: Coliform counts were determined by the MPN method using MacConkey's broth[10]. For counting fecal coliform bacteria the tubes were incubated at 44-45°C for 24 hr.

Enterococci and lipolytic bacteria: These were estimated by the procedure described by Sharf[11] and Seeley and Vandemark[12] respectively.

Salmonella: This was tested on a 10 g sample according to the method of Thatcher and Clark[13].

Vibrio cholerae and V. parahaemolyticus: These organisms were detected on Thiosulphate Citrate Bile salt Sucrose Agar medium by their characteristic colonies[14].

Staphylococcus aureus: Coagulase positive *S. aureus* were noted by their golden yellow colour colonies on Milk Salt Agar[13] medium. Suspected colonies were tested for coagulase activity.

Biochemical reactions: The isolated organisms were identified on the basis of cultural and biochemical characteristics[15,16].

Serotyping: The isolated strains of *E. coli* were sent to the National Salmonella and Escherichia Centre, Kasauli, India for serotyping.

Antibiotic resistance: The antibiotic resistance of the isolates was studied by disc diffusion method against the commonly used antibiotics as per the procedure of Anderson[17]. Reference grade antiobiotics used in the study were obtained from Central Drugs Laboratory, Calcutta. Antibiotic impregnated discs (6 mm diameter,

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punched out from Whatman No. I paper) were prepared by soaking them in appropriate concentration of antibiotic solution and followed by drying in vacuum. Penicillin, ampicillin, streptomycin, each at 10 μg erythromycin at 15 μg and neomycin, chloramphenicol, oxytetracycline and tetracycline each at 30 μg were used in the study.

Results and Discussion

Table I gives the quantitative enumeration of different groups of bacteria with the minimum and maximum found in 100 samples of ice cream manufactured by ten different manufacturers in Calcutta. The overall average of each of standard plate count, psychrophilic plate count, lipolytic bacterial count, yeast and mould count per gram of the product was 0.41×10^6, 0.120×10^6 15.4×10^3, 3.77×10^3 and 3.11×10^3 respectively. The average standard plate count exceeds 250,000/g—the maximum limit laid down by Indian Standards specifications[18]. 52 per cent of the samples in winter and 50 per cent in summer were within the limits of the Indian standard specifications. Only 12 per cent of the samples can be classed as Grade A (standard plate count per gram (50,000) as per United States Public Health Service Code[19].

The psychrophilic bacterial count was determined because the importance of this group of bacteria has increased with the increasing use of cold storage facilities for preservation of raw and pasteurised milk and also of other dairy products. Though these bacteria are not pathogenic, they may produce a variety of offflavour as well as physical defects. The observed psychrophilic count was lower than that reported by other investigators[8].

The seasonal variation of these groups of bacteria were also noted. The average standard plate count and psychrophilic plate count were higher in summer than in winter. Similar observations were also made by Singh *et al.*[8] But the seasonal variations were not statistically significant in case of standard plate count[20] on these sets of data but it was highly significant in case of psychrophilic plate count ($P<0.1$ per cent).

The counting of lipolytic bacteria in ice cream is important in view of high fat content of the product. The lipolytic count was slightly higher in winter than in summer but the difference was statistically not significant ($P>0.5$ per cent). The overall average count found was lower than that reported in other studies[7,8].

Yeast and mould counts of the product did not show any significant difference due to seasonal variations.

The enumeration of coliforms, fecal coliforms and *E. coli* in food products is generally employed as a sanitation index. The presence of these organisms in food beyond certain numerical limit is generally considered to indicate that the food in question is exposed to conditions that might introduce or allow proliferation of pathogenic microorganisms and hence there is the possibility of occurrence of public health hazard when such food is consumed by people. Average coliform and fecal coliform counts noted were 425.8 and 36.5 per gram of the product respectively. Fecal coliform was detected in 71 per cent of the samples. Average coliform count was much higher than 90/g—the maximum ISI[18] limit. Twenty six per cent of the samples in winter and 36 per cent in summer were conforming to ISI specifications. The various coliforms isolated included *Klebsiella aerogenes*, *K. ozaenae*, *K. oxytocum*, *K. scleroma* group, *K. freidlander* group, *Enterobacter cloacae*, *Enterobacter aerogenes* and *Citrobacter* species. The seasonal difference in coliform and fecal coliform count (Table 2) were not statistically significant ($P>0.5$ per cent).

The presence of *E. coli* was confirmed on the basis of biochemical and serological reactions in 37 per cent of the samples. Ninteen different serotypes namely 05, 011, 017, 022, 025, 029, 034, 036, 038, 039, 042, 044, 046, 055, 060, 0101, 0126, 0141 and 0143 were isolated and 13 strains were found to be untypable. In five samples more than one serotype was detected. Out of the 19 serotypes isolated eight serotypes namely 011,

TABLE 1. MICROBIAL POPULATION OF ICE CREAM

Season	Standard plate count/g ($\times 10^6$)	Range of psychrophilic plate count/g. ($\times 10^6$)	Range of Lipolytic bacterial count/g. ($\times 10^3$)	Range of Yeast count/g ($\times 10^3$)	Range of Mould count/g ($\times 10^3$)
Winter	0.019–2.17 (0.299)	0.014–0.425 (0.075)	1.0–77.0 (17.5)	0.0–13.5 (4.6)	0.0–4.7 (2.97)
Summer	0.018–4.8 (0.462)	0.0025–0.47 (0.142)	0.80–68.0 (14.5)	0.10–15.7 (3.41)	0.05–28.0 (3.17)

Figures in parenthesis indicate average value.

TABLE 2. INDICATOR ORGANISMS IN ICE CREAM

Season	Coli form t/g	Fecal coliform t/g.	Enterococci t/g (X 10^3)
Winter	0.0-2400 (756.2)	0.0-240 (56.5)	0.0-40.0 (6.4)
Summer	0.0-2400 (284.2)	0.0-240 (27.9)	0.5-30.0 (6.0)

Figures in parenthesis indicate average value.

TABLE 3. RELATIOSHIP BETWEEN STANDARD PLANTE COUNT AND INDICATOR ORGANISMS

Standard plate count/g	Coliform t/g	Fecal coliform t/g	Enterococci t/g (X 10^3)
5×10^4 <(12%)	89.7 (0–240)	13.1 (0–79)	2.09 (0.45–16)
5×10^4–25×10^4 (40%)	283.4 (0–1600)	14.97 (0–240)	4.98 (0.6–17.7)
$>25\times10^4$	628.47 (0.2–2400)	62.7 (0–240)	7.56 (0.25–40)

Percent in parenthesis indicate % of samples within the range. Figures in parenthesis indicate range value.

022, 025, 039, 044, 055, 0126 and 0143 are considered to be enteropathogenic[19-21]. The occurrence of such enteropathogenic serotypes of *E. coli* in ice cream indicates the poor hygienic quality of the product. However, coagulase positive, *Staphylococcus aureus Vibrio cholearae*, *Vibrio parahaemolyticus* and *Salmonellae* could not be detected in any of the sample.

The enumeration of enterococci in foods is used as an index of sanitary quality and shelf life for frozen foods since enterococci survive for longer periods than the coliform group of organisms[22]. The average enterococcial count was 6.1×10^3/g. No significant difference in count was noted for the sample analysed in winter and in summer (Table 2). The count was lower than that reported by Singh *et al.*[8] The type of fecal streptococci isolated and identified were *Streptococcus faecium*, *S. bovis*, *S. faceium* var. *durans*. It is also interesting to note that as standard plate count increased, the count of indicator organisms also increased (Table 3).

Antibiotic sensitivity of coliform organisms isolated from ice creams is shown in Table 4. Most of the strains were resistant to penicillin, ampicillin and erythromycin but sensitive to streptomycin and chloramphenicol. Tetracycline was less effective than streptomycin and chloramphenicol on inhibiting the growth of coliforms.

Klebsiellae in foods are commonly associated with vegetation but in recent times it is being isolated from a variety of human infections[23], thereby serving as a source of potential danger for the health of man. Though the *Klebsiellae* from the botanical environment are often genetically more diverse than isolates of clinical origin, evidences are available when environmentally derived *Klebsiellae* were of faecal origin and some possessed transmissible R-factors as well[24].

Most of the *E. coli* strains (95.1 per cent) were resistant to penicillin, erythromycin (90.3 per cent), neomycin (72.6 per cent), ampicillin (62.8 per cent) and tetracycline group (53.2 per cnet). Resistance to chloramphenicol and streptomycin was noted only in 8.1 per cent and 9.7 per cent of the isolates respectively. Multiple resistance of *E. coli* strains to different antibiotics has been given in Table 5. Out of 14 enteropathogenic strains isolated two were resistant to six antibiotics, 6 strains to 5 antibiotics and 6 strains were found resistant to two or more than two antibiotics. Out of 62 strains of *E. coli* isolated one was resistant to all the eight antibiotics used in the study, 3 to 7 antibiotics, and 41 to four or more than four antibiotics in various combinations.

