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Protein Quality Evaluation of Popped Barley Grains *(Sattu)*

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Protein quality evaluation of the popped barley grains of normal and high lysine cultivars/lines revealed that the processing of the grains caused non-availability of the amino acids; dye binding capacity (DBC) method could be used to evaluate this non-availability; the high lysine lines with a greater content of free amino acids were more prone to damage; threonine content in the *sattu* material increased due to a change in the relative composition of the amino acids; and since the eating quality of *sattu* is improved when it is taken in combination with roasted Bengal gram flour the biological value of the combination will be high.

One of the several forms in which barley is consumed in India is *sattu*. It is a sieved flour of roasted (popped) barley grains. This is consumed either in the form of a drink consisting of the flavour, water, fresh lemon and sugar added in the required proportions, or in the form of a dough consisting of *sattu* and flour of roasted Bengal gram with salt added to taste. The prevalent belief is that *sattu* is a valuable food and when consumed along with Bengal gram it forms a wholesome diet. However, there is no information available regarding this aspect. Evaluation of the nutritional quality of *sattu* is, therefore, of relevance.

In the present study, the *sattu* prepared from normal and high lysine barley grains was evaluated and compared with quality characters of the whole meal prepared from unpopped grains.

Materials and Methods

Grain material: Grains of eleven normal barley cultivars viz. '*Clipper*', '*C* 164', '*RS* 6', *Ratna*, *Vijaya*, *DL* 3, *DL* 69, *RD* 31, *NP* 113, *DL* 70 and *Jyoti*, and four high lysine lines viz. 1098-2, 1098-7, *Notch* 1 and *Notch* 2 were grown, in the experimental fields of the Indian Agricultural Research Institute, under recommended cultural practices.

Preparation of sattu: Clean river bed sand was taken in an iron vessel and placed over a gas oven regulated to medium heat. Preliminary investigations indicated that 5 min. were sufficient to bring the sand to the required temperature. Triplicate samples of grains of each cultivar, weighing 40 g each were roasted (popped) separately. Preliminary investigations showed that 30 sec of roasting gave popped grains of the best taste and flavour. The taste and the flavour were assessed by requesting persons at the Institute who hail from traditionally barley growing regions of Bihar, UP and Haryana to make an evaluation by tasting the popped grains processed for different periods.

Grinding of unpopped and popped grains: Representative samples of each cultivar/line were ground to 60 mesh size using a hammer mill.

Determination of moisture: Moisture was determined in the unpopped grain whole meal and '*sattu*' flour by taking a known quantity and drying to a constant weight at 105°C in a hot air oven.

Determination of crude protein: Nitrogen percent was determined, in duplicate, in each of the samples by using the Technicon Autoanalyser[1]. Protein percentage was then computed by multiplying by the conversion factor of 6.25.

Dye binding capacity (DBC): The DBC value of the samples was determined by the procedure described earlier[2]. For comparison between samples, optical density values itself were used.

Amino acid spectra: One representative sample of the normal and all the four high lysine barleys were analysed. Amino acid composition was determined on the acid hydrolysate of the samples using a Technicon Sequential Multisample Amino Acid Analyser[3].

Estimation of tryptophan: Tryptophan was determined colorimetrically[4].

Free amino acid content: A known amount of ground material was extracted with chilled 80 percent ethanol and centrifuged for 15 min. at 10,000 rpm to get a clear supernatant. The extraction was repeated 4-5 times. The pooled supernatant was dried on a rotary flash evaporator. The residue was dissolved in 0.01 N HCl and made to a known volume. The amino Nitrogen was estimated following Rosen's method[5].

Amino acid score: The FAO reference pattern was used to calculate the amino acid score and to determine the limiting amino acids[6].

All the values have been presented on moisture free basis.

Results

Protein content and DBC value of unpopped and popped barley grains: There were no significant differences in the crude protein content of unpopped and popped barley grains (Tables 1 and 2). However, the DBC value of the samples showed some interesting differences. In the case of barley grains of normal cultivars, the differences in DBC value of unpopped and popped ones was not of large magnitude, except in the case of *NP* 113 where the reduction in DBC value of popped grains over that of the unpopped grains was 13 per cent. In other varieties, the reduction varied from 2 to 6 per cent. The variety, *Vijay* did not show any change at all.

In the case of the high lysine lines, 1098-2 and 1098-7 obtained in a breeding programme using *Hiproly*[2], and *Notch* 1 and *Notch* 2 obtained by induced mutation[7], the crude protein content did not show a significant change (Table 2). However, on the basis of the amount of amino acids recovered, the protein content showed a reduction of 9,8,18 and 22 per cent, respectively in 1098-2, 1098-7, *Notch*-1 and *Notch*-2 (Table 3).

The DBC value varied considerably in the high lysine lines (Table 2). *Sattu*, prepared from the grains of 1098-2 and 1098-7 showed a reduction of 7 and 6 per cent, respectively, over the DBC value obtained for the unpopped grains. *Notch* 1 and *Notch* 2 showed a greater decrease being 17 and 21 per cent, respectively.

Amino acid composition of unpopped and popped grains (sattu): Popping barley grains caused destruction and non-availability of almost all the amino acids. However, the extent of reduction varied with the amino acid. This resulted in a change in the relative proportion of amino acids in *sattu* as compared to that in the unpopped grains (Table 3). For instance, the proportion of essential amino acids, lysine, histidine and arginine decreased while the proportion of the essential amino acids, threonine, valine, methionine and isoleucine increased.

The free amino acid content in unpopped grains and *sattu* showed marked differences (Table 4). The normal barley sample had less free amino acids as compared to the high lysine ones. Among the high lysine lines,

TABLE 1. PROTEIN CONTENT (N × 6.25) AND DBC VALUE OF UNPOPPED AND POPPED BARLEY GRAINS (*SATTU*)

Cultivar	Protein % (N×6.25)	DBC Value (Optical Density)
	Mean±S.D.	Mean±S.D.
Clipper	13.0±0.2	0.210±0.002
	(12.8±0.3)	(0.200±0.004)
C 164	12.5±0.2	0.185±0.002
	(12.2±0.3)	(0.180±0.004)
RS 6	11.4±0.2	0.205±0.002
	(11.4±0.2)	(0.195±0.003)
Ratna	13.7±0.2	0.200±0.002
	(13.8±0.4)	(0.190±0.003)
Vijay	11.0±0.2	0.210±0.002
	(11.0±0.3)	(0.210±0.004)
DL 3	12.0±0.2	0.205±0.001
	(12.0±0.4)	(0.200±0.003)
DL 69	11.8±0.2	0.215±0.002
	(11.7±0.2)	(0.210±0.004)
RD 31	13.2±0.2	0.203±0.002
	(13.0±0.2)	(0.195±0.003)
NP 113	13.1±0.3	0.213±0.002
	(13.0±0.3)	(0.186±0.003)
DL 70	12.0±0.2	0.180±0.002)
	(11.8±0.2)	(0.170±0.003)

Each mean is from three observations.
Figures in the parentheses are the values for popped grain.

TABLE 2. PROTEIN CONTENT AND DBC VALUE OF NORMAL AND HIGH LYSINE BARLEY UNPOPPED AND POPPED GRAINS (*SATTU*)

Characters	Variety/Line*				
	Jyoti	1098–2	1098–7	Notch 1	Notch 2
Protein % (N×6.25)	13.3±0.2 (13.1±0.3)	15.5±0.3 (15.3±0.4)	15.2±0.3 (15.3±0.4)	16.8±0.4 (16.5±0.5)	16.9±0.4 (16.8±0.5)
Protein % (actually recoverable)**	12.9±0.3 (12.7±0.3)	14.9±0.4 (13.9±0.4)	14.7±0.4 (14.1±0.4)	16.0±0.5 (13.6±0.4)	16.1±0.5 (13.3±0.4)
DBC Value (Optical density)	0.200±0.002 (0.195±0.003	0.285±0.002 (0.265±0.004)	0.270±0.003 (0.255±0.004)	0.280±0.003 (0.232±0.004)	0.290±0.003 (0.228±0.004)

*Average value and standard deviation of three separate estimations.
**upDlicate estimations were made in this case. Figures in parenthesis are for popped grains.

TABLE 3. PROPORTION OF THE AMINO ACIDS IN THE PROTEIN OF NORMAL AND HIGH LYSINE BARLEY UNPOPPED AND POPPED GRAINS (*SATTU*)

Amino acid	Amino acids (% of the total recovered) in the indicated variety/line									
	Jyoti		1098-2		1098-7		Notch 1		Notch 2	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Lysine	3.07	2.99	4.11	3.89	3.67	3.53	4.02	3.51	4.01	3.64
Histidine	2.32	2.06	2.35	2.29	2.78	2.53	2.40	1.98	2.62	2.11
Arginine	5.20	4.45	4.67	4.14	4.99	4.62	5.39	3.71	5.39	3.62
Aspartic acid	6.62	7.55	6.82	7.60	6.43	7.71	6.52	7.46	6.70	7.68
Threonine	2.75	3.03	2.89	3.06	2.80	2.90	3.08	3.14	3.09	3.15
Serine	3.60	4.17	4.26	4.05	4.37	4.03	3.82	4.37	3.47	4.38
Glutamic acid	25.67	23.08	23.51	23.39	25.04	22.89	25.42	23.68	24.37	21.53
Proline	11.38	10.30	9.51	10.46	9.73	10.00	9.75	10.37	10.28	9.16
Glycine	4.01	3.71	4.21	4.02	4.11	3.88	4.23	4.38	4.22	4.42
Alanine	4.43	4.62	5.12	4.86	4.61	4.83	4.37	4.89	4.64	5.12
Cystine	2.23	3.04	2.19	2.44	2.18	1.82	1.90	2.24	2.30	2.35
Valine	4.57	5.47	5.18	5.57	5.16	5.93	5.11	6.12	5.05	6.74
Methionine	1.80	2.31	1.78	1.97	1.72	1.95	1.76	2.09	1.69	2.20
Isoleucine	3.89	4.15	3.83	4.45	3.63	3.78	3.58	3.82	4.04	4.97
Leucine	6.87	6.99	7.03	6.89	6.53	6.81	6.55	7.42	6.63	7.83
Tyrosine	3.22	3.16	3.26	2.27	2.78	3.70	3.38	2.36	3.24	2.61
Phenylalanine	5.21	5.14	5.48	4.85	5.58	5.44	4.89	4.76	4.52	4.64
Tryptophan	1.16	1.18	1.20	1.20	1.14	1.15	1.07	1.10	1.08	1.09
Ammonia	2.00	2.60	2.60	2.60	2.50	2.50	2.60	2.60	2.69	2.69
Recovery (% of crude protein)	97	96	96	91	97	92	95	82	96	78
Amino acid score %	56	55	72	71	67	64	73	64	73	66
1st limiting amino acid	Lys	Lys	Thr	Lys	Lys	Lys	Lys	Lys	Lys	Lys
Amino acid score (%)	69	76	72	76	70	73	77	79	77	79
2nd limiting amino acid	Thr	Thr	Lys	Thr	Thr	Thr	Thr	Thr	Thr	Thr

a: average value of duplicate estimations of unpopped grains.
b: average value of duplicate estimations of popped grains.

TABLE 4. TOTAL FREE AMINO ACID CONTENT IN NORMAL AND HIGH LYSINE BARLEY UNPOPPED AND POPPED GRAINS (*SATTU*)

Variety/Line	Total free amino acids (mg/100 g flour)*	
	Popped	Unpopped
Jyoti	110	10
1098-2	175	12
1098-7	155	5
Notch 1	215	10
Notch 2	240	9

*Average value of three separate estimations.

1098-2 and 1098-7 had a lower content of free amino acids as compared to *Notch* 1 and *Notch* 2. There was considerable reduction in the content of free amino acids in the *sattu* samples.