Antibiotic resistance of the *E. coli* strains observed

TABLE 4. ANTIBIOTIC SENSITIVITY OF COLIFORMS

Organism	Penicillin	Ampicillin	Strepto-mycin	Erythro-mycin	Neomycin	Chlora-mphenicol	Tetracycline	Oxytetra cycline
Klebsiella aerogenes	*15	20	75	20	40	100	60	55
Klebsiella ozaenae	17	17	100	17	17	83	17	17
Other *Klebiella*	0	0	100	0	67	100	67	67
Enterobacter cloacae	14	43	72	14	54	71	57	44
Enterobacter aerogenes	33	0	67	100	100	67	33	0
Citrobacter spp.	0	0	67	22	33	78	11	33

*Results are expressed as percentage of strains sensitive to the antibiotic tested.

TABLE 5. MULTIPLE RESISTANCE OF E. COLI STRAINS

Antibiotic combination	No. of resistant strains
EN; PATO; PNATO; PENTOC; PNATOC; PENATS; PEATOS; PENATOS; PENATOSC.	1
PE; PNTO; PENAT; PENAO; PENAS; PENATOC	2
PEA; PENTO	3
PENA	6
PEATO	7
PENATO	8
PEN	11

E = Erythromycin, N = Neomycin, P = Pencillin, A = Ampicillin, T = Tetracycline, O = Oxytetracycline, C = Chloramphenicol, S = Streptomycin.

in the present report raises a warning because of the importance of *E. coli* in transferring R-factors in *vivo*. *E. coli* strains with R-factor plasmids have been causing various human infections. The presence of *E. coli*, specially the enteropathogenic variety with R-factors is therefore undesirable in ice cream.

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RESEARCH NOTES

ANTIBIOTIC RESISTANT ENTEROBACTERIA OF MUTTON

Sheep reared on natural grazing grounds were screened for drug resistant gram negative facultative intestinal flora. *E. coli*, *Klebsiella* and intermediate groups isolated from deep rectal and visceral swabs of freshly slaughtered sheep were screened for resistance to antibiotics. Resistance patterns with ampicillin (A), tetracycline (T) and streptomycin (St) were determined. Of the total number (1040) of isolates tested, 7.8% were sensitive to all three antibiotics; 22.7% showed resistance to all three antibiotics 35.7% showed resistance to AT and 5.3% to ASt. Resistance to other antibiotics such as chloramphenicol, septran, neomycin, garamycin, kanamycin and doxycycline were also studied.

Antibiotic resistant bacteria in the environment are of ecological and epidemiological significance[1]. The causes for the emergence of resistant bacteria have been described[2]. Reports on public health significance of feeding low levels of antibiotics to animals as is done through animal feed supplements are contradictory[3]. As part of a programme for isolating and studying the drug resistant enterobacteria in foods[4] and food materials, we have investigated the antibiotic resistance of isolates derived from sheep reared on natural grazing grounds to evaluate the naturally present resistant types in mutton before it was processed.

Twelve healthy male sheep, 1 to $1\frac{1}{2}$ year old and with a body weight of 20-22 kg raised on natural fodder, slaughtered for the preparation of processed foods were selected for the isolation of their facultative intestinal gram negative flora. The anal area was cleaned with alcohol dampened cotton. Deep rectal swabs were taken and immediately dropped into tubes containing 1 ml of 0.1 per cent peptone water. The viscera of the dressed carcasses were also swabbed with cotton swabs and collected in peptone water. After incubating the tubes for 1 hr at 37°C, the swabs were streaked on MacConkey agar plates and incubated at 37°C for 16 hr. Coliform colonies from each sample (24 rectal and 24 visceral swabs) were confirmed on eosine methylene blue agar and classified by the IMVIC test. All media for the isolation and performance of different tests were made according to the Oxoid manual[5]. Antibiotic sensitivity test on these isolates was also performed[4] using discs made with solutions of commercially availabe ampicillin (10 μ g), tetracycline (30 μ g), streptomycin (10 μ g). Neomycin (30 μ g), septran (25 μ g), chloramphenicol (30 μ g), doxycycline (10 μ g), garamycin (10 μ g) and kanamycin (30 μ g) were also tested.

E. coli biotype 1 (12 per cent), *Klebsiella* (8.5 per cent), *Enterobacter* (19 per cent) and organisms of indeterminate group between *Enterobacter-Klebsiella* and *Enterobacter-E. coli* (58 per cent) comprised the total genera among the 1040 isolates investigated. Non-lactose fermenters were not detected. Sixty per cent were drawn from the rectal swabs and about 31 per cent from the viscera of the carcasses. Table 1 shows the types of resistance patterns exhibited by the organisms against ampicillin, tetracycline and streptomycin. Multiple resistance (A-ampicillin, T-tetracycline and St-streptomycin) was shown by 22.7 per cent, whereas resistance to 2 antibiotics (AT and ASt) was shown by 35.7 and 5.3 per cent respectively. Of the remainder, 19.3 per cent were resistant to ampicillin only. Resistance to streptomycin and tetracycline was generally present linked to at least one other antibiotic viz., ATSt, AT and ASt. Nearly 90 per cent of the isolates were resistant to ampicillin either singly or in combination with other antibiotics. Organisms were also resistant to chloramphenicol (93.2), neomycin (37.2 per cent), septran (49.3 per cent), garamycin (10 per cent), kanamycin (24.3 per cent), and doxycycline (65.72 per cent). Interestingly, the same isolates showed resistance to tetracycline and doxycycline (a tetracycline analogue) simultaneously.

Thus it is seen that the pool of resistant bacteria is high in animals reared even under natural grazing conditions. These animals although not given the feed supplements would have ingested some household refuse or edible remains of food, etc. In feed formulations streptomycin and tetracycline are added at minute levels to obtain growth and weight increase in meat animals.

TABLE 1. ANTIBIOTIC RESISTANT PATTERNS OF COLIFORMS ISOLATED

Antibiotic*	% of organisms resistant
ATSt	22.7
AT	35.7
ASt	5.3
A	19.3
St	5.7
T	1.4

*A = ampicillin, T = tetracycline, St = streptomycin.
Among all the organisms isolated, 7.8% were found sensitive to all these antibiotics tested.

In India although a few products such as Auropac (Cyanamid) containing chlorotetracycline and TM-5 (Pfizer) containing oxytetracycline are marketed, only large farm holdings perhaps use these; mainly because they are still out of reach of the small holdings. Our study indicates that even without using any artificial feeds, there is a high level of drug resistant bacteria in the animals. Resistance to drugs appears due to selection pressure and constant use of miniscule amounts of antibiotics in feeds and higher doses as prophylactics will cause a positive pressure for the ultimate high level build up of drug resistance in such animals. This has far reaching effects. Firstly as tetracycline in animal foods is, in a small way implicated in the selection of ampicillin resistant *Salmonella typhimurium*[6], it is not entirely inconceivable that other drugs may preferentially select a proportion of the multiple resistant bacteria thus causing an increase in such types. Secondly, most of such bacteria possess transferable drug resistance[7] and may pass the resistance to other more dangerous organisms. Thirdly it has been found[8] that due to antimicrobials, drug resistance appeared in gram negative obligate anaerobes of human and animal faeces and persisted over a period of time despite withdrawal of some drugs. Thus they are more important than the transient facultative flora, which give us only an indication of the general level of resistance.

As mutton is an important food item, presence in it of such resistant bacteria will lead to a general spread due to cross contamination during cooking or processing.

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MICROBIAL STUDIES ON INPACK PROCESSED CHAPATIES

Six species of *Bacillus* were present among the 20 distinctive cultures isolated from chapaties preserved with sorbic acid (0.2%), NaCl (2.5%) and an inpack heat treatment at at 90°C for 2 hr. All *Bacillus* strains were starch hydrolysing and proteolytic; many tolerated high levels (5-10%) of NaCl and 0.1% of sorbic acid and the spores of most of them survived heating at 90° for 2 hr. The total spore load was however low (2.2×10 org/10 g) and a combination of several factors may have contributed to the perservation of chapaties over a long period.

Microbiological quality of preserved chapaties containing 0.4 per cent sorbic acid (SA) and 2.5 per cent salt has been reported earlier[1]. Since the Prevention of Food Adulteration Act[2] limits the concentration of SA in foods, this method was modified with a reduced SA content and an inpack heat treatment[3]. We have re-examined the microbiological quality of inpack processed chapaties (heat treatment at 90°C for 2 hr subsequent to baking) containing 0.2 per cent SA. The results of these studies are reported in this communication.

Chapaties were made from unleavened whole wheat flour dough containing whole milk powder (2 per cent), salt (2.5 per cent), sugar (3 per cent), fat (10 per cent), citric acid (0.4 per cent) and SA (0.2 per cent). Soon after baking on a hot griddle they were packed (four to a pack) in paper foil laminated flexible pouches and heated at 90°C for 2 hr which was determined[3] as the optimum period for inpack heating for organoleptic reasons and keeping quality.

Sampling, analysis for total counts, yeasts and moulds and primary isolations were done as described earlier[1]. Presence or absence of staphylococci, *Salmonella* and coliforms were also carried out utilizing standard methods and media[4,5,6]. Sorbic acid (E Merck) tolerance was tested by growing cultures on SA incorporated nutrient agar (NA) and nutrient broth[4] (NB) as well as by the agar cup assay method[7]. Zones of inhibition of 16 mm diameter and more were taken as being sensitive. Heat tolerance of cultures was tested in peptone water and salt tolerance in NaCl broth[4,5]. Growth at different pH levels was tested on NA adjusted to different pH levels with 0.1N HCl. Growth temperature studies were carried out on NA slopes.

In the 20 batches examined total aerobic, mesophilic (37°C) and thermophilic (55°C) plate counts did not exceed 2.4×10^3 organisms/10 g and 4.2×10^2 organisms/10 g respectively. The total aerobic spore load on Dextrose tryptone agar was not more than 2.2×10^3 org/10 g. Staphylococci, coliforms, *Salmonella* and moulds were not present in the finished product.

TABLE 1. GROWTH RESPONSE OF BACILLUS SPECIES TO NACL, SA* AND HEAT TREATMENT

Isolate No	Assigned to Group	Growth in NaCl (%) 2.5	5	7	10	Growth in SA (%) 0.05	0.1	0.2	Tolerance of spores to 90°C for 2 hr.
1		+	+	+	+	+	+	—	+
2	*B. licheinformis*	+	+	+	+	+	+	—	+
14		+	+	—	—	—	—	—	+
17	Gr. 1	+	+	+	+	(+)	(+)	—	+
18		+	+	+	+	+	+	—	+
3		+	+	+	+[c]	—	—	—	+
4	*B. megaterium*	+	+	+	(+)	+	+	—	+
9	Gr. 2	+	+	+[c]	(+)	—	—	—	+
13		+	+	+	(+)	+	+	—	+
16	*B. megaterium*	+	+	+	—	—	—	—	+
19	(non mucoid)	+	+	+	+	+	+	—	+
20	Gr. 3	+	—	—	—	—	—	—	+
6	*B. subtilis*	+	—	—	—	+	+	—	+
7	(Mucoid)	+	+	(+)	—	+	+	—	+
15	Gr. 4	+	—	—	—	—	—	—	—
10	*B. brevis* Gr. 5	+	+[c]	—	—	—	—	—	+
11	*B. polymyxa*	+	—	—	—	—	—	—	—
12	Gr. 6	+	—	—	—	—	—	—	+
5	*B. cereus* Gr. 7.	+	+	+	—	+	+	—	+
8	Plant coryneform type Gr 8.	+	+	+	—	—	—	—	0

*SA = Sorbic acid
+ = Good growth with the formation of a film at top
— = No growth
(+) = Delayed growth after 7-14 days incubation
+[c] = Growth in clumps, no film at top.
0 = No spore formation

Several morphologically different colonies from the total aerobic count plates and spore count plates were purified and examined. These were all gram positive and catalase positive rods. Twenty distinctive cultures based on colony appearance and growth characteristics on NA were isolated and characterised. Nineteen cultures belonged to the genus *Bacillus* and one isolate in which spore formation could not be demonstrated was placed in the plant coryneform group[9]. The *Bacillus* strains were identified by standard methods[9,10] as belonging to six different species viz. *B. licheniformis* (5 strains), *B. megaterium* (4 mucoid and 3 nonmucoid strains), *B. subtilis* (3 mucoid strains), *B. brevis* (1 strain), *B. polymyxa* (2 strains) and *B. cereus* (1 strain). All the 20 cultures hydrolysed starch and excepting *B. brevis*, all were proteolytic. *B. brevis* and *B. cereus* showed lipase and lecithinase activities respectively. All strains of *B. licheniformis*, *B. polymyxa* and *B. cereus* showed anaerobic growth in glucose broth. Twelve strains utilized citrate as sole source of carbon, while 4 showed poor growth and 4 showed no growth on citrate. *B. licheniformis* strains were facultative thermophiles (25-55°C) growing over a pH range of 5 to 7.5, their optimum being 6.8. *B. megaterium*, *B. subtilis*, *B. cereus* and the coryneform type showed good growth between 25 to 45°C with an optimum of 30°C. *B. brevis* and *B. polymyxa* however grew well between 25-37°C. None of the strains were psychrotrophic. For all these strains excepting the nonmucoid *B. megaterium* and *B. cereus* the growth pH lay between 6.8 and 7.5. Thirteen strains were thermoduric while spores of all but *B. polymyxa* (1 strain) and *B. subtilis* (1 strain) were able to withstand heating at 90°C for 2 hr. Levels of salt (NaCl) tolerance (Table 1) was generally high (10 per cent) among strains of *B. licheniformis* and *B. megaterium*. The coryneform type and *B. cereus* tolerated upto 7 per cent NaCl. SA at 0.2 per cent inhibited growth of all strains while at 0.1 per cent, only

10 strains were inhibited. *B. brevis*, *B. polymyxa*, the Coryneform, 4 strains of *B. megaterium*, 1 strain of *B. subtilis* and 1 strain of *B. licheniformis* were inhibited by even 0.05 per cent SA. It may be mentioned here that the effective concentration of sorbic acid in the chapaties after heat treatment varied between 0.08 and 0.15 per cent. Sensitivity to SA of spores of one strain of *B. licheniformis* (isolate 1) was tested in liquid (NB) and plate media using a concentration profile ranging from 0.01 to 0.5 per cent. SA at 0.05 per cent did not have any marked effect on the growth and sporulation but at 0.17 per cent both growth and sporulation were delayed. There was no synergistic effect of SA and NaCl (at 0.1 and 2.5 per cent respectively) on the inhibition of this isolate. Probably this may hold good for other isolates as well.

Usually *Bacillus* strains have been reported[11-12] as common contaminants in wheat and bakery products present in the flour, air and equipments. The normal cooking eliminated the gram negative rods, cocci and the vegetative cells of the *Bacillus* and pathogens. The inpack heat treatment of chapaties eliminated the contamination picked up subsequently from handlers, equipment surfaces, processing atmosphere, etc. It had been observed that when the inpack heating was not done, slime formation and in rare instances, mould growth occurred. In maize prepared tortillas[13] higher total counts 24 hr after cooking were attributed to workers' hands, water and the general unhygienic conditions. *B. cereus*, *B. macerans*, *B. megaterium* and *B. polymyxa* were reported in the same study.

Freshly baked chapaties were found to have low total counts and most of the load was due to subsequent contamination. Immediate packaging prevented secondary contamination. Spores present in the dough or picked up along the production line survived the heat treatment at 90°C for 2 hr, partly due to uneven heat penetration during the cooking and heat processing and partly due to their inherent heat resistance. The fat present in chapaties could have contributed to increased heat resistance as was found in the case of *B. licheniformis* spores suspended in lard and tallow[14].

The pH of the chapaties varied between 5.5 and 5.8 whereas the optimum pH for growth of all the 20 strains studied was 6.8, with poor or no growth at 5 and 7.5. Many of the isolates were not inhibited by 0.1 per cent SA. The slow growth observed at 0.1 per cent SA could be attributed to the resistant types which have built up sufficient numbers. Hence if the initial spore load is high even with 0.1 per cent SA, spoilage could occur at ambient temperatures but not at cooler temperatures. *B. cereus* although detected was found not to contribute materially to the spore load.

The distinguishing features of the organisms (present in low numbers) in chapaties are that they are: *a*) starch and protein hydrolysing, *b*) can grow fermentatively, *c*) can dissimilate citrate used for maintaining low pH, *d*) tolerate appreciable levels of salt much higher than those obtaining in the chapaties, *e*) can grow in the presence of 0.1 per cent SA, the concentration of which lies between 0.08 and 0.15 per cent in chapaties, *f*) form spores that can withstand the time-temperature treatment the packages are subjected to, *g*) grow over a wide temperature range but not below 20°C and *h*) grow poorly at low pH.

Thus the factors contributing to shelf life of preserved chapaties are: *a*) low initial load after cooking, *b*) low pH, *c*) immediate packaging to prevent secondary contamination, *d*) heat treatment (baking), *e*) subsequent inpack treatment to remove post baking contamination during handling, *f*) most of the spores not germinating perhaps due to some form of injury and *g*) the presence of SA arresting any possible growth of survivors.