Discussion

In cereal protein wherein lysine forms the most limiting amino acid, the DBC method has found popular acceptance. The principle of the method is that at low pH value the azo-sulphonic groups of the dye Orange G react with the basic imidazol, guanidine and amino groups of the protiens. These groups chiefly originate from the basic amino acids, histidine, arginine

and lysine. Some secondary mechanisms could also be involved[8]. Correlation co-efficient obtained between DBC value and the basic amino acids or lysine alone are highly significant[9,10]. Significant positive correlation has been obtained also between DBC value and protein[10,11]. Moreover, the DBC method unlike the Kjeldahl nitrogen value, does not measure the non-protein nitrogen. In the case of lysine it measures only the nutritionally available sites. Evidently, if the biological value of a food protein is limited by its content of a basic amino acid, its DBC value should correlate with the results from practical feeding trials.

From the results of the present study it is evident that processing of grains to yield '*sattu*' causes a certain amount of non-availability of amino acids. This, however, does not get reflected in the crude protein value obtained by conventional procedure of nitrogen determination. On the other hand, the DBC value is indicative of the loss of availability that occurs. Since the DBC method is faster and cheaper than the biological methods of estimation of protein quality, and since it is fairly reliable, it can be used to evaluate the loss of proteins due to food processing.

The chemical changes occurring in the grain protein during processing appears to be irreversible as evidenced from the lower recovery percentage of amino acids from hydrolysis of popped grains.

Difference in the extent of damage due to processing could be due to variability in the chemical make up of the grains of the different varieties and the high lysine lines. The biochemical mechanisms operative in the high lysine lines results in an enhancement in the content of free amino acids and lysine rich proteins in the grains.[7,12].

The chemical score of the *sattu* material showed a decrease, lysine being the first limiting amino acid. However, since threonine, which is the second limiting amino acid, shows an increase, the chemical score of the protein in a combination, where *sattu* is mixed with Bengal gram, would be high. The low content of lysine in *sattu* will be adequately compensated for by the high content of the amino acid in Bengal gram.

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Microbiological Aspects of Sangak Bread

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Sangak bread is a sour dough type bread which is nutritionally improtant in Iranis. Eighteen samples of sour dough starter and fresh dough were collected from fourteen bakeries in the Tehran area, and analysed for their total yeast and bacteria. Yeast and bacteria were isolated and taxonomically characterized. Morophology of yeast and bacteria is discussed.

Seventy seven per cent of the total bacteria found in the starter were *Leuconostoc* and *Lactobacillus* species, which are essential in fermentation of Sangak dough, for giving the desirable flavour and sour taste. The small number of yeasts present in the starter is responsible for leavening of the dough.

Sangak is a sour dough-type bread which is nutritionally important in Iran.

It is a long, thin sour dough bread, the starter or the leavening consisting of a portion of dough from previous batches. This starter is added to the newly prepared dough and after 1½ or 2 hr relaxation, a ball of 400-500 g of dough is spread on a wide wooden paddle and placed on the floor of an oven which is covered with gravel. The bread is baked on this hot gravel for 2-3 min[1].

The basis of the sangak process is the starter which contains the yeast and bateria necessary to leaven the bread and produce the characteristic flavour. In Iran starter is called "Torsh" which means sour, and consists of dough from a previous batch held for 5 to 10 hr in an open container. It was found that methods of starter preparation and dough formulation varied widely from area to area depending on the facilities available in the shop and the methods the baker has learned. But the starter is important regardless of what modifications the baker has made in the process. If a baker desires to start making sangak he usually goes to an established baker to get an initial starter. This starter provides the large inoculum of microorganisms necessary to accomplish the fermentation in the short time (1½—2 hr) allowed and reduces the pH of the bread dough (about pH 4.1-4,6). The acidic environment then inhibits the growth of all but acid-tolerant species of microorganisms.

The nature of the process suggests the presence of specific bacteria and yeasts which produce the charachteristic flavour of the product. A system of this kind has been described for an American sour-dough French bread process[2].

In this paper the microorganisms found in the Sangak dough and starter are characterized.

Materials and Methods

Eighteen samples of sour dough starter and fresh dough were collected in sterilized plastic containers from fourteen bakeries in Tehran area. To isolate and enumerate the microorganisms in the samples, 22 g of dough were aseptically mixed for 2.5 min. in a sterile Waring blender with sterile 0.25 M phosphate buffer.[3] Serial dilutions were made and pour plates prepared using the appropriate dilution.

Total yeast counts reported here were made on sterilized Sabouraud Maltose Agar (Difco) with 1.5 ml of 10 per cent sterile citric acid (sterilized at 45°C) per 100 ml of media. Plates were incubated at 20°C (room temperature) for 72 hr. Yeast cultures isolated from these plates were carried on Sabouraud Maltose Agar slants and held at 4-5°C. Transfers were made once in every two weeks.

Total bacterial counts were made using *Lactobacillus* selection Agar (B.B.L.) with 1 per cent fresh yeast extract (FYE) added. The FYE was prepared as described by Kline *et al.*[4] Incubation was at 30°C for 27 hr. Bacterial isolates were carried on LBS Agar or Nutrient Agar slants depending on the nature of the organism.

Morphology of bacteria from LBS or Nutrient Agar slants was examined after 72 hr of incubation at 30°C under gram stain. Yeast morphology was studied using wet mounts from cultures grown in yeast extract-peptone-glucose (YPG) Composition: Glucose 2.00; Yeast ext. 1.00; Peptone 0.5 g and water 99.5 ml.

Cycloheximide (Upjohn Company, Kalamazoo, Michigan) tolerance was tested using W.L. Differential Agar Medium (BBL). Comparative growth tests were done using a commercial baker's active dry Star). Pure cultures of *Saccharomyces cerevisiae* were isolated from this dry yeast product.

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** Head of the Institute of Nutrition sciences and Food Technology.

For the identification and grouping of bacterial isolates dextran formation on sucrose agar, Tryptone, 1% yeastral, 0.5%, K_2HPO_4 0.5%, a ammonium citrate, 0.5% and sucrose, 5%. Litmus milk reaction and fermentation of different sugars were carried out as follows: 2 ml. of 30 per cent sterile sugar solutions were added to tubes containing sterile nutrient broth to give a 5 per cent sugar solution, used phenol Red as indicator.

Typical colonies from each group were characterized according to the procedure outlined by Rogosa and Sharpe[5] and Bread *et al.*[6] The ability of *L. plantarum* to grow in NaCl 5.5 per cent were examined, the basal medium being yeast glucose broth. The growth after 2 days at 30° C was considered. The temperature test was carried out in nutrient broth incubated at different temperatures (15°—45°C) and cultures observed daily for one week.

Leuconostoc was classified on the basis of its ability to ferment different carbohydrate sources, according to the procedure outlined by Garvie[7].

The methods of Gaswell and Burnett[8] and Lodder[9] were used for the study of morphology and identification of yeast species and their role in the learning process. For comparative purposes a standard culture of *Torulopsis* was used to leaven Sangak bread.

Results

All the bacteria isolated from Sangak starters fell into three general groups except for four *Alcaligenes* species in two samples. The bacteria present were Gram positive lactic types, and could be grouped as (*a*) dextran-forming *Streptococci*, (*b*) *Lactobacillus*-type and (*c*) cocci or terrads (Table 1).

The *Streptococci* were the most common types isolated. These organisms varied in shape from cocci to oval forms in pairs to coccobacilli. They fermented the six sugars but produced no reaction in litmus milk.

TABLE 2. CHARACTERISTICS USED TO CLASSIFY 31 BACTERIA ISOLATES FROM GROUP "A"—AS *LEUCONOSTOC MESENTEROIDES*

Species Garvie Group	*Leuconostoc mesenteroides*		
	III	V	VI
Litmus milk	—	—	Slight acid
Growth at 37°C	+	+	+
Dextran	—	+	+
Acid from: Arabinose	+	—	+
Xylose	±	+	+
Sucrose	+	+	+
Lactose	±	±	±
Trehalose	+	+	+

* As described by Garvie (Dextran formation was tested on 5% Sucrose Agar).

— = —ve Reaction, + = +ve Reaction, ± = Variable Reaction.

The most characteristic reaction of the group was formation of dextran on 5 per cent sucrose agar. They were identified as *Leuconostoc mesenteroides* Garvie group V and VI (Table 2).

The species isolated from *Lactobacillus* type organisms were *Lactobacillus plantarum and Lactobacillus brevis.* The homo-fermentative streptobacterium, *L. planatarum* was recognized by its tolerance of 5.5 per cent NaCl and fermentation reactions and hetero-fermentative streptobacterium, *L. Brevis* was recognized by its production of both acid and gas (Table 3)

The homofermentative cocci which were found in small numbers were identified as *Pediococcus cervisiae* with an optimum temperature of 18 to 25° C, and high salt tolerance (group C Table 1).

Yeasts were divided into two groups on the basis of morphology in yeast extract-peptone-glucose (YPG) and maltose fermentation (Table 4). The predominant

TABLE 1. GROUPING OF BACTERIA ISOLATES

Bacteria	Morphology	No. of isolates in this group	Dextran formation on Sucrose Agar	Litmus milk	Fermentation					
					Glucose	Sucrose	Maltose	Lactose	Arabinose	Xylose
Group A	Gram +ve Streptococci or Cocoobacilli	31	+	N.R.	+	+	+	±	±	+
Group B	Gram+ve Rod usually short	23	—	variable	+	+	+	+	±	±
Group C	Gram+ve Cocci Tetrads	8	—	Acid	0	0	0	0	0	0

Key; 0 = Not Tested, + = +ve Reaction, ± = Variable Reaction, N.R. = No Reaction.

group (group 2) was unable to assimilate lactose but utilized glucose, sucrose and maltose even in the presence of cycloheximide. The other group (group 1) was unable to assimilate maltose and lactose but utilized glucose and sucrose vigorously. A strain of baker's yeast was also tested for comparative purposes. The sangak yeast cells were oval showing multipolar budding; pseudomycelium was absent and no ascospores, endospores or ballistospores were formed. No pigments were produced and they fermented some carbohydrates. These characteristics place them in genus *Torulopsis*. The species identified were *Torulopsis candida* and *Torulopsis colluculosa* (group 2 Table 4)

The role of the *Torulopsis* yeast was suggested by holding starters at refrigerator temperatures until the number of viable yeast became very low and a test dough was produced by this starter. Then, starter help for 12 days at approximately 4°C lost the ability to form sufficient CO_2 but acid production was normal. So the yeast must be responsible for most of the leavening power.

The number of bacteria in newly inoculated dough varied from 1×10^7 to 2×10^7 cells per gram, and in fully developed starter the number increased to 10×10^8 organisms per gram of which *Leuconostoc* Sp, *Lactobacillus* sp and *Alcaligenas* Sp comprise 48 per cent, 19 per cent and 2 per cent respectively.

In some samples the *Leuconostoc* species made up 85 per cent of the bacterial population but this varied widely. After five or six hours, the microbial count stopped increasing.

Yeasts were present in much smaller numbers. Freshly inoculated dough contained about 4×10^6 cells per gram and starters contained $1.1\text{-}1.3\times10^7$ yeast per gram after several hours incubation in which *Torulopsis candida* was 50 per cent of the yeast population.

TABLE 3. CHARACTERISTICS USED TO CLASSIFY 23 BACTERIAL ISOLATES FROM GROUP "B" AS *LACTOBACILLUS PLANTARUM* AND *LACTOBACILLUS BREVIS**

	Lactobacillus plantarum	*Lactobacillus brevis*
Morphology	Gram +ve Rods, Small rounded ends under Opt conditions	Gram +ve Rods or Coccobacilli
Growth at 15°C	+	+
Growth at 45°C	±	—
% Acid in Milk	0.3–1.2	0
Fermentation of		
Arabinose	±	++
Lactose	+	+
Melibiose	+	+
Raffinose	±	—
Sucrose	+	+
Xylose	±	+

* as described by Rogosa *et al*[5]
0 Not tested
+ +ve Reaction
— —ve Reaction
++ Vigorous Reaction

Discussion

Unbleached sangak flour usually contains 8 to 12,000 bacteria per gram[10]. The species present are varied, but *Leuconostoc* and *Lactobacillus* are usually not isolated. The isolation of a certain species of bacteria from a fermented food product does not prove the organism is involved in the fermentation. However, the large number of *Leuconostoc* species and *Lactobacillus* species in Sangak dough (and the absence of others) suggests they must be essential in the fermentation.

The incidence of *Alcaligenas* species was very low so they may have been contaminants *Lactobacillus* species found were difficult to culture. Among those isolated, *L. plantarum* is homofermentative and *L. brevis* is hetero-fermentative producing both acid gas when fermenting carbohydrates and these two species are often

TABLE 4. GROUPING OF YEAST ISOLATES

Yeast	Morphology on YPG*	No. of isolates in in group	Growth in presence of 100 mcg/g of Cycloheximide	Fermentation of			
				Glucose	Sucrose	Maltose	Lactose
Group 1	Small ovals, may form Pellicle	2	+	++	++	—	—
Group 2	Larger ovals	16	+	+	+	+	—

*Morphology and Fermentation reactions determined using the method of Lodder[9].
++ +ve Reaction
++ Vigorous Reaction
— —ve Reaction.

*This is, however, not enough to produce the desirable fermentation.

isolated from the same sources as *L. mesenteroides*[11] These lactics share the same optimum temperature of 30°C and are capable of producing both CO_2 and acidity necessary in a Sangak bread* and the yeasts are responsible for the leavening power.

As mentioned, after six hours the microbial count stops increasing. This is due to the depletion of nutrients and the accumulation of acid. The fermentable carbohydrates make up approximately 2 per cent of the flour and consist of glucose, fructose and large amounts of low molecular weight glucofructance[2]. The bacteria and yeasts present in the dough compete for these sugars.

A specific symbiotic relationship of one yeast species and one bacterial species has not been formed in the sangak process, as was described for some sour dough breads[4,12] but rather, a group of compatible, low-temperature lactic bacteria have been established in a system favouring growth of *Torulopsis* yeasts. The unique feature of these yeasts seems to be their cycloheximide resistence. The yeast isolated grew in the presence of 100 mcg of cycloheximide per gram and this indicates they may co-exist with the sour dough bacteria because of their resistance to an antibiotic substance produced by the lactic organisms[12].

Commercial bakers yeast is now available to sangak bakers and there is interest in using this dry yeast for sangak. Preliminary studies show that bakers' yeast can be used to leaven sangak bread, but the distinctive flavour is not developed. Fermentation with Bakers' yeast produces a few flavour compounds. Bacteria produce many such as diacetyl, aldehydes, iso-alcohols, acids and their ester. To produce sangak bread, the unique balance of yeasts and bacteria is necessary and any attempt at developing this process will require close attention to the fermentation.

The occurrence of a typical low-temperature lactic flora in sangak starter suggests a similarity with European rye bread. These low-temperature lactics (*Lactobacillus plantarum*, *Lactobacillus brevis* and *Leuconostoc mesenteroides*) are among the species added as pure cultures to produce the desired acidity and flavour in rye breads. A study of European sour dough bread and of the use of prepared pure cultures (as are used in yogurt production) will provide useful information for the development of the sangak bread process.

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Evaluation of Bajra *(Pennisetum typhoides)* for Malting and Brewing

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Bajra (*Pennisetum typhoides*), has been evaluated for malting and brewing purposes under standard conditions used for barley malting. Optimum steeping time at 17°C for 92% germination was 8 hr. Compared to barley malt, bajra malt has a lower amylolytic activity and the wort prepared from such malt has a low reducing sugar content which is inadequate for brewing purposes. It is concluded that bajra, although rich in starch and low in proteins, is unsuitable for brewing purposes.

Bajra, is a crop that is cultivated under rainfed conditions during the summer months in the State of Haryana, India. At present, it is mostly used as a fodder crop. With the increasing demand for the limited quantity of barley malt available, it was desired to evaluate this surplus grain for brewing purposes. Being rich in starch and low in proteins, it appeared to be ideal for brewing. Sheorain and Wagle[1] and Pal *et al.*[2] have reported the possibility of malting this grain. However, conditions employed for malting were not ideal to derive conclusions. This paper reports our studies carried out to prepare Bajra malt under standard conditions used for malting barley and to examine its suitability for brewing purposes.

Materials and Methods:

Barley, variety 'C-164' and bajra, variety 'HB-3' used in the present studies are commercially grown varieties and were from the Department of Plant Breeding, Haryana Agricultural University, Hissar. These grains were analysed for various ingredients by the methods described by AOAC[3]. Total nitrogen was estimated as described by the Cereal Laboratory methods[4]. The starch content was determined by the method of Hassid and Nuefeld[5].

Germination studies: Seeds (100 g) were washed in tap water to remove all the extraneous materials and soaked in distilled water (500 ml) at 17°C for 8 hr (bajra) or 48 hr (barley). The seeds were then allowed to germinate at 17°C in BOD incubator in petridishes (8 in. diameter) spread on wet filter paper. Samples (5g) were withdrawn at intervals of 24 hr upto 120 hr during germination for analysis.

Total amylases were extracted and assayed according to the method of Nason[6] and α-amylase activity was measured by the method of Swain and Dekker[7]. Enzyme activity is expressed as mg of maltose released/ml enzyme extract at 37°C in 3 min.

Preparation of malt: Grains were germinated as above and those showing optimum germination (acrospire 1/2-3/4 of the length of grain) were sorted out manually and kilned at 45°C for 24 hr and then at 55°C for 8 hr. The cured grains were screened to remove the rootlets, cooled and stored in a cool dry place (25°C). The malted grains were coarsely ground in a coffee grinder and analyzed by the method of AOAC[3].

Analysis of wort: Wort was prepared according to the procedure described by Weissler[8]. The samples were equilibrated at 20° C and filtered through Whatman No. 1 filter paper before analysis.

Specific gravity was determined using a boot type pycnometer[8] (50 ml capacity) at 20°C. Extract content of wort was calculated from the plato tables of the American Society of brewing Chemists[9] and relating to the specific gravity of the wort.

Total reducing sugars in the wort were estimated as maltose by the dinitrosalicylic acid method[10]. The pH of wort was determined using an Elico pH meter Model Li-10 and the total acidity of wort was determined as per cent lactic acid by weight according to the procedure described by Hortwitz[9]. Colour of wort was estimated by the Spectrophotometic method of Weissler[8] and total protein content by the Micro-kjeldahl method as described by Piper[11].

Results and Discussion

Chemical composition: Grains used for brewing must have a low protein content but should be rich in starch. The chemical composition of the varieties of barley and bajra used in these studies are shown in Table 1. The starch content of these grains varied slightly. However,

TABLE 1. CHEMICAL COMPOSITION OF BAJRA* AND BARLEY*

	Bajra	Barley
Moisture (%)	9.2	10.1
Crude protein (%) (N×6.25)	12.5	8.8
Ether extract (%)	4.4	2.0
Starch (%)	50.0	55.8
Germination (%) (at 17°C)	92.0	99.9
1000 grain wt. (g)	6.4	50.9

*dry wt. basis.

the crude protein and ether extractives were more in bajra.

Effect of temperature and time on amylase content of bajra: Since it was necessary to determine the time period for germination and also to determine the stage at which the grains have maximum amylolytic activity, germination studies were conducted at 17°C (optimum temperature for barley) and at 35°C[1]. The total amylase activity increased rapidly at 35°C germination (Fig 1) and reached a maximum at about 30 hr after which it declined. On the other hand, ∝-amylase activity reached a maximum at 18 hr and thereafter, rapidly declined. As compared to this, at 17°C, the increase in both total and ∝-amylases is gradual and reaches the maximum value in about 120 hr. The amount of total and ∝-amylase activity in grains germinated at 17°C was 2 and 4 times more respectively, than at 35°C. The grains had attained a moisture content of 35.6 per cent at this stage. The germination was terminated at this stage since the grains had attained the appropriate germination stage.

Amylase content of bajra and barley: The amylase

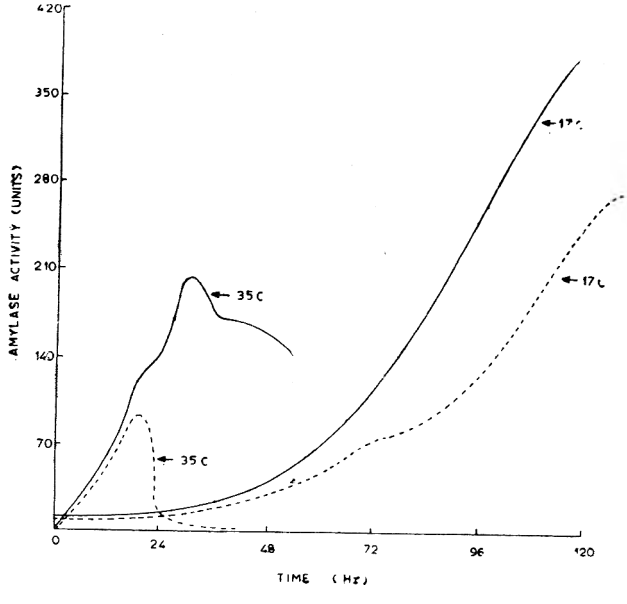

TABLE 2. AMYLASE FORMATION DURING GERMINATION OF BAJRA AND BARLEY

Time (hr)	Total amylases*		∝-amylase*	
	Bajra	Barley	Bajra	Barley
0	11.6	4939.0	10.2	371
24	18.2	4218.5	15.6	752
48	38.4	7118.5	28.4	822
72	114.8	12912.5	72.2	1263.5
96	260.0	27981.2	120.0	2801.6
120	390.0	34119.0	250.0	3415.5

*Units/g dry wt.

content of malt is a major parameter in brewing and a good malt must have a high amylase content. The amylolytic acivity in both bajra and barley when germinated at 17°C increased continuously during the five days of germination (Table 2). The increase in amylolytic activity in bajra is about 40 folds over the ungerminated grain while in barley it is only about 7 folds. However, barley had a very high initial and final enzyme level compared to bajra.

Malt composition: The malt prepared from barley and bajra were analyzed for various components such as moisture, crude protein, ether extractives and starch (Table 3). The over all composition of the malts was similar to that of the grains. Both malts had a satisfactory flavour and desirable taste.

Wort analysis: To examine whether the amylolytic activity in bajra malt is adequate for its use in brewing, wort was prepared as described by Weissler[8] and analysed. The major parameters which finally reflect in the quality of beer are fermentable sugars, the total protein content (∝ amino N), pH, total acidity and colour of the wort. Table 4 shows the analysis of the bajra and barley worts. Reducing sugars which play an important role in establishing the quality and identity of beer, are too low (1.6 per cent) in the bajra wort while the other ingredients are comparable to that of barley wort and are within the range of average American wort[8]. The pH of bajra wort was higher than barley wort while the total acidity was lower

TABLE 3. CHEMICAL COMPOSITION* OF BARLEY AND BAJRA MALTS

	Bajra malt	Barley malt
Moisture (%)	6.9	8.1
Crude protein (%) (N×6.25)	14.3	9.8
Ether extract (%)	4.9	2.4
Starch (%)	43.6	48.5

*dry wt. basis

TABLE 4. ANALYSIS OF BAJRA WORT AND BARLEY WORT

	Bajra wort	Barley wort	Av range American barley wort
Sp. gr.	1.051	1.0358	1.0476 — 1.0497
Extract (0 Plato)	12.62	8.98	11.80 — 12.30
Colour	12.29	8.13	3.00 — 5.00
Total acidity (%)	0.06	0.12	0.11 — 0.12
Reducing sugars (%)	1.60	8.19	7.00 — 8.50
Protein (%)	0.40	0.48	0.38 — 0.50
pH	6.12	5.55	5.20 — 5.80

and beyond the acceptable range for average American barely wort. The colour of the bajra wort was also darker than Barley wort and the extract of bajra wort was higher than the barley wort.