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AFLATOXIN IN GROUNDNUT OIL, GROUNDNUT CAKE AND HYDROGENATED OIL IN HAPUR (UTTAR PRADESH, INDIA) MARKET

The extent of aflatoxin contamination in groundnut oil, groundnutcake and hydrogenated oil sold in the market of Hapur, Uttar Pradesh, India was investigated. Nearly 66.7 per cent of the total samples of oil contained toxin. Out of these, 70 per cent contained as much as 2660 ppb of aflatoxin B_1. Seventy percent of the cakes were contaminated with aflatoxin which ranged from 1135 to 2250 ppb. Refined oil or Vanaspathi did not contain any aflatoxin.

Oil Seeds and various foodgrains are good substrates for the growth of micro-organisms specially fungi such as *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. These fungi, if present, cause grain heating, decompose the cereal grains and produce mycotoxins under suitable environmental conditions. An investigation was carried out at the Institute to find out the extent of contamination by aflatoxin in groundnut oil, groundnut cake and hydrogenated oil sold in the market of Hapur, U. P., India.

Unrefined groundnut oil is a major cooking oil used in India. Of the total production of groundnut oil, 32 per cent is utilised to produce *Vanaspathi* (hydrogenated fat) and the remaining quantity is mainly used in an unrefined form. It has been observed by earlier workers that considerable quantities of aflatoxins percolate during extraction of oil from affected groundnut kernels in the expellers. Giridhar *et al.*[1] reported that 44.2 per cent samples of groundnut oils were contaminated in Andhra Pradesh by aflatoxin of which 25.7 per cent had a level of toxin more than 100 ppb and a few samples contained as much as 5000 ppb of aflatoxin. Shanta *et al.*[2] have also reported high contamination of groundnut oil with aflatoxin (0.1 to 2.6 ppm) in India.

It has been shown by Dwarkanath *et al.*[3] that the toxin in the oil is fairly heat stable and being carried away in considerable quantity when any material is fried in it. Thus food fried in contaminated unrefined groundnut oil could become an important source of aflatoxin toxicity to the consumer. It was of interest, therefore, to study the extent of aflatoxin contamination in peanut oil and other groundnut products collected at Hapur, U.P., where a large section of population use it for cooking and as cattle feed respectively. Samples of unrefined and refined groundnut oils, hydrogenated fats and groundnut cakes were collected from Hapur market and aflatoxins were extracted and purified according to the procedure described by Pons *et al.*[4] The cleaned up extract was dried by the passage through a bed of anhydrous sodium sulphate and after evaporation, the residue was dissolved in chloroform and made upto 5 ml; 0.2 ml of the aliquots, was spotted on T.L.C. plates coated to 500 microns thickness with silicagel G and developed in toluene + iso amyl alcohol + methanol mixture (90 + 32 + 3)[5] followed by development in diethyl ether[6] to remove interfering materials. Chromatographic plates were examined under long wave UV-light (365 mm) for evidence of flourescent compounds. Further confirmation of aflatoxins was made by spraying plate with sulphuric acid (1 + 3) as suggested by Michael *et al.*[7] For the determination of aflatoxin concentration, spectrometric procedure of Nebney and Nesbit[8] was followed.

TABLE 1. AFLATOXIN CONTENT IN GROUNDNUT/OIL, CAKES AND HYDROGENATED FATS

Samples	No. of samples analysed	No. of samples contaminated	Aflatoxin B_1 (Range) ppb
Crude groundnut oil	30	20, (66.7%)	4.43-2660
Refined groundnut oil	15	Nil	Nil
Hydrogenated fats (Vanaspathi Ghee)	15	Nil	Nil
Groundnut cake	10	7, (70%)	113.5-2250

Figures in parenthesis indicate percentage

The aflatoxin content (as aflatoxin B_1) of oils and cakes are given in Table 1. It is of interest to note that some samples contained very high amount of toxin. On an average, 66.7 per cent of total samples of oils contained toxin. Out of these, 70 per cent contained as much as 2660 ppb of aflatoxin B_1. Seventy per cent of the total samples of groundnut cakes were found to be contaminated with aflatoxin, the range being 1135 to 2250 ppb. No aflatoxin was detected in any of the samples of refined oils and Vanaspathi, which confirms that these products are free from mycotoxins.

Analysis of market samples of groundnut oil revealed that the unrefined oil sold in the market is invariably contaminated with significant quantities of aflatoxin, the well known hepato carcinogen. Surveillance and monitoring is therefore, necessary to screen samples of unrefined groundnut oil over larger areas, in view of the increasing supply and consumption of this edible oil. This study also reveals the need to encourage the consumption of refined groundnut oil which is free from aflatoxin contamination.

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CELL MACERATING ACTIVITY OF FUNGAL CULTURE FLUIDS: STANDARDISATION OF ASSAY PROCEDURE

The possibility of a colorimetric procedure for the assay of cell macerating activity of fungal culture fluids was studied in relation to suitablity of the substrates, enzyme concentration and incubation period. Potato or apple tissues can be used as substrates and the enzyme activity was linear with respect to substrate and enzyme concentrations and the incubation period.

Cell macerating (also called cell separating) enzymes have been described in connection with processes for softening of vegetables, and fruits and increasing their digestibility[1]. The cell macerating activity has been found in microorganisms[2] as well as in higher plants[3] and was attributed to various enzymes or enzyme combinations comprising pectinolytic, hemicellulolytic and cellulolytic functions. The assay procedures so far described, take into account the time required for disintegration of vegetable tissues based on visual observation[4]; obviously this procedure is subject to errors due to personal variations in the experimenters' observations. In the present work, attempts have been made to evolve a suitable assay procedure for a more quantitative determination of enzyme activity.

The culture fluids of the two fungal cultures (*Monilia* sp. and *Aspergillus* sp.) isolated earlier in this laboratory possessed considerable xylanase activity accompanied with some amount of amylolytic, pectinolytic and cellulolytic activities[5]. The above culture fluids were employed as sources of the cell macerating enzyme in the present studies.

Cell macerating activity was assayed by suspending a piece of the vegetable tissue of known weight in the enzyme solution and measuring the increase in turbidity of the suspension fluid. The weight of the residue of plant tissue after attack by the enzyme was also determined.

a) Selection of suitable substrate: Potato, apple, sugar beet, carrot and raddish were cut into small discs (2.0 cm. dia. $\times$0.5 cm. ht. weighing 1.0-2.0 g) using a cork borer. The pieces were soaked in distilled water for 30 min and excess water was removed by pressing each piece between folds of absorbent paper. Each piece was then transferred to a wide mouth tube containing 8 ml of crude xylanase preparation having approximately 625 units xylanase ml^{-1} in 0.0125M acetate buffer of pH 6.0. (Crude xylanase was prepared by growing xylanase producing fungal cultures, *Monilia* sp. and *Aspergillus* sp. by surface cultivation on wheat bran containing 1 per cent groundnut meal wetted to 60 per cent moisture with a solution containing $MgSO_4$, 0.05 g; KCl, 0.05g; KH_2PO_4, 0.1; $FeSO_4$ 0.001 per cent for 7 days. Fifty grams of mouldy bran were extracted with 200 ml of tap water. One unit of xylanase is equal to the amount of enzyme which generated 1 μ mole of xylose from xylan[5]). The tubes were kept at 55 °C for 24 hr. The supernatant fluid was decanted off for measurement of absorbance and the weight was determined. The results given in Table 1 indicate that apple, potato and carrot were acted upon more efficiently by the *Monilia* sp. culture fluid while *Aspergillus* culture fluid was effective on apple, sugar beet and radish tissues. In the case of sugar beet considerable colour was also extracted which interfered with measurement of absorbance. The absence of a correlation between absorbance and tissue weight loss was also due to the extraction of these coloured pigments. Based on these criteria, potato and apple were selected as suitable substrates. Since the culture fluid of *Monilia* sp. was more potent in disintegrating the plant tissue, it was used in subsequent studies for determining optimum conditions for assay.

TABLE 1. CELL MACERATING ACTIVITY* OF *Monilia* SP. AND *Aspergillus* SP. CULTURE FLUIDS ON DIFFERENT PLANT TISSUES

Substrate	Increase in absorbance of supernatant (660 nm) per g. plant tissue		Wt. loss of tissue (%)	
	Monilia sp. enzyme	*Aspergillus* sp. enzyme	*Monilia* sp. enzyme	*Aspergillus* sp. enzyme
Apple	0.24	0.11	70.6	44.8
Potato	0.29	0.13	46.1	5.0
Sugar beet	0.55	0.21	15.5	17.7
Radish	0.28	0.07	22.3	15.3
Carrot	0.18	0.04	46.3	8.1

*Incubation for 24 hr at 55°C
Substrate: 1-2 g.

b) Optimum conditions for assay: The effect of various levels of enzyme on the absorbance of suspension fluid for both apple and potato is indicated in Fig. 1. In case of both the substrates, increase in absorbance of the suspension fluid with higher levels of the enzyme was observed.

The velocity of the cell macerating activity measured as absorbance of the suspension fluid against the amount of potato and apple tissue showed a linear relationship, as can be seen in Fig. 2.