The reducing sugar content of bajra wort is too low and this is perhaps due to the low amylolytic activity in bajra. This is inadequate to produce the minimum amount of alcohol (3.10–3.90 per cent by weight) prescribed for beer[12]. These experiments have been repeated several times. It is, hence, concluded that bajra malt alone, because of its low amylolytic activity cannot be used for brewing purpose. However, as reported elsewhere[13], bajra starch can be effectively used as an adjunct in association with Barley malt for brewing.

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Biochemical, Physical and Organoleptic Studies on Production of Enzyme Tenderized A. F. D. Goat Meat Chunks

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A study was carried out to commercially produce improved quality, enzyme tenderized accelerated freeze dried (AFD) mutton chunks. Tenderization was measured in terms of (a) protein extractability, (b) total volatile nitrogen (TVN), (c) extract release volume (ERV), and (d) juice expressed by press fluid method. Products were evaluated both organoleptically and by chemical analysis. All the enzyme treated products were found better than their controls prior to freeze drying. Ficin tenderized AFD mutton chunks were found to be the best in overall quality and uniformity of the tenderness. Enzymatic tenderization resulted in reduction of cooking time by half an hour at 5 p.s.i.

Tenderizing effect of enzymes is known for a long time[1]. The enzymes important to meat tenderization are proteases.[2,3] The main commercial sources of plant enzymes are papain from papaya, bromelin from pineapple and ficin from figs.[4] A close relationship between enzyme tenderization and tenderness of meat has been reported.[5] The enzymes can be applied to meat either by dipping, spraying or injecting method[6] but the uniform distribution of enzyme depends upon many factors.[7] Approximately 5 to 7 mm deep penetration of enzyme has been reported in 'dipping' process and hence its use is limited to relatively thin cuts of meat.[8]

In the present paper results of the attempts made to explore the feasibility of the production of enzyme tenderized accelerated freeze dried meat chunks using plant proteases namely ficin, bromelin and papain have been reported.

Materials and Methods

(*a*) *Goat meat:* The meat carcasses of good quality were obtained from local market. Only male goats from two to two and a half years in age were used. All the carcasses were almost of the same weight. Immediately on receipt (approximately 4 to 6 hr post slaughter) the superficial fat of the carcasses was trimmed off and each carcass was anterioposteriosly divided into two similar halves which formed the test and corresponding control group. The meat was deboned and cut into 10 to 15 mm cubes and hand mixed. For preliminary experiments the meat was divided into six groups for standardizing the optimum conditions for enzymatic action. Subsequently for production of enzyme treated batches and their control on pilot plant scale, two to three carcasses which were longitudinally divided into two equal halves, constituted one group as control and the other for enzyme treatment. All operations were done under strict hygienic conditions.

(*b*) *Enzymes:* All the enzymes used were of commercial/laboratory grade. Ficin and Bromelin, E.Merck Germany and Papain, P.C.I. Delhi, India and sodium chloride L.R. BDH were used.

(*c*) *Method of treatment and cooking:* Enzyme treatments were carried out for 30 minutes following 5 min preincubation at 60°C and pH 6.1 $\pm$ 0.2 in 0.5 per cent saline broth. The pH of the meat chunks was not altered. Enzymes were used at different levels from 0.001 to 0.25 per cent (w/w) on dressed meat basis in saline during preliminary experiments. Ratio of the dressed meat to saline broth was kept 2:1 (w/v). After the action was over, the meat was cooled to room temperature in water. Samples were drawn for analysis, these contained corresponding muscles from control and treated meat, as well as random sampling was done. The enzyme broth was discarded and meat was cooked alongwith respective controls at 5 psi for 45 min as against 75 minutes required during normal cooking at this pressure[9]. The gravy was concentrated in open pans over flame and mixed with respective meat. The cooked samples were curried and organoleptically evaluated to select the optimum enzyme level for further experiments.

Based on the results of above preliminary experiments the optimum concentration of enzymes (0.001 per cent) was used under the experimental conditions for production of full load (approx. 12 kg wet material) of tenderized mutton and their controls for further studies. Samples in triplicate were drawn at all stages for analysis i.e. after treatment, after cooking and after freeze drying.

(*d*) *Analysis of the samples:* The chemical analysis of the samples in triplicate was carried out as per AOAC[10] methods. Determination of protein extractability was done as per the method of Herring *et al.*[11] The samples were homogenized mechanically for 10 min. in 0.1 M KCl in 1:10 ratio using Thomas (Teflon) tissue grinder having clearance 0.005 to 0.007 in. Extracted protein was determined according to the method of Lowry *et al.*[12]

Total volatile nitrogen (TVN) was determined as a measure of the degree of protein breakdown and the extract release volume (ERV) was determined for exploring the acceptability of the product as per the method of pearson.[13] Higher the TVN value, higher being the acceptability of the product.

Expressed juice measurement was done as a measure of juiciness according to the procedure of Wierbicki and Deatherage[14] on 10 replicates using the Texture Test System, TP-1 of the Food Technology Corporation[15], U.S.A. (A patent of Kramer Press). 0.5 gm samples were used on a 9.0 cm dia. Whatman No.1 filter paper and 100 lb force was applied for one minute. The relative humidity, moisture content of the paper and regression coefficient of water were determined accordingly.

Reconstitution time, rehydration ratio and per cent moisture were calculated and SH content of the meat protein was determined as per the method of Jozef and Raymond[16] using Ellman's reagent.

(*e*) *Evaluation of the products:* The products in triplicate were evaluated by a laboratory panel of six judges on a nine point hedonic scale and graded in the order of choice.

Results and Discussion

Results of the preliminary experiments have been presented in Table 1. It is seen that the maximum enzymatic action is over at 0.001 per cent enzyme concentration under the experimental conditions and further increase in the enzyme concentration does not produce any appreciable change in TVN or ERV. At this enzyme level pH of the broth also remains unaffected. Higher enzyme concentrations in ficin treatment produce pH shift towards acidity whereas in papain treatment towards mild alkalinity and lead to overtenderization/sogginess in the product. Total solids in the broth are not affected as a result of enzyme action. The gravy production remains as much as in control meat or becomes slightly less. 0.001 per cent concentration of enzymes was chosen for further experi-

TABLE 1. EFFECT OF VARYING ENZYME CONCENTRATION UNDER THE EXPERIMENTAL CONDITIONS UPON TENDERIZATION

Enzyme used	Enzyme concn. (%, W/W to mu ton in the broth	pH		Total solids in broth (%)	Total volatile N in (ml. of std. H_2SO_4/10g or ml)		Gravy released cooking volume (%)	Extract release	Organoleptic quality
		Before action	After action		Broth	Mutton			
Ficin	0.001	6.4	6.4	0.05	0.40	0.8	16.94	26.0	Good/tender
	0.01	6.4	6.3	—	0.45	0.85	—	24.0	V. tender
	0.1	6.4	6.1	—	0.5	1.0	—	15.5	Over tender little soggy
Control	Nil	6.4	6.4	0.05	0.3	0.7	18.17	24.0	slightly hard
Bromelin	0.001	6.0	6.0	0.065	0.4	0.7	10.4	16.0	Good/tender
	0.01	6.0	6.0	—	0.45	0.7	—	12.5	Very tender
	0.1	6.0	6.0	—	0.5	0.75	—	6.5	Over tender
Control	Nil	6.0	6.0	0.06	0.3	0.65	14.5	9.5	slightly hard
Papain	0.001	6.0	6.1	0.045	0.6	0.8	19.75	19.5	Good/tender
	0.01	6.0	6.1	—	0.8	0.8	—	18.5	Over tender
	0.1	6.0	6.1	—	0.9	0.8	—	18.5	Over tender
Control	Nil	6.0	6.0	0.045	0.6	0.7	20.64	19.0	Slightly hard

—Not determined

ments as it gave an organoleptically good/tender product with highest ERV and slightly higher TVN values over control meaning thereby optimum protein breakdown and acceptability[13] in relation to organoleptic quality. These findings correlated with organoleptic evaluation of the freeze dried products also. Effect of enzymes treatment upon protein extractability have been shown in Table 2. Maximum protein breakdown is observed in case of ficin treatment as has been reported also[17]. It resulted in 22.34 per cent excess extractable protein over control. Subsequently upon cooking and freeze drying the extractability of the proteins was lowered slightly possibly due to heat denaturation. Bromelin and papain treatments produced 8.0 and 5.64 per cent excess extractable protein over control, respectively. Upon cooking and freeze drying the protein extractability in case of papain treated mutton was unexpectedly reduced to 0.5 per cent over control. Possibly it is one of the causes of its low acceptability as judged by poor juice expression and organoleptic score.

TABLE 2. EFFECT OF ENZYME TREATMENT UPON PROTEIN EXTRACTABILITY OF MEAT

Product	% extractable protein			% gain over control
	Before cooking	% gain over control	After cooking and freeze drying	
Ficin treated meat	43.50	22.34	37.0	20.1
Control	21.16		17.1	
Bromelin treated meat	29.16	8.00	23.1	6.1
Control	21.16		17.0	
Papain treated meat	23.64	5.64	17.0	0.5
Control	18.00		16.5	

Data on the effect of enzyme tenderization upon juice expression have been presented in Table 3. It will be seen that ficin treatment produced 14.4 per cent extra juice over control, as against 7.2 per cent in case of papain after freeze drying. Since ficin is the most powerful proteolytic enzyme[17] among these and degrades both collagen as well as muscle proteins, it produced optimum protein breakdown which, it appears helped in forming a more expanded gel upon reconstitution with retention of higher amount of "immobilized" water and therefore higher degree of swelling and juiciness[18].

Rehydration ratio of the ficin tenderized AFD meat chunks was found to be comparatively higher (1:2.52) than bromelin or papain tenderized AFD meat chunks which were 1.88 and 1.98 respectively. No difference in SH groups content was observed as a result of enzymatic tenderization in any of the groups. The freeze dried tenderized meat could be reconstituted by simmering it in boiling water for 15 minutes.

TABLE 3. EFFECT OF TENDERIZATION UPON JUICE EXPRESSION UNDER THE EXPERIMENTAL CONDITIONS*

Meat treated/control	After enzyme treatment		After cooking		After freeze drying and reconstitution	
	EJA (sq cm)	% extra juice over control	EJA (sq cm)	% extra juice over control	EJA (sq cm)	% extra juice over control
Ficin	22.53	16.03	11.60	24.58	7.0	14.4
Control	19.19		6.88		4.0	
Bromelin	22.01	15.74	12.01	17.90	6.0	7.2
Control	18.73		8.26		4.0	
Papain	20.95	19.6	10.75	10.32	4.1	1.44
Control	16.75		8.60		3.8	

*RH 60%, Moisture content of the filter paper 6.7±0.1% and regression coefficient of water 60.95 mg/sq cm.
EJA—Average expressed juice area i.e. (average total area—average meat film area)

Results of the organoleptic evaluation have been presented in Table 4. Though the papain treatment resulted in considerable protein breakdown as indicated by TVN and ERV values suggesting tenderization however, after cooking and freeze drying it had low preference due to poor juice expression. The reasons are not well understood. Bromelin treated freeze dried mutton was found to be comparatively more tender and juicy. The ficin treatment gave best product which was graded first in the order of choice. These findings agree with the data on juice expression and protein extractability of the treated meats.

Conclusion: The study indicates that the ficin treatment being the best can be used in meat tenderization with a limited chunk size for preparation of meat or meat based convenience foods.

TABLE 4. ORGANOLEPTIC EVALUATION OF THE DIFFERENT ENZYME TENDERIZED MEAT CHUNKS

Parameter	Ficin	Bromelin	Papain	Control
Colour	6.3	6.1	5.7	6.1
Flavour	5.3	5.8	5.5	5.7
Juiciness	5.3	4.5	3.7	3.3
Tenderness	5.5	4.0	2.7	2.5
Total score	22.4	20.4	17.6	17.6
Remarks	—	—	Sl. hard	—
Grading	I	II	IV	III

(Average score out of 10)

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Development and Application of Fungistatic Wrappers in Food Preservation. Part II. Wrappers Made by Coating Process

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An effective fungistatic wrapper can be economically made by coating grease proof paper with an aqueous dispersion of sorbic acid in 2% carboxymethyl cellulose solution. A simple technique is described whereby foods which are generally amenable to spoilage by mould attack can be preserved for short periods of minimum 10 days by simply wrapping them with the sorbic acid treated paper and then enclosing in a polyethylene bag.