The increase in absorbance of suspension fluid was measured during different periods of incubation and the data are indicated in Fig. 3. In the case of both the substrates upto 7 hr a good linear relationship of enzyme activity to period of incubation is evident.

From the above data, it is clear that an assay procedure based on the measurement of absorbance of the suspension fluid can be easily undertaken with a fair degree of accuracy and either potato or apple could be used as the substrate. In a few cases where disintegration of plant tissues resulted in formation of smaller fragments, the suspension fluid contained a range of particles from very fine to somewhat clumpy bits of tissue. In such cases, the suspension fluid was homogenized in a teflon homogenizer and even then the results obtained were linear in relation to concentrations of enzyme and substrates and incubation period upto 7 hr.

In contrast to the procedure now available for the assay of cell macerating activity which relies on visual observation for disintegration of tissues, the present procedure is more reliable as it is based on changes in the absorbance of the suspension fluid.

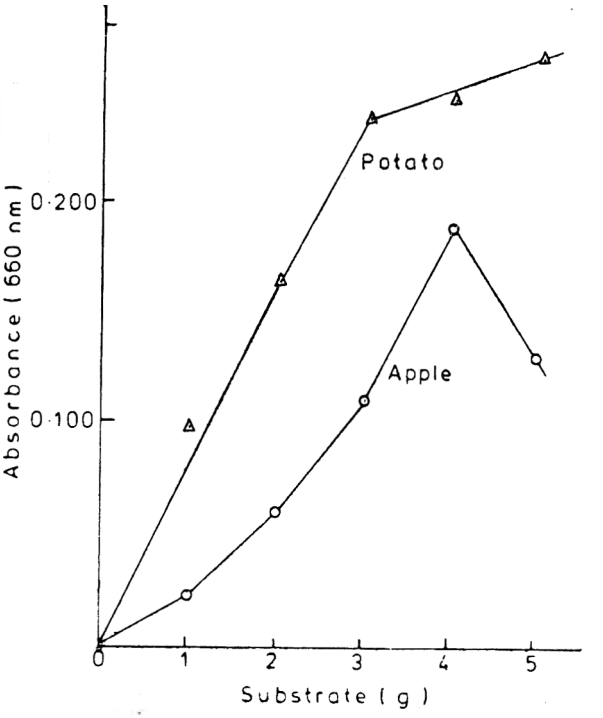

Fig. 2. Effect of substrate concentration on the rate of cell macerating activity of *Monilia* Sp culture fluid.

It will be of interest to determine the relative potencies of various enzymes present in the culture fluids for the cell macerating activity. It is reasonable to expect that all the enzymes viz. pectinase, hemicellulase and cellulase will have their own cell macerating activities as the interlamellar spaces of plant tissue contain a heterogenous

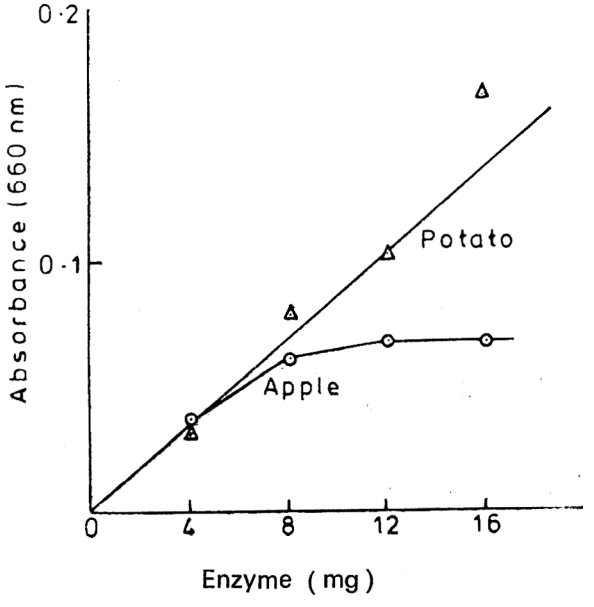

Fig. 1. Effect of enzyme concentration on cell macerating activity. (Substrate: 1g)

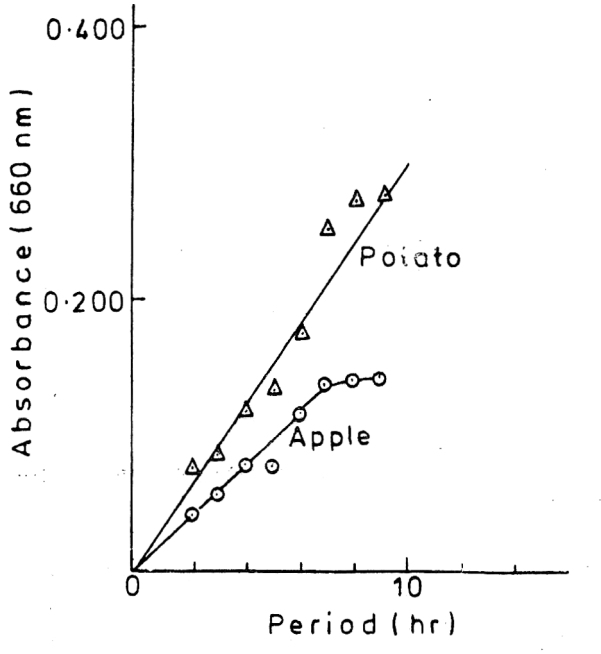

Fig. 3. Effect of incubation period on cell macerating activity. (Substrate: 1 g)

complex of pectins, hemicellulose and cellulose. The degradation of any component of this complex could disintegrate the cell cementing matrix. In the present case, since culture fluid contained all the above mentioned enzymes, the break down products as detected by paper chromatography were glucose, maltose galacturonic acid and xylose.

The authors are thankful to Dr. V. Sreenivasa Murthy, Project Coordinator, Discipline of Microbiology, Fermentation and Sanitation for helpful suggestions and critical scrutiny of the manuscript and to Shri C. P. Natarajan, Director of the Institute for facilities and encouragement given during the work. The first author (Anil Sharma) is a recipient of a Junior Research Fellowship of the Council of Scientific and Industrial Research, New Delhi.

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DETECTION OF PONGAM OIL USING ACETIC ANHYDRIDE SULPHURIC ACID REAGENT

A simple and rapid colour reaction for detection of pongam seed (*Pongamia glabra*) oil in binary oil mixtures using a modified acetic anhydride-sulphuric acid reagent is reported. The reaction produces an instant red to deep red colour sensitive at 1-2 percent level of pongam oil in simple mixtures.

A colour reaction for the detection of pongam oil using antimony trichloride was reported in 1969[1], and a TLC method on the same principle was developed by Sreenivasulu *et al.*[2] Subsequently a TLC method of identifying a characteristic spot, under UV light was reported by Rao *et al.*[3] In this paper, a colour reaction involving acetic anhydride sulphuric acid is described. The apparatus and chemicals required for the test are: a dropper, 5 ml capacity, graduated in 0.1 ml; cylinders, 5 ml capacity, graduated in ml; dropping tubes, 0.03 mm interior diameter; and test tubes, 16 mm × 125 mm. Sulphuric acid, chloroform and acetic anhydride, are all of analytical reagent grade.

The procedure is applicable to pongam oil exclusively or in admixture with other oils and fats at ambient temperature. Dissolve 0.2 ml oil or fat (or 5 drops from the dropping tube) in 2 ml chloroform and add 5 ml acetic anhydride. Shake well and add one or two drops of sulphuric acid. Observe the colour development in the first 30 sec. An immediate characteristic red to deep red colour indicates the presence of pongam oil in the sample.

The present method differs from earlier tests like the Liebermann-Storch test[4], Fitelson's test[5,6], and modified Liebermann-Burchard reaction[7]. The Liebermann-Storch test was developed chiefly for the detection of rosin and rosin oils. Fitelson's test and the modified Liebermann-Burchard reaction were meant for detection only of tea seed oil in olive oil and other oils. The colour developed by pongam oil in the present test is distinct and characteristic and the technique is simple, rapid and reproducible.

The reaction is sensitive to the presence of raw pongam oil in other vegetable oils at 1 percent level; pongam oil that has been refined and bleached gives a red colour of lesser intensity, and the test is sensitive to 2 percent of pongam oil in other vegetable oils. Raw pongam oil obtained either by pressure or extraction methods, and alkali-neutralized, alcohol-refined, alcoholic alkali-refined and bleached pongam oils all respond to this test. Binary mixtures of pongam oil in common oils such as sunflower, safflower, soybean, groundnut, sesame, rapeseed, mustard, niger, *ambadi*, and castor, all respond to the test.

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BOOK REVIEWS

Sugar: Science and Technology: Edited by G. G. Birch and K. J. Parker, Applied Science Publishers Ltd., London 1979, pp xii+475. Price: 80 $

This book records twentytwo papers presented at an Industry—University Co-operation Symposium held at the University of Reading in April 1978.

The opening paper (by J. A. C. Hugill) is more of historical nature. The first section (papers 2 through 6) is on the general perspective of sugar: its production economics and technological aspects. The chapter on colour in the sugar industry (by M. J. Kort) is well presented and provides a comprehensive account of natural occurrence of colour and its formation during many of the processing conditions. The removal of colour, is also of great importance in sugar refining particularly when an universally acceptable product is desired. Paper 5 presents the progress in the technology of beet sugar and lists briefly some of the new products such as liquid sugar, instant sugar and jellifying sugar. Paper 6 deals with the potential for industrial uses of sucrose. It is tempting to know that sucrose, a simple disaccharide of glucose and fructose, is capable of introducing a new thread of sucrochemical research!

Section II opens with an article on advances in sucrose chemistry by R. Khan which discusses the various types of reactions and their mechanisms in relation to their structure-sweetness. The next three papers (Nos. 8-10) record several physico-chemical standardisations of sucrose including the food quality and storage effects on sugar beets and potatoes. Dr. Howling in his paper narrates the technological problems of glucose syrups and also lists their colligative properities. Section III deals with hydrogenated products of glucose syrups (No. 12) and xylose (No. 14) and their applications. In paper 13, is given a vivid description of the properties, manufacture and use of fructose as an industrial raw material.

Section IV concentrates on some of the analytical aspects of food carbohydrates and carbohydrate sweeteners. The discussion by Southgate on food carbohydrate analysis is informative. The article on the role of structure-activity relationships in sweetness research is well presented.

The final section is on the medicinal aspects of sucrose. This section is complete with four papers (No. 19 through 22) and in general the articles describe some of the merits and demerits of sucrose and other carbohydrates in the normal dietary conditions.

To sum up, the use of sucrose, a regenerable chemical resource of considerable commercial potential, in the production of new derivatives for use as surfactants, in plastics and polymers, in paint, resins, etc. apart from very many food uses is well documented. The book is essentially a timely review for greater understanding of the chemistry and technology of sucrose. The presentation and getup of the book are impressive.

R. N. THARANATHAN
CFTRI, MYSORE.

Freezing, Frozen Storage and Freeze Drying: International Institute of Refrigeration, Paris, 1977; pp 493; Price: 80FF.

This is a compilation of 53 papers discussed in the yearly meeting of the International Institute of Refrigeration at Ettlingen, Federal Republic of Germany on September 6-8, 1977. 47 of the papers are in English and 6 are in French.

Out of these, the effects of freezing and frozen storage on animal tissues was the subject matter of 15 papers, industrial aspects of freezing and frozen distribution, (10 papers) freeze drying, (9 papers) freezing and frozen storage of vegetables and ready-to-serve products (8 papers) mathematics treatment of the freezing and thawing process (5 papers) physico-chemical phenomena 4 papers and basic phenomena (2 papers.)

The papers of fundamental and theoretical nature range from structural and physiological features of cold resistance, postulated mechanisms of freezing and frozen storage damage, physico-chemical phenomena during freezing process as measured by refractometer in sucrose solutions, sugar concentration gradient in quick frozen thin flakes of orange juice, etc.

The papers on foods of animal origin deal with changes affecting the use of frozen stored and thawed meat/fish regarding WHC, cathepsin activity, rigor changes, micrographic and x-ray diffraction, mineral (ionic) and formaldehyde interaction.

The section on long term frozen storage discusses the effect of intense freezing on cell structure, blanching prior to freezing, quality of green beans, cooked fish flesh, foods for aeroplane and industrial catering and combination of irradiation and cold storage.

The section on mathematical treatment comprises of discussion on heat transfer and heat capacity during

freezing and thawing, heat capacity *vs* time, time-temperature *vs* weight loss. All the above discussions concern meat as the experimental material.

Freezing and frozen storage, industrial conditions of freezing, frozen storage and distribution of products, temperature maintainenance during transporation and distribution, storage effects in retail outlets and domestic freezers, methods of thawing and use of time, temperature indicators in quality control of frozen products form the subject matter of the section on industrial freezing and distribution of frozen foods.

The last section deals with industrial freeze drying of different commodities, microbiological and chemical aspects during freeze drying and subsequent storage, use of carbonmonoxide enriched nitrogen atmosphere to improve the colour of freeze dried beef, techno-economic improvements in the freeze-drying cycle, electrical resistance measurements and crymicroscopy to measure rates of freezing and freeze-drying, mass diffusivity and tendency to structure collapse in freeze-dried model systems.

The different papers are of interest to research institutes, fundamental as well as applied. Food processors handling freezing, frozen storage, frozen distribution and freeze-drying could obtain many points to improve their products.

B. R. BALIGA
CFTRI, MYSORE.

Chemical Toxicology of Food: C. L. Galli, R. Paoletti and G. Verrorazzi, Elsevier/North-Holland Biomedical Press, Amsterdam, New York, Oxford.

The present volume of chemical toxicology of food is the proceedings of a symposium held in Milan (June 1978). The contributions cover general principles of safety assessment, legal aspects, methods of toxicological assessment of food additives, interrelationship of nutrition and toxicology and applied aspects of toxicology of food. With increased use of food additives, colours etc. concern for the safety of these additives as they affect the health and well being of man is currently of utmost importance. The food technologists, nutritionists and clinicians are vitally involved in such discussions.

These proceedings provide an insight into the multi-faceted problems of safety assessment and the legal implications. Truhant has lucidly outlined the general principles of toxicological evaluation of food additives. The concept of benefits Vs risks in recommending AID as guidelines to health authorities is discussed. It is intriguing however, to note his statement—"It is obvious that with a malnourished population with an expectation of life less than 40 years it is justified to take greater risks than for populations with an over abundance of food! (page 18, para 3). To me it appears to be perverted logic! The difficulties involved in recommending an 'acceptable risk' for a food additive are well delineated by Darby. Grice states that it is difficult to over emphasize the importance of an informed public opinion in ensuring that the consumer's point of view is taken into account before decisions on acceptable risks are taken. Elias also concludes by stating that the actual selection of an acceptable risk is no longer the task of the toxicologist but a soceital decision.

The legal aspects of food additives, pesticide residues, etc. for EEC countries are discussed in depth by Alain Gerard. Gnauck points out that in case of drugs with side effects the question is whether to run a risk or not. Whereas in case of foods one should never run a concrete risk or accept noxious side effects.

The second section deals with toxicological evaluation of food additives. The problems associated with the testing for mutagenic potential are extremely well presented and discussed. Attention is drawn to the fact that some intestinal microbes can convert certain dietary components to mutagens *in vivo*. Further it is suggested that tests for mutagenic metabolites of the additives in urine and feces of animals fed the compound under investigation may be useful. Cecal contents of such animals could also be tested.

Munro and Willes elucidate with examples the importance of studies on pharmaco kinetics, tissue distribution and metabolism of a compound prior to conducting studies on possible adverse effects on reproductive performance. In new approaches to mutagenicity and carcinogenicity testing *in vivo* mammalian systems Zbinden and Schlatter have presented data on binding of aflatoxin, benzpyrene etc. to DNA in an attempt to correlate hepato carcinogenicity with DNA alkylation. Such approaches hopefully lead ultimately to useful viable short term toxicity tests.

The influence of nutritional status on the metabolism of toxicity of food additives or pesticides is well recognised. The perils of extrapolating, tolerance levels determined in healthy animals fed nutritionally adequate diets to human populations in developing countries where dietary intake of protein and calories are low are rightly highlighted (Almeida *et al*). Newberne and McConnel have provided extensive and excellent data to show that newer sources of proteins (single cell protein, plant protein concentrates) and carbohydrates (modified starches, gums etc.) cannot be included in experimental diets with 1000 to 1 or even 100-1 margins of intended use. The protagonists for Algal or Leaf protein find this disturbing, to say the least! Rightly

they stress that cautious clinical studies in man should be done in one or more animal species with newer types of protein developed from novel or conventional sources.

Epidemiological evidence linking dietary factors with types of cancer are also discussed by Gori and the allergenic potential of food additives is briefly enumerated by Zanusi.

The imperative need for data on metabolism and pharmaco kinetics of the compounds in safety evaluation is stressed by Parke. Species differences in rates of metabolism and dosage of a given chemical that affect species of animals differently are illustrated with examples (paraacetamol toxicity in mouse hamster rat; differences in microsomal hydroxylation of biphenyl among species etc.)

A number of natural colours extracted from fruits, vegetables and their synthetic equivalents have been used. Many of them have not been evaluated for their safety. Kojima has lucidly summarised this data. It is of particular interest for the food technologists to note the reported presence of a potential convulsant 4-methyl-imidazole in caramel colour produced by process using ammonia or its salt. Specific data on pharmocokinetics of styrene monomer (Packaging material), marine biotoxins in sea food mutagenic activity of carmenic acid, chronic lead poisioning and studies on saccharin are other topics discussed.