A fungistatic wrapper developed for food preservation can have wide application if it is economically and conveniently made and can also be used at all places without needing any special technological facilities for its application. The wrapper and its method of use previously described[1] do not fully conform to these requirements since its manufacture requires the use of alcohol which raises its cost and the method also requires post packaging heat treatment of the food in thermostatically controlled ovens. Investigations were, therefore, undertaken to find out whether instead of alcohol water based sorbic acid formulation could be used for coating paper to give an equally effective wrapper and also if under suitable situations the wrapper could be used without needing subsequent heat processing of the packaged food in an oven. As a result a new coated fungistatic wrapper was developed and a new and simple technique for its application was evolved.

Materials and Methods

Grease proof paper: Super white grease proof paper of 45 g/sq m substance, indigenously manufactured.

Sorbic acid: Sorbic acid (E Merck) of 99 per cent purity.

Carboxymethyl cellulose: "Cekol HVP" brand, produced by M/s Cellulose Products of India Ltd.

The coating formulation was composed of Sorbic acid, 30 g; Carboxymethyl cellulose, 20 g; Embanox-6, 0.8 ml; and Water, 1l

Sorbic acid was added little by little to carboxymethyl cellulose solution and mixed thoroughly using a mechanical stirrer. Antioxidant, "Embanox-6" was finally added and the homogenous mixture was used up within 2 to 3 hrs.

Coating method: The coating could be carried out on a laboratory model, two roller coating machine, one of the rollers acting both as pick up and applicator roller and the other by suitable adjustment of the nip acting in effect as a doctor (Fig. 1). The coated paper was allowed to dry under room conditions by laying on a flat clean surface. The exact thickness of the coating to give the concentration of sorbic acid per square meter of the coated paper to 1.8 to 2.2 g was suitably adjusted.

The process can be carried out more efficiently and on a large scale in a continuous coating machine provided with drying channel and unwind and rewind rolls (Fig 2) The drying was carried out at a channel temperature of 55° to 60°C.

Estimation of sorbic acid: For rapid estimation of sorbic acid in coated paper a 10 cm × 10 cm piece was cut into small bits, soaked in a stoppered flask with 50 ml of freshly boiled and cooled distilled water, adding 20 ml of N/10 NaOH solution and back titrating the excess alkali with a standard N/10 oxalic acid solution to phenolphthalin indicator. Sorbic acid transferred to the food materials was estimated by the method of Schimdt.[2]

The following food materials were used in the preservation experiments.

(*i*) Breads, both ordinary Modern Bakery breads and ordinary local bakery breads: Moisture content varied between 30 to 33 per cent and water activity between 0.90 to 0.94. (*ii*) Puries, made out of good quality wheat flour adding 2 per cent salt on the weight of the flour and

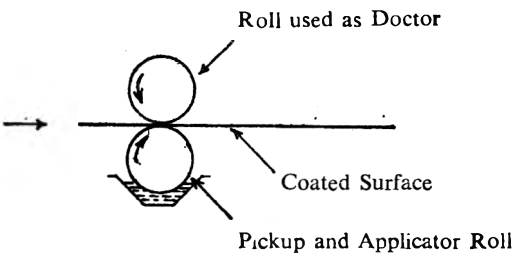

Fig. 1. Two roller coating

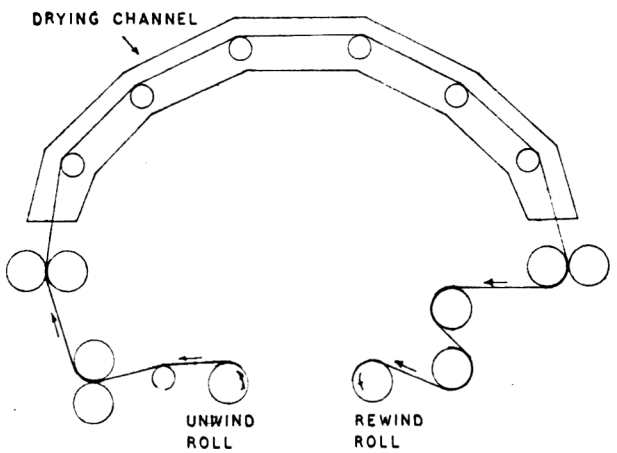

Fig. 2. Continuous coating, drying and rewinding machine

using hydrogenated oil or refined groundnut oil for frying in the usual manner. Moisture content was approximately 25 per cent and water activity between 0.86 and 0.90. Each puri was of 40 g with diameter of 5.5. inches. (*iii*) Chapaties, made from good quality wheat *atta* adding 6 per cent oil hydro and 2 per cent salt on the weight of the *atta*, baking and puffing in the normal manner. The moisture content was approximately 30 per cent. Each chapati was of 45 g and of 6 inches in diameter. The water activity of different batches varied between 0.86 and 0.90. (*iv*) Agra petha, obtained from local market with sugar syrup of 75° Brix. The water activity varied between 0.80 and 0.86. (*v*) Khoa, freshly made from full cream milk to a moisture content of 27 per cent and water activity between 0.86 and 0.90. (*vi*) Intermediate moisture fruit salad prepared from banana slices by the process of osmotic dehydration[3] using gradually increasing concentration of sugar syrup till the final syrup strength was 75° Brix.

Results and Discussion

Paper for coating process: In a coating process using an aqueous formulation of sorbic acid, the paper to be used should be light and at the same time strong, enough to run on a coating machine. It should also be nonporous as far as possible so that it does not soak water immediately on being coated and thereby lose strength and dimensional stability while it is running on the machine. Grease proof paper of 45 g substance has been found to be satisfactory from these points of view and in fact, has presented no problem on a continuous coating, drying and rewinding machine. The paper is also suitable for use in contact with food materials.

Coating formulation: Clacium sorbate was previously reported to have been used[4] in coating composition. For reasons of convenience, economy and storage property, however the use of sorbic acid itself was favoured. As binding and dispersing agent high viscosity grade carboxymethyl cellulose proved to be quite satisfactory. Using a 3 percent dispersion of sorbic acid in a 2 percent solution of carboxymethyl cellulose solution and adjusting the coat thickness properly it was found that sorbic acid concentration in the coated paper could be adjusted to 2 ± 0.2 g per square meter without much difficulty. As in case of alcoholic solution, in the coating composition also Embanox-6 was added to the extent of 0.8 ml per litre to minimise oxidative changes in sorbic acid. The coated paper was found to release

TABLE 1. STORAGE STABILITY OF VARIOUS FOODS WRAPPED WITH COATED FUNGISTATIC WRAPPER AND SUBSEQUENTLY HEAT PROCESSED AFTER PACKAGING

Food items	Details of packaging	Period of heating at 90-95°C (min)	Shelf life based on microbiological stability (months)	Shelf life based on organoleptic acceptability (weeks)
Unsliced Modern Bakery Bread, 400 g	Individually wrapped and enclosed in laminated paper/polyethylene bags	30	>4	4
Chapaties	5 nos wrapped together and enclosed in a paperfoil-polyethylene laminated pouch	30	>3	8
Puri fried in refined groundnut oil	,,	30	>3	4
Banana fruit salad	50 g made into a disc shape of 2 cm thickness, wrapped and enclosed in laminated foil pouch	30	>2	6
Agra petha	30 g wrapped and enclosed in laminated foil pouch	30	>6	>24

sorbic acid readily on heating at 90°–100°C. The cost of producing the coated paper in the above manner works out to be considerably cheaper than the impregnated paper made from alcoholic solution of sorbic acid. The coated paper is also more stable. After storage for 4 months at ambient conditions the concentration of sorbic acid was found to be reduced from 1.95 to 1.88 g per square meter.

Effectivity of the coated wrapper: To verify the effectivity of the coated paper, it was used for preservation of breads, chapaties, puries and Agra pethas and banana fruit salad in the same manner as previously described i.e. by first wrapping and subsequently heat processing of the wrapped and packaged items at 90° to 95°C for 30 to 60 min. The results of storage studies of these packages are given in Table 1 from which it will be seen that the coated paper is as effective in preservation against microbiological spoilage as the paper made by soaking with alcoholic solution. The actual shelf life of foods thus preserved is determined by other forms of deterioration. In case of breads and puries the onset of off flavour, in case of chapaties, the onset of bitter taste and in case of banana fruit salad, intense browning restrict the shelf life of these products upto 1 to 2 months. In case of Agra petha, which does not develop discolouration or rancidity a very long shelf life, i.e. beyond 6 months is achieved.

Elimination of post packaging heat process: The distinctly separate roles of heat processing and of the fungistatic wrapper in the preservation of the packaged food was clearly brought out in the earlier paper[1]. However, it was realised that the necessity for heat processing of the packaged food under controlled conditions considerably restricts the ready application of the method. In a bid to make the method simpler, it was thought that this step could be eliminated without any detrimental effect, if the food during its preparation is subjected to sufficiently high temperature to bring down its microbiological load to an insignificant level and is also available for wrapping with fungistatic wrapper immediately after the heating process is over without allowing it to cool down by normal atmospheric exposure. Experiments which were carried out in this connection proved that this contention is indeed correct. 400 g local bakery breads which were wrapped within 1 to 2 min after removal from oven and then packed in 200 gauge polyethylene bags remained free from mould and yeast and rope formers after 12 days. Puries were also similarly wrapped in groups of five within 1 to 2 minutes of their removal from the frying pan and were

TABLE 2. STORAGE STUDIES OF HEAT PROCESSED FOODS PRESERVED BY FUNGISTATIC WRAPPER UNDER DIFFERENT CONDITIONS OF WRAPPING

Food item	Wrapper used			Time lag in wrapping (min)	Microbiological condition on ambient storage for			Useful shelf life (days)
	Paper type	Sorbic acid in paper (gsm)	Treatment method		7 days	10 days	>10 days	
Unsliced Modern Bakery Bread, 800 g	Crepe	1.5	Soaking	2-3	Good	2% spoiled by mould attack	Mouldy after 15 days	—
Unsliced local breads, 800 g	,,	,,	,,	6-7	20% spoiled by mould attack	—	—	—
,,	,,	2	,,	6-7	Good	25% spoiled by mould attack	—	—
Unsliced Modern Bakery Breads, 800 g	,,	,,	,,	2-3	Good	Good	Mould free for > 1 month	15
,,	Grease proof paper	,,	Coating	Max. 4	,,	,,	A few spoiled by mould after 20 days	15
Unsliced local breads, 400 g	,,	,,	,,	,,	,,	,,	No mould spoilage upto 3 wks	15
Puries fried in hydrogenated oil	,,	,,	,,	Max. 2	,,	,,	Mould free for >2 months	
Chapaties	,,	,,	,,	,,	,,	,,	,,	30
Khoa	,,	,,	,,	,,	,,	,,	Mould free for > 2 wks	10

Note:—The outer packaging used for the wrapped items was 200 gauge polyethylene in all cases except for puries and chaptaties where paper/foil/polyethylene pouch was used.

subsequently packed in paper-foil-polyethylene laminate pouch. These also remained free from mould and yeast upto 90 days and the total plate count at 55°C increased initially from 0 5 to 30-50/g at the end of the 90-day period. At the processing temperature of about 100°C or more the only surving organisms are the thermophyls and under aerobic conditions of storage, their subsequent growth inside the packages does not pose either spoilage or health hazards if the storage life is limited to one to two weeks. It was also observed in these experiments that the amount of sorbic acid transferred to food from the wrapper is well within the limit of 0.1 percent allowed by the statutory authorities.

For adopting the above simple technique for minimum 10 days, two more facts had to be ascertained viz., whether the optimum concentration of 2 gsm of sorbic acid in the wrapper previously established was still valid for this method also and secondly, whether the time lag between the stoppage of heating process of the food and its wrapping could be relaxed and, if so, to what extent. Large scale experiments using 200 bread loaves in each, were carried out in which sorbic acid treated wrappers had concentrations of both 2 gsm and 1.5 gsm. The time gap of both 3 min and 6 to 7 min between the removal of the bread from the baking oven and their wrapping were used. The results are given in Table 2. It can be concluded from these results, that even for short periods of preservation of 3 to 4 weeks, sorbic acid concentration in the wrapper cannot be lowered beyond 2 g per square meter and it will be equally unsafe to increase the time gap of wrapping after the heating stage to more than 4 minutes in case of the breads used, the maximum time gap permissible in other cases clearly depending upon factors such as size, weight etc., of the items.

Table 2 also shows the results of application of the simple technique on other foods such as puries, chapaties and Khoa. The application to khoa was especially carried out in view of the current interest in its preservation[5]. After 10 days the khoa was still found to possess good aroma and flavour. After 20 days though no mould growth was observed a distinct rancid off-flavour was perceptible.

The above simple technique, because of the possibilities of microbiological contamination, however, small especially in large scale manual operation, can evidently be looked upon as a method of preservation for short periods not exceeding 10 to 15 days. But even this short period of preservation can be of immense utility in certain situations when a food item prepared at one place has to be used at some time afterwards, probably at a different and distant place. Also, in many cases a too long period of preservation against microbiological deterioration does not serve any useful purpose since the onset of other types of deteriorations such as staling, off-flavour due to rancidity, browning etc., might restrict the shelf life to a much shorter period.

In conclusion, it may be pointed out that the method of preservation of foods by fungistatic wrapper is suitable only to those which are prone to fungal spoilage, that is foods with a water activity of generally less than 0.90 (or equillibrium relative humidity of less than 90 per cent)[6] although sometimes foods (e.g., bread) with water activity higher than 0.90 can also be preserved. Since a heating process is involved in both the long term and the short term preservation methods, the foods so preserved may be taken to be free from pathogens and under aerobic conditions of storage they can also be taken to be safe against toxin producing bacteria.

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Determination of Iron Particle Content of Black Tea

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Four different methods involving both colorimetric and gravimetric procedures were tried to find out a suitable method for estimation of iron particles in black tea. Direct ashing of the sample before and after magnetic separation of free iron particles by magnet, acid digestion of the residue and ammonium thiocyanate colorimetric method seem to be relatively simpler in procedure and more reproducible than other methods.

It is well known that during black tea manufacture some quantity of metallic iron particles due to wear and tear of machinaries are imparted to tea and these are almost entirely removed by means of powerful magnets at all stages of manufacture. However, it becomes necessary to determine the exact quantity of iron particles that are retained in tea by following certain assay procedures.

Standard methods are available for determining the different constituents of black tea[1]. However, no standard method is so far available for the determination of metallic iron content of black tea. The AOAC recommend gravimetric[2], permanganimetric[3] and colorimetric methods, the latest involving orthophenanthroline[4] and ammonium thiocyanate[5] reagents for determining iron in ashed samples of food materials. In the present investigation both colorimetric and gravimetric methods were tried to find out a suitable and relatively simpler and more reproducible method to estimate the iron particle content of tea.

Materials and methods

For the purpose of comparative studies on analysing iron particle content of tea by four different methods involving gravimetric and colorimetric procedures, two grades of CTC (Crush, Tear and Curl) processed tea samples, viz., Broken Orange Pekoe—a leaf grade and Super Fine Dust—a dust grade, were obtained from one of the tea garden factories in South India.

Preparation of sample: Tea samples were ground in a glass mortar and pestle to pass through 500 micron mesh sieve and mixed thoroughly to get a homogeneous sample.

Moisture: Five grams of prepared samples were dried to constant weight at 100±2°C over night. The loss in weight as moisture was recorded.

Method I

Ashing, acid digestion and analysis: Five grams of the samples were dry ashed at 525±20°C for 30 min. Ash of the residues was treated with 30 ml of iron-free 1:1 (v/v) HCl-water, boiled for 20 min. cooled and then the volume made to 50 ml with distilled water. The iron content of the solution was estimated by following the ammonium thiocyanate colorimetric method[6].

Magnetic separation[2] Iron particles from 5 g of sample were removed by playing a powerful magnet several times at 1 cm height over uniformly spread sample on a white grease-free glazed paper, covering an area of 25 cm × 25 cm.

The iron particle-free samples were ashed, acid digested and analysed for their residual iron contents by using the above mentioned thiocyanate method[6]. The difference in values of iron contents of samples before and after removal of iron particles was reported as free iron particle content.

Method II

Floating of tea in carbontetrachloride: To 10 g of well mixed ground samples in a 250 ml beaker, 150 ml of carbon tetrachloride was added, and the mixture allowed to stand for 30 minutes with occasional stirring. Tea and carbontetrachloride were decanted leaving heavy residue of sand and iron in the beaker. Three successive portions of carbontetrachloride were used until practically all tea was floated off and separated by decantation. The residual solvent was evaporated by keeping the beaker on a hot plate for 5 min. and cooled.

Estimation of total iron: After acid digestion the total iron content in the residue was estimated by thiocyanate colorimetric method[6]. Similarly, after magnetic separation of iron particle from the residue the non-magnetic part of the residue was acid digested and estimated colorimetrically for its total non-magnetic iron content. The difference in iron content of the residue before and after magnetic separation was reported.

TABLE 1. IRON PARTICLE CONTENT OF CTC-BOP AND CTC-SFD TEA SAMPLES BASED ON FOUR DIFFERENT METHODS OF ESTIMATION

Sample	Trial No.*	Method I Iron content (ppm) Magnetic separation Before C_1	After C_2	Magnetic iron content (ppm) C_1-C_2	Method II Total iron content (ppm) Magnetic separation Before FC_1	After FC_2	Magnetic iron content (ppm)FC_1-FC_2	Magnetic iron content Method III (ppm) FG	Method IV (ppm) G
BOP	1	168.61	125.60	43.01	30.88	13.33	17.55	243.90	530.83
	2	165.00	129.04	35.96	42.02	13.33	28.69	121.95	592.27
	3	175.49	130.76	44.73	25.73	10.75	14.98	365.85	730.58
	4	151.41	130.76	20.65	27.44	12.90	14.54	132.11	354.80
	Mean	—	—	36.09	—	—	18.94	215.95	552.12
	S.D.	—	—	9.50	—	—	5.75	98.89	134.95
SFD	1	145.11	108.84	36.27	42.15	13.39	28.76	234.69	193.00
	2	162.39	122.66	39.73	42.15	9.07	33.08	153.06	81.63
	3	169.30	122.66	46.64	29.25	8.21	21.04	275.51	243.90
	4	141.66	105.38	36.28	24.95	12.09	12.86	397.96	335.37
	Mean	—	—	39.73	—	—	23.94	265.31	213.48
	S.D.	—	—	4.23	—	—	7.71	88.37	91.63

*Figures given under each trial are average values of four determinations.

Method III

Floating of tea in carbontetrachloride and weighing the residue: The weight of the solvent-free residue obtained after floating 10 g of well mixed ground tea samples in carbontetrachloride was determined by weighing the beaker containing the residue. The magnetic iron particles from the same residue were removed by magnet and again weighed. The difference in weight was noted for magnetic iron particle content of the residue.

Method IV

Direct weighing of tea sample before and after magnetic separation of iron particles: About 10 g of ground sample was accurately weighed in a watch glass. Iron particles from the weighed sample were removed by magnet and the weight of the residue determined. The difference in weight was the quantity of magnetic iron particles present in the tea sample.

Correction for moisture absorption: Correction for moisture absorption was effected by finding out the increase in moisture content of the sample when it was allowed to absorb moisture within the stipulated time of weighing.

Results and Discussion

The scheme of four different methods for determination of iron particles in CTC-BOP and CTC-SFD grade tea is given in Fig 1 and the results obtained on the basis of four methods are presented in Table 1.

On comparing the magnetic iron particle content of CTC-BOP and CTC-SFD tea samples as determined by the four different methods (Table 1), the results obtained from method IV shows higher values of magnetic iron particle content than that obtained by methods III, I and II. The higher values obtained by method III and IV can be explained on the basis that both the methods are gravimetric in nature and a change in third decimal during weighing causes an error to an extent of 100 ppm. In method IV, particularly, analysts find often a serious problem of moisture absorption by tea samples during weighing (Table 2). However judicious and fast the assay may be, a correction factor to this source of exaggerated values is not easily possible. On the other hand, in methods I and II such problems are not encountered since a very sensitive

TABLE 2. MOISTURE ABSORPTION OF CTC-BOP AND CTC-SFD TEA SAMPLES DURING WEIGHING AND MAGNETIC SEPARATION BY METHOD IV

Sample	Trial No*	Sample wt (g)	Moisture absorbed (g)
BOP	1	11.303	0.030
	2	10.299	0.028
	3	10.676	0.029
	4	9.301	0.025
SFD	1	10.533	0.018
	2	10.021	0.018
	3	10.024	0.022
	4	9.776	0.022

*Figures given under each trial are average values of four determinations.

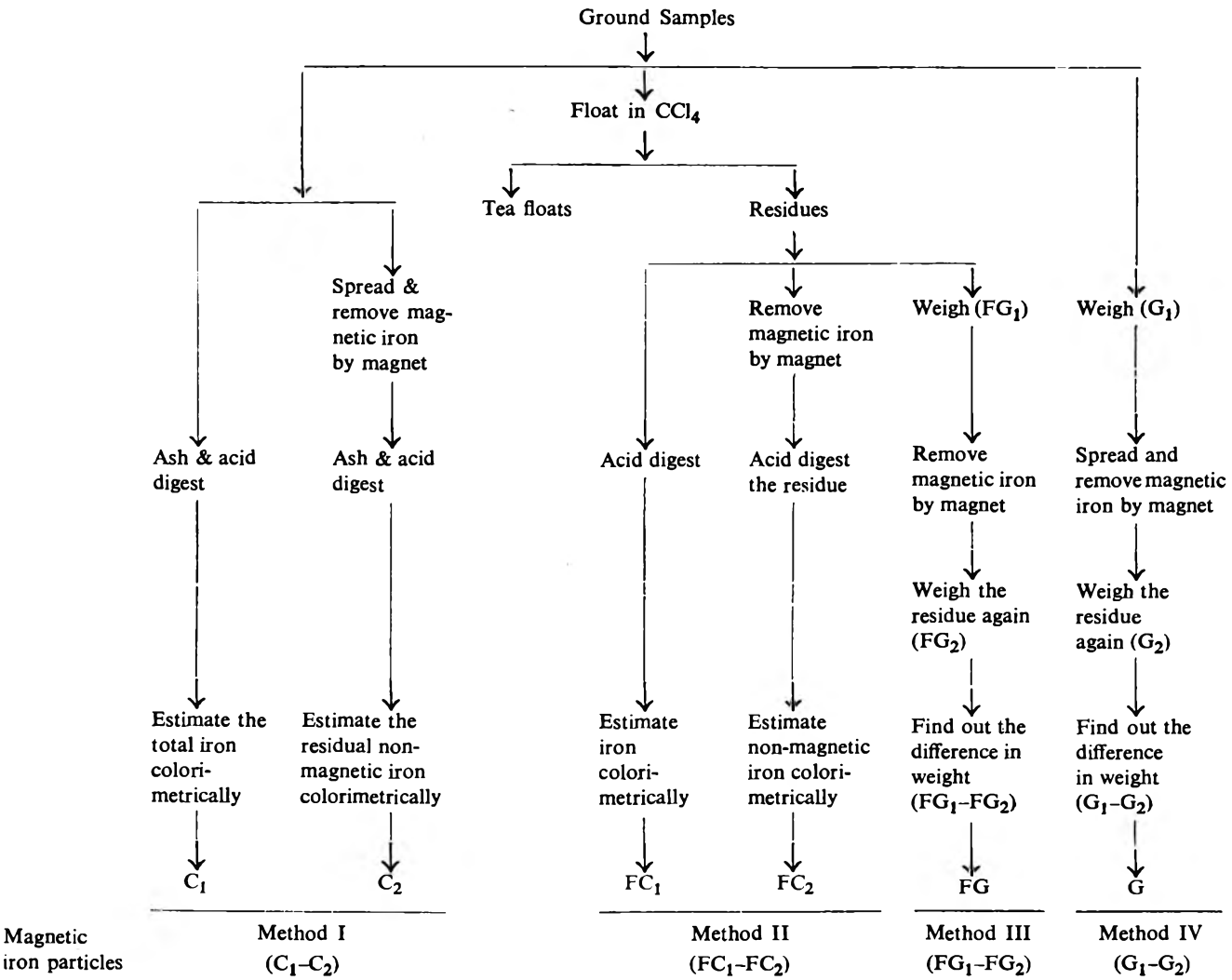

FIG. 1. SCHEME OF DIFFERENT METHODS OF IRON PARTICLE DETERMINATION IN TEA

technique of colorimetric method is employed. The lower value of iron particle content as shown by method II is explained by the fact that during floating of tea with carbon tetrachloride, very fine particles of metallic iron stick to tea and as a result of this the actual iron particle content in the residue goes down. The same thing is reflected in method III and in this method while removing the magnetic iron by magnets, siliciceous materials also come along with the iron particles showing a very high value of the magnetic iron particles.