This proceedings is a welcome addition to library of any food research/toxicology Institute and certainly useful for Toxicologists, Nutritionists and Food Technologists.

P. B. RAMA RAO
CFTRI, MYSORE.

ERRATA

Following correction is suggested for the paper, "Stability of Groundnut oil during continuous deep-fat frying at plant level" by T. Nataraja Murthy *et al.*, published in *this Journal* 1979, **16** (2), 75–77.

The first line "Sev or Muruku (Bengal gram dhal dough extruded and fried in oil)" should be read as "Sev or Muruku (Cornsoy—milk powder dough extruded and fried in oil)

ASSOCIATION NEWS

Annual General Body Meeting

The 14th Annual General Body Meeting of the Association was held on 1st July 1979 at Taj Mahal Hotel, Bombay, Dr. B. P. Baliga, President of the Association, presided over the meeting. The meeting was attended by 65 members.

The Secretary in his report for 1978–79, highlighted the major activities of the Association which included adoption of the new constitution by the Extraordinary General Body Meeting held on May 25th 1979. It was also resolved that the new constitution will come with effect from 1st Janury 1980. The Association, he said has brought out for the first time a technical directory. Several symposia were organised during the year by Head quarters, different Zones and Chapters, which included 'Status and Prospects of Confectionery Industry in India' jointly organised by the Association, Central Food Technological Research Institute and the Indian Confectionery Manufacturers' Association, New Delhi, at Mysore on May 25–26, 1979; 'Quality Control in Processed Food' organised by Trivandrum Chapter on December 15-16, 1978; 'Development of Meat Industry in India' organised by Western Zone and 'Small scale Food Industry for Rural India', organised by Eastern Zone in September 1978. Several seminars were also arranged by Headquarters, various zones and chapters on current topics of interest.

The Secretary announced the institution of two new awards—'Young Scientist Award' for scientists below the age of 35 years for excellence in the area of food science and technology and two 'Best Student Awards' for the merited students in post-graduate course in food science and technology. The report was unanimously adopted.

Representatives were also present from Headquarters, Western Zone and Bangalore Chapter.

The Secretary presented the audited statement of accounts and the budget proposals on behalf of the Treasurer. This was adopted unanimously by the General body.

Presentation of Awards

Prof. V. Subrahmanyan's Industrial Achievement, Award: The award for the year 1978 was presented to Dr. P. K. Kymal, Executive Director, Food and Nutrition Board, New Delhi. The award consisted of a cash grant of Rs. 2500, a plaque and a citation. It was received on behalf of Dr. Kymal by the Northern Zone Secretary, Sri A. K. Sachdev.

Gardner's Award: The award for the best research paper published in the *Journal of Food Science and Technology*, during the year 1977 was presented to Dr. A. Sreenivasan and Dr. D. R. Bongirwar, Bhabha Atomic Research Centre, Bombay, for their paper entitled "Studies in Osmotic Dehydration of Banana".

Suman Food Consultant Travel Award: This award for the best essay on "Role of Food Additives in Food Processing and Public Health" was given to Sri S. S. Deshpande, B.Sc., Food Technology Student, CFTRI, Mysore.

Young Scientist Award: This award which was introduced this year (1978) for excellence in research in the area of food science and technology for scientists below the age of 35 was presented to Dr. S. Govindarajan, Manager, Research and Development, The East India Hotels Limited, New Delhi, for his work on frozen meals for air-line catering and dehydration of on herbs.

Best Student Award: The best student awards, two in number for merit in post-graduate courses in food science and technology was awarded to Shri S. S. Deshpande, B.Sc., Food Technology Student, CFTRI, Mysore and Miss Usha Grover, B.Sc., Food Technology Student, GB Pant University, Pantnagar, U.P.

Office Bearers for 1979

The Secretary announced the names of the office bearers of the Association for 1979.

President	— Sri Dayanand
President-elect	— Dr. K. T. Achaya
Vice-president (HQ)	— Sri S. K. Majumder
Hon. Exe. Secretary	— Sri J. D. Patel
Hon. Joint Secretary	— Dr. K. R. Sreekantiah
Hon. Treasurer	— Sri K. Lakshminarayana Rao
Councillor (HQ)	— Sri K. Vidyasagar.

No nominations were received for the offices of Vice-presidents and Councillors from the Zones. The General Body authorised the Executive Committee to get in touch with the various zones and chapters for nominations to these offices.

The President in his concluding remark briefly reviewed the activities of the Association. He recalled that the Association in these 22 years, has contributed considerably towards the development of food science and technology through arranging symposia and by publication of the Journal. He stressed the need for the Association

to play a larger role in the national planning of research and development work connected with food production and processing.

The meeting ended with a vote of thanks by Dr. S. R. Padwal Desai.

Ludhiana Chapter

The Third Annual General Body Meeting of the Chapter was held on 2nd July 1979, in the college of Agricultural Engineering, Punjab Agricultural University Ludhiana. The President, Dr. J. S. Pruthi was in the Chair.

Shri A. K. Bharadwaj, the Hon. Secretary presented the Annual Report for 1978.

In the absence of Shri A. K. Saxena, Hon. Treasurer, the Hon. Joint Secretary, Shri B. L. Raina, presented the statement of accounts and budget proposals.

The Hon. Secretary, then announced the elected Office-bearers for 1979. They are:

President	— Dr. J. S. Pruthi
Hon. Secretary	— Shri B. L. Raina
Jt. Secretary	— Dr. V. K. Thaper
Hon. Treasurer	— Shri M. S. Teotia
Executive Councillors	— Dr. K. N. Singh
	Prof. M. M. Kashyap

The meeting ended with a vote of thanks by Hon. Secretary, Shri B. L. Raina.

Trivandrum Chapter

The Annual General Body Meeting of the Trivandrum Chapter was held at Trivandrum Hotel on 6th April 1979. Shri R. Hariharan, President of the Trivandrum Chapter, welcomed the members and delivered the opening address. Shri A. V. Bhat, Hon. Secretary, read the report on the activities of the Chapter during the period 1978–79. In the absence of Shri C. Balachandran, Hon. Treasurer, Shri A. V. Bhat read the statement of accounts for the year 1978. The President announced the elected office bearers for 1979–80. They are:

President	— Dr. A. G. Mathew,
Hon. Secretary	— Dr. K. Rajaraman
Hon. Treasurer	— Shri M. Gopalakrishnan.

After induction of the new President, Shri A. V. Bhat proposed a vote of thanks.

LIST OF NEW MEMBERS

Ordinary Members

Miss Ranjita Maitra, Scientist, FoSTIS, C.F.T.R.I., Mysore

Mr. Chalvada Jyothirmayee, C/o G. Raja Rao Plot No. 50, Indiranagar, Hyderabad-500 0890.

Mr. Gyanendra Upadhyay, Food Crafts Institute, Vidyanagar, Hyderabad-500 768

Mrs. B. Kumar C/o Deputy Tech. Adviser, Mistry Bhavan (4th floor), D. W. Road, Church Gate, Bombay-400 020.

Mr. Vilas Pandurang Sinkar, Ramnarain Ruia College, Matunga, Bombay-400 019.

Mr. R. Pandurang Padke, Dept of Microbiology. R. Raja College, Matunga, Bombay-400 019.

Mr. Azeez P. Khambatta, Pioura Industries, Asarva Bridge, Northend, Ahmedabad-380 016.

Dr. Jayam Subramaniyam, No. 11, Subbaraya Madali, street, Nungambakam, Madras-600 034.

Dr. P. V. Khandwekar, House No. 265, Old Dharampeth Nagpur.

Dr. N. Kripalani, 16E, Community Centre, Basant Lake, Vasant Vihar, New Delhi-110 057.

Mr. B. P. Ram, Discipline of Biochemistry, CFTRI, Mysore-13.

Mr. H. Vinod Kumar, UNU, CFTRI, Mysore-13.

Mr. M. M. Patel, B-3, CFTRI Hostel, Mysore-13.

Mr. Nagin Chand, Discipline of Sensory Evaluation, CFTRI, Mysore-13.

Mr. B. Bihari Lal, 28, Vasant Hostel, Indian Agrl. Res. Institute, New Delhi-110 012.

Mr. S. K. Kalra, S-2 (Fruit Technology), Central Mango Research Institute, B-53, Sector A, Mahanagar, Lucknow-226 006.

Dr. B. Ranganathan, Head, Southern Regional Station, N.D.R.I., Bangalore-560 030.

Dr. V. Unni Krishnan, Dairy Chemistry Section, National Dairy Research Institute, Bangalore-560 030.

Mr. V. Venkatesan, Paddy Processing Res. Centre, Tiruvarur-610 108.

Sri A. Ranga Sai, 1-9-286/2-6A, Vidyanagar, Hyderabad-500 044.