Of the two colorimetric methods, viz., Method I and Method II, the former seems to be relatively simpler in procedure, more reproduceible and less expensive than the latter. The gravimetric methods *in toto* are not suitable for determination of iron particles in tea.

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Fatty Acid Composition of Marine Fish Body Fat

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The fatty acid composition of the body fat of 11 species of marine fish was determined. Lipids were found to be rich in 20:5 and 20:6 polyunsaturated fatty acids, 18:1 is generally the dominating monounsaturated acid and saturated acids account for over 40 percent of the total. Lipids of squid and cuttlefish are distinctly different from marine fish lipids in fatty acid composition.

The fatty acid composition of marine lipids is impotant from the point of view both of nutrition and of processing of these fish. This report presents the fatty acid composition of a number of marine fish, most of which are not commercially exploited so far, and are obtained as a by-catch from shrimp trawlers.

Materials and Methods

Lipids were extracted from edible meat of the fish with a mixture of chloroform-methanol (2:1 V/V) following the method of Bligh and Dyer[1].

Preparation of methylesters: The extracted lipids were saponified by the AOCS method[2] in an inert atmosphere of nitrogen. The fatty acids recovered were esterified with methanol-HCl reagent under nitrogen, concentrated, filled in sealed ampoules under nitrogen and kept at—15°C before analysis.

Gas-liquid chromatography: Fatty acid methyl esters were analysed in a gas chromatograph equipped with flame ionisation detector, temperature controller and stripchart recorder (10 mv) (Toshniwal India, Model KL04/04). A stainless steel column 6 ft × 1/4 in (O.D) packed with Chromosorb W (60-80 mesh, acid-washed) coated with 15 per cent DEGS was used. The samples were analysed at a column temperature of 196°C (isothermal), detector temperature of 275°C and an injection port temperature of 250°C using nitrogen at 40 ml/min as carrier gas.

Fatty acid esters of fish oils were identified, as earlier described[3], against reference standards either provided by National Institute of Health, U.S.A. (unsaturated esters) or from A. G. Fluka, Switzerland (saturated esters). Peak areas were determined by triangulation and results expressed as area percentages.

Results and Discussion

Details of the fish species analysed and their lipid contents are given in Table 1. The fatty acid composition of the fish lipids is listed in Table 2. The total amount of saturated fatty acids (37-61 per cent) is slightly higher than for other marine oils[3-5]. The 14:0 acid shows wide variation, from 1.6 (skate) to 10.0 per cent (gizzard shad), as does also the 16:0 acid levels (16.3 to 31.5 per cent). All species contain significant proportion (1.6 to 9.3 per cent) of oddchain 13:0, 15:0 and 17:0 acids.

Total monounsaturated acids (15.0 to 31.9 per cent)

Table 1. Fish studied and their body fats

Common name	Species	Lipids (g/100 g wet tissue)	I. V. of lipids
Cuttle fish	*Sepia*	0.6	178
Squid	*Loligo*	0.8	213
Kalava	*Serranus hexagonatus*, Day	0.5	162
Ribbon fish	*Trichurus savala*, Cuvier	0.7	145
Skate (granulated short noseray)	*Rhinobatos granulatus*, Cuvier	0.6	148
Knobby flathead	*Suggrundus tuberculatus*, Cuvier	0.7	100
Rusty smalltoothed jobfish	*Aphareus rutilans*, Cuvier	0.22	175
Shortnose gizzard shad	*Anodontostoma chacunda*, Hamilton Buchanan	2.25	90
White pomfret	*Stromateus sinensis*, Day	0.5	143
Silver jewfish (young ones)	*Johnius argentatus*, Houttuyn	0.35	116
Madura anchovy	*Thrissocles Kammalensis*, Bleeker	0.6	150

TABLE 2. FATTY ACID COMPOSITION OF BODY FATS OF ELEVEN MARINE FISH

	Cuttle fish	Squid	Kalava	Ribbon fish	Skate	Knobby flat head	Job fish	Gizzard shad	White pomfret	Silver Jew fish (Young ones)	Madura anchovy
Saturated acids											
13:0	1.7	0.9	0.9	0.5	0.4	1.8	2.3	—	—	—	—
14:0	3.3	5.9	2.6	4.9	1.6	6.4	4.0	10.0	8.1	4.6	8.0
15:0	1.7	—	—	1.2	1.5	2.2	0.3	0.8	1.0	1.6	1.2
16:0	16.3	18.0	18.0	19.5	18.8	22.8	23.7	31.5	20.8	27.3	28.8
17:0	5.9	0.9	1.1	2.0	0.4	2.0	1.5	0.8	1.0	0.7	0.9
18:0	11.8	11.4	13.6	12.8	15.7	11.9	4.6	17.6	14.1	21.0	9.4
19:0	—	0.3	0.2	—	0.4	0.6	0.6	0.6	1.0	0.8	0.6
Total	40.7	37.4	36.7	40.9	38.8	48.4	37.0	61.6	46.0	56.0	48.9
Mono unsaturated acids											
13:1	—	—	—	0.2	—	1.2	1.4	—	—	—	—
14:1	0.5	0.9	0.6	0.5	2.5	0.7	0.2	5.5	1.0	2.1	1.8
15:1	—	—	—	—	—	—	—	—	—	—	—
16:1	3.1	1.6	2.0	4.2	1.4	2.8	1.9	3.0	1.5	3.2	5.3
17:1	—	—	0.5	0.9	0.7	1.7	—	—	—	—	—
18:1	8.3	4.6	16.6	18.0	14.0	15.6	16.3	7.5	13.5	9.0	4.0
20:1	9.8	5.9	9.0	4.6	10.0	3.5	—	0.6	4.0	0.7	4.0
22:1	0.3	1.5	—	—	0.7	0.9	3.0	8.3	1.0	—	1.8
24:1	—	1.8	—	—	2.6	4.3	—	—	—	—	—
Total	22.0	16.3	28.7	28.4	31.9	30.7	22.8	16.9	21.0	15.0	16.9
Polyunsaturated Acids											
18:2	0.7	0.5	0.6	2.7	0.7	3.5	2.9	2.6	3.9	2.2	3 5
18:3	0.3	1.6	2.0	1.5	—	0.7	3.7	0.7	2.5	2.3	2.2
18:4	0.9	1.6	1.5	2.2	0.4	0.7	0.7	0.6	4.0	0.9	2.0
20:2	—	—	—	—	—	—	—	0.7	—	—	—
20:3	0.5	—	0.8	—	—	—	0.9	0.9	1.9	0.7	0.8
20:4	3.5	1.1	1.4	1.0	1.4	2.3	1.8	4.3	1.0	7.0	1.0
20:5	10.3	11.5	3.2	5.7	3.5	2.9	4.2	4.9	8.0	7.1	11.8
22:2	0.2	0.5	—	0.3	0.3	0.3	—	—	—	0.5	—
22:3	0.6	0.5	2.6	1.2	1.3	—	—	0.7	0.7	0.5	0.3
22:4	0.7	0.7	4.9	1.7	1.5	0.9	1.0	0.7	0.9	0.5	0.3
22:5	1.9	1.1	1.6	1.9	2.1	0.9	11.0	3.5	1.6	6.0	0.3
22:6	17.7	27.2	16.0	12.5	18.1	8.7	14.0	1.9	8.5	1.3	12.0
Total	37.3	46.3	34.6	30.7	29.3	20.9	40.2	21.5	33.0	29.0	34.2
Unsaponifiable matter lipids.	4.6	7.6	4.0	6.0	10.0	10.0	8.0	0.5	3.5	4.9	3.3

are lower than for the marine body fats earlier reported[3,4] but similar to that in prawn lipids. The chief constituents are 18:1, 20:1 and 16:1 acids.

Total polyenoic acids constitute 20.9 to 46.3 per cent of the body fats, 22:6 and 20:5 being the major constituents. Jobfish, a common trashfish, is quite distinct from other marine fish body fats in having an exceptionally high level (11.0 per cent) of 22:5 acid.

A significant feature of the present fats is the low values of the 18-C acids. It has been pointed out[6,7,8] that with increasing depth of water there is a corresponding increase in the quantity of 18-C fatty acids in marine organisms. The present results support this hypothesis, since the species are all from shallow waters.

The composition of squid and cuttlefish fats, with high contents of 18:1 and 20:1 and 20:5 and 22:6 fatty acids, is different from the marine fish species. The present tropical squid fat is similar in composition to Atlantic squid studied by Japanese workers[9], but with higher levels of 18:0 and 18:1 and slightly lower levels of 20:5 and 22:5 acids. The cuttlefish lipid now studied closely resembles that reported by the same workers[9].

The composition of the present fats from by-catch fish is similar to that of shrimp. This could reflect the effect of food and environment on fatty acid make-up.

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Influence of Maturity and Pretreatments on Quality of Canned Okra *(Abelmoschus esculentus)* (L.) Moench)*

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The okra cultivars–*Pusa Sawani* and *Dwarf Green Long Pod* were best suited for canning on the seventh day from the time of flowering. The canned product from the former was comparatively better than the latter. Of the various pretreatments, cutting the pods into pieces 3.75 to 5.0 cm long, blanching in boiling water for 2 min and calcium firming for 30 min in 0.5 per cent calcium chloride solution was found to be the best. The mucilage content of the covering liquid and the drained weight decreased with maturity and in addition, depended on pretreatment.

Okra (*Abelmoschus esculentus* (L.) Moench) is a popular vegetable grown throughout the country and successfully raised all through the year in southern parts of India. Okra pods, canned in brine, become soft in texture and mucilaginous making the covering liquid thick and viscous[1]. Ranganna and Siddappa[1] found calcium treatment to be effective somewhat in reducing the mucilage of the covering liquid. The actual maturity stage and variety of the okra pods used for their study were, however, not defined. The firness and texture of vegetable products vary with maturity and processing operations[1,2].

In this paper, are presented the findings of an investigation carried out to study the effect of maturity and pretreatments on the quality of canned product of two okra cultivars, *Pusa Sawani* and *Dwarf Green Long Pod.*

*Part of M.Sc. (Agri.) thesis submitted by the first author to the University of Agricultural Sciences, Hebbal, Bangalore.

Materials and Methods

The pods of two okra cultivars, *Pusa Sawani* and *Dwarf Green Long Pod* obtained from the crop raised in Gandhi Krishi Vignana Kendra, Bangalore, during January-April were used for this study. The experiment was laid out in a split split plot design. The flowers from the two cultivars were tagged in late March and the pods 6, 7, 8 and 9 days after flowering were used for canning. The following different treatments were used. Whole pods blanched in water for 2 min, cut into pieces (3.75-5.00 cm long) and canned (t_1). Pods cut into pieces (3.75-5.00 cm long). blanched in boiling water for 2 min and canned (t_2). The cut pieces were soaked in calcium chloride (0.5 per cent) solution for 30 min, blanched in boiling water for 2 min and canned (t_3). Same as 3 except that calcium treatment was given after blanching (t_4). The cut pieces were blanched in 0.5 per cent calcium chloride solution for 2 min and canned (t_5). The treatments were replicated thrice.