Mr. K. Nagaiah Setty, 3-5-930, Himayat Nagar, Hyderabad-500 029.

Sri D. C. Krishna Murthy, 1-8-168/2, Chikkalapalli, Hyderabad.

Sri M. V. Ramana Rao, 1-1-336/114, Vivekanagar, Hyderabad-500 020.

Sri M. Amarbabu, 2-2-1118/3/A, Hyderabad-500 044.

Sri K. Prabhakara Rao, 4-7-71/2, Esamia Bazar, Hyderabad-500 027.

Sri P. R. Rajendranathan Nair, Chief Quality Control Officer KLD & MM Board, Mani Bhavan, Sasthamangalam, Trivandrum 10.

Sri Rajagopalan, CSIR, PTC, Pappanamcode, Industrial Estate, Trivandrum.

Sri S. Govindaswamy, Sowbagiyalakshmi Rice Mills, 13A, Kuppamane Thope, Red Hills, Madras-600 052.

Mr. Gajendra Kumar, Jain Ayurvedics, Cash Bazar, Coowoor-643 102.

Mr. Satpal Gera, M/s. Vicky Food Corpn, C-38/1, Lawerence Road, Industrial Area, Delhi-110 035.

Mr. Joseph Selvam, M/s. Kamala Sugar Mills, Amaravathi Nagar, P. O. Udumalpet, Coimbatore-642 102.

Mr. K. K. Kalsi, Milkfood Limited, Bahadurgarh, P.O. Dist. Patiala (Punjab).

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ANNOUNCEMENT OF

Prof. V. Subrahmanyan Industrial Achievement Award for the Year 1979

Nominations for the above award for the year 1979 are invited. The guidelines for the award are as follows:

1. Indian Nationals engaged in the field of Food Science and Technology will be considered for the award.

2. The Nominee should have contributed to the field of Food Science and Technology, for the development of Agro-based food and allied industries or to basic food science and technology with immediate prospect and/or future potential for industrial application.

3. The nomination should be proposed by any member of the Association; the bio-data of the candidate together with his consent should be given in detail including the work done by him and for which he is to be considered for the award.

4. The Awardee will be selected (from the names thus sponsored) by an Expert Panel constituted by the Executive Committee for the above purpose.

Nominations along with bio-data and contributions, should be sent by **Registered Post,** *so as to reach Shri J. D. PATEL Honorary Executive Secretary, Association of Food Scientists and Technologists (India), Central Food Technological Research Institute, Mysore-570 013 latest by 31st of January 1980*

ANNOUNCEMENT OF

Young Scientist award for the year 1979

Association of Food Scientists and Technologists (India), announces with pleasure the institution of the YOUNG SCIENTIST AWARD for distinguished scientific research and technological contributions to the field of Food Science and Technology.

The award consists of a cash prize of Rs. 1,000/-, a plaque, and a citation.
Nomination for the Award is open to aspirants fulfilling the following conditions:

1. The candidate should be an Indian National below the age of 35 years on the date of application working in the broad area of food science and technology.
2. The candidate should furnish evidence of either,
   (*a*) Original scientific research of high quality, primarily by way of published research papers, and (especially if the papers are under joint authorship) the candidates own contribution to the work:

   OR

   (*b*) Technological contributions of a high order, for example in product development, process design etc., substantiated with documentary evidence.

The application along with details of contributions and bio-data (in triplicate) may be sent by REGISTERED POST, so as to reach Sri J. D. Patel, Hon. Exec. Secretary, Association of Food Scientists and Technologists (India), CFTRI, Mysore-13 before 31st January, 1980.

ANNOUNCEMENT OF

Best Student award for the year 1979

Association of Food Scientists and Technologists (India) announces the institution of the BEST STUDENT AWARD for students with a distinguished academic record undergoing post-graduate courses in Food Science and Technology. There are two awards, each comprising a book grant of Rs. 500/-.

The award is open to candidates fulfilling the following conditions:

1. The candidates must be Indian nationals undergoing Post-graduate courses in the area of Food Science and Technology in any Institution in India.
2. The Head of the Post-graduate Department may put up for the award, the name of one candidate from each institution, who has at least completed one year of study, supported by the following information prepared by the candidate:
   (*a*) Graduate record
   (*b*) Post-graduate performance to date.

Nominations may be sent by REGISTERED POST so as, to reach Sri J. D. Patel, Hon. Exec. Secretary, Association of Food Scientists and Technologists (India), Central Food Technological Research Institute, Mysore-570 013 before 31st January 1980.

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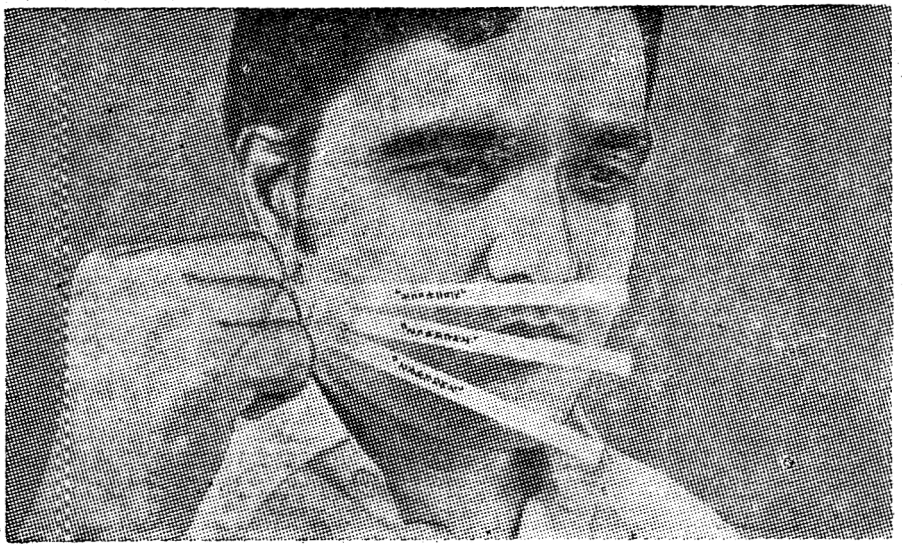

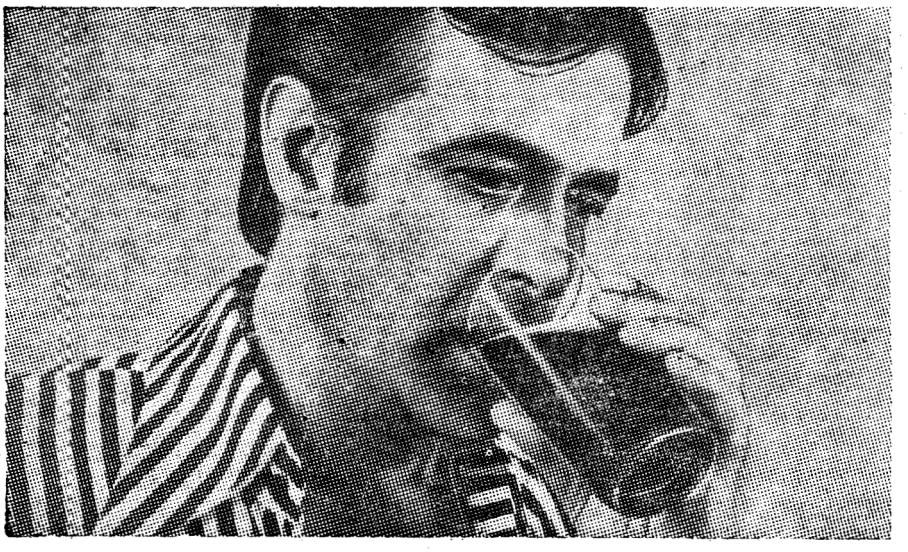

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1. Manuscripts of papers should be typewritten in double space on one side of the paper only. They should be submitted in **triplicate.** The manuscripts should be complete and in final form, since no alterations or additions are allowed at the proof stage. The paper submitted should not have been published or communicated anywhere.

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. Superscript and subscripts should be legibly and carefully placed. Foot notes should be avoided as far as possible.

4. **Abstract:** The abstract should indicate the scope of the work and the principal findings of the paper. It should not normally exceed 200 words. It should be in such a form that abstracting periodicals can readily use it.

5. **Tables:** Graphs as well as tables, 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. Nil results should be indicated and distinguished clearly from absence of data.

6. **Illustrations:** Line drawings should be made with *Indian ink* on white drawing paper preferably art paper. The lettering should be in pencil. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size. Photographs must be on glossy paper and contrasty; *two 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 should be cited completely in each reference. Abbreviations such as *et al.*, should be avoided.

   In the text, the references should be included at the end of the article in serial order.

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

   (*a*) *Research Paper:* Menon, G. and Das, R. P., J. sci. industr. Res., 1958, **18,** 561.

   (*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:* As in (*c*).

   (*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.

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