The pieces of pods treated with calcium chloride were washed well with water to remove traces of calcium chloride. Canning was done in plain cans of 301 × 309 size. In each can, 125 g of the prepared vegetable was filled and covered with 200 g of hot 2 per cent brine. The cans were exhausted to can centre temperature of 82°C, sealed, processed for 30 min at 116°C and cooled in water[1]. The canned product was stored at room temperature for four months and then examined. The cans of each replication were examined on successive days in the order they were canned.

Drained weight was determined by pouring the contents of the can on to a sieve and allowing to drain for 2 min. To determine the mucilage content, 20 ml of the drained liquid was mixed with 60 ml of absolute alcohol and the precipitate formed was dried and weighed[3].

A panel of five judges evaluated the canned product. The quality was adjudged adopting the following score card.

Quality		*Score*
Fibrousness	..	30
Mucilage	..	20
Aroma	..	15
Colour and appearance	..	15
Taste	..	15
Seediness	..	5

Lower score values were assigned to greater fibrousness, higher mucilaginous nature, higher seediness and product with split and disintegrated pods. Higher scores were given to freshness in colour, soundness in appearance of the vegetable pieces and taste. Between each tasting, the judges washed their mouth and chewed a piece of bread to overcome the fatigue of tasting. The scoring by judges was analysed statistically by the method of co-efficient of concordance utilizing the rankings based on the mean scores[4].

Results and Discussion

Maturity of pods and pretreatments given had significant effect on mucilage content and drained weight (Tables 1 and 2). The two cultivars did not differ significantly between themselves in their response to the pretreatments. When the drained weight was less, the pods were less mucilaginous and more firm and crisp.

Among the pretreatments, okra pods blanched whole and cut into pieces thereafter (t_1), recorded the highest mucilage content in the drained liquid (Table 1) and caused an increase in the drained weight (Table 2) of the product. Apparently, these changes were due to the higher amount of mucilage retained in the vegetable during blanching which leached out when cut and canned. On the contrary, when the pods were cut before blanching, some amount of mucilage evidently leached out into the blanching water, resulting in bringing down the mucilage content of covering liquid and the drained weight of canned product. Ranganna and Siddappa[1] made similar observations. They reported that the covering liquid became more viscous with an increase in the mucilage content, which resulted in the lowering of the quality of the canned product.

Treatment with calcium chloride reduced the mucilage content and produced a firm product (Tables 1 and 2). Of the pretreatments studied, blanching of cut pieces

TABLE 1. MUCILAGE CONTENT IN DRAINED LIQUID OF CANNED OKRA AS INFLUENCED BY MATURITY OF PODS AND PRETREATMENTS[a] (MEAN OF CULTIVARS *PUSA SAWANI* AND *DWARF GREEN LONG POD*)

Pretreatments	Mucilage content (g/100 ml of drained liquid) in different maturity pods (days after floweing)				
	6	7	8	9	Mean[b]
t_1	1.329	0.978	0.905	0.737	0.990[d]
t_2	0.909	0.934	0.780	0.634	0.814[c]
t_3	0.905	0.843	0.638	0.580	0.742[b]
t_4	0.713	0.733	0.544	0.505	0.626[a]
t_5	1.066	0.921	0.700	0.613	0.825[c]
Mean	0.984[c]	0.884[bc]	0.715[ab]	0.614[a]	0.799
	Maturity of pods				*Pretreatments*
S.Em	±0.066				±0.023
C.D. at 1%	0.203				0.061

a) examined after 4 months of storage

b) Means not having a common letter are significantly different at 1 per cent probability level.

TABLE 2. DRAINED WEIGHT PER CENT OF CANNED OKRA AS INFLUENCED BY MATURITY OF PODS AND PRETREATMENTS[a] (MEAN OF CULTIVARS *PUSA SAWANI* AND *DWARF GREEN LONG POD*).

Pretreatments	Drained wt % in different maturity of pods (days after flowering)				
	6	7	8	9	Mean[b]
	Drained weight per cent				
t_1	68.01	61.88	56.76	48.94	58.90[d]
t_2	61.72	57.24	55.47	46.14	55.14[c]
t_3	58.27	56.48	51.38	43.76	52.43[b]
t_4	57.05	54.09	45.95	41.19	49.57[a]
t_5	60.51	57.93	51.64	44.83	53.70[bc]
Mean[b]	61.09[d]	57.52[c]	52.24[b]	44.97[a]	53.95

	Maturity of pods	*Pretreatments*
S.Em	±0.941	±0.729
C.D. at 1%	2.875	1.933

a) Examined after 4 months of storage

b) Means not having a common letter are significantly different at 1 per cent probability level.

TABLE 3. ORGANOLEPTIC EVALUATION OF CANNED OKRA PODS OF DIFFERENT MATURITY STAGES

	Mean score and rank for different maturity Stages (days after flowering)							
	Pusa Sawani				Dwarf Green Long Pod			
	6	7	8	9	6	7	8	9
Mean score	55.80	63.00	52.40	31.00	56.80	61.00	48.40	24.40
Rank	3	1	5	7	4	2	6	8

W (Coefficient of concordance) = 0.831)

and soaking thereafter in calcium chloride (0.5 per cent) for 30 min yielded canned product having lower drained weight and less mucilage in the covering liquid (Tables 1 and 2).

In the canned product, the drained weight and the mucilage content of the drained liquid decreased with maturity from 6 to 9 days, but the fibrousness increased (Tables 1 and 2). The canned product prepared from 7-day old pods was adjudged as the best in organoleptic evaluation (Table 3). The 6-day old pods though younger and more tender got the second ranking as they were more mucilaginous. The product of 8-and 9-day pods got lower scores because they were more fibrous. Between the two cultivars, *Pusa Sawani* secured better ranking owing to its better appearance. texture, less pronounced ridges and less mucilage. From these studies it may be inferred that the 7-day old pods of *Pusa Sawani* and *Dwarf Green Long Pod* okra cultivars, cut and blanched in boiling water for 2 min and soaked in calcium chloride solution (0.5 per cent) for 30 min gave a less mucilaginous and firmer canned product.

Acknowledgement

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RESEARCH NOTES

HISTOCHEMICAL LOCALIZATION OF LIPASE IN THE RICE GRAIN

Histochemical study of the rice grain indicated that the bulk of the lipase activity was present in the testa layer in both raw and parboiled rice and to a smaller extent in the pericarp layers. The oil is distributed in the aleurone and in the testa and in the bran layers to some extent. It is concluded that since the lipid and the lipase are spatially distributed in aleurone and testa layers respectively in intact rice grain, there is more stability of the oil in the intact rice grain.

Earlier we reported that lipase was present mostly in the bran layers of the paddy grain, although rice germ was also a good source[1]. The present note pertains to its histochemical localization.

Paddy grain was dehusked by hand and the grain was soaked in sterile distilled water for 24 hr at room temperature. Then 10 thin sections (of the mid-portion of the grain) were made and incubated for 5 hr at 25°C in a lipase-assay medium (containing 1 ml sodium taurocholate (containing 8.5 mg/ml), 5 ml of emulsified β-naphthyllaurate in 50 mM phosphate buffer (pH 7.4) containing 0.1 mg of the substrate/ml). They were then washed with water and 2 ml of diazonaphthanil blue B (4 mg/ml) were added to the sections and incubated for 30 min. The sections were then washed with water and observed under a microscope (Fig IA and IB). Fig IA corresponds to raw rice and Fig IB corresponds to parboiled rice. The testa layer had a deep pink colour; the mesocarp layer also had pink colour, but the endo-and epicarp layers had very much less pinkish colour: the aleurone layer had none. Thus the bulk of lipase activity seems to be present in the testa layer of both raw and

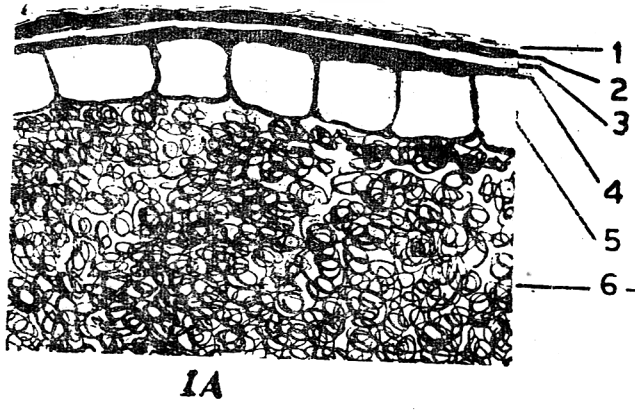

Fig. IA—Cross-section of hand dehusked rice stained for lipase activity

1. Epicarp; 2. Mesocarp; 3. Endocarp;
4. Testa; 5. Aleurone; 6. Endosperm.

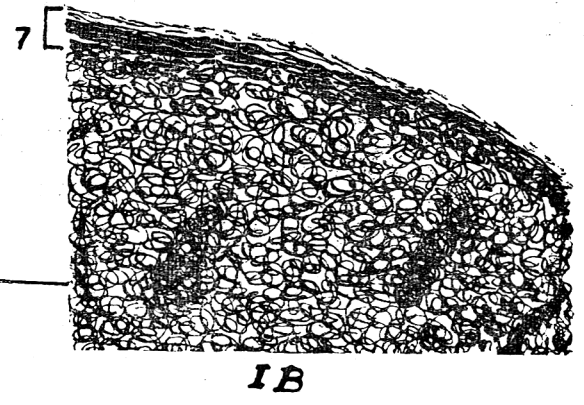

Fig. IB—Cross-section of parboiled rice stained for lipase activity

6. Endosperm; 7. Bran layer (Endo, Meso and Epicarp)

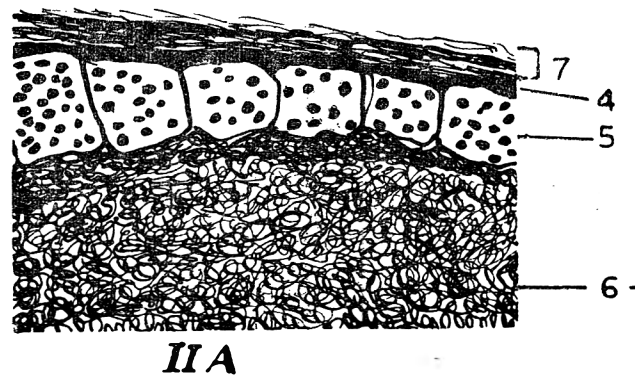

Fig. IIA—Cross-section of hand dehusked rice stained for fat
4, 5, 6, 7 are as in IA and IB

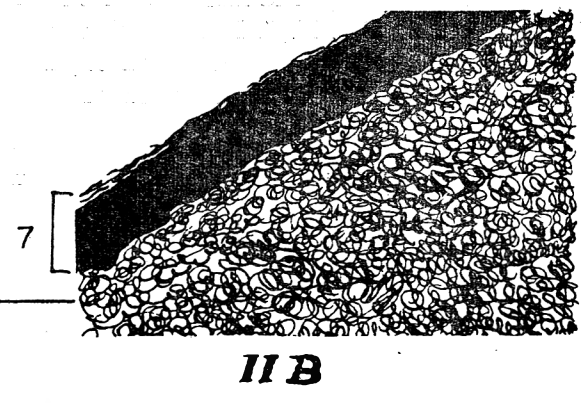

Fig. IIB—Cross-section of parboiled rice stained for fat
6 and 7 are as in IB.

parboiled rice and also distributed, though to a much less extent, in the pericarp layers; parboiled rice had much less lipase.

When fresh sections were treated with Sudan Black-4 for 3-4 min, the testa layer as well as the aleurone layers appeared red (Fig. 2A). In the case of parboiled rice (Fig. 2B), fat is distributed into all layers because of partial gelatinization and spread due to parboiling. In order to ascertain whether the lipid was free or bound to protein, 10 to 20 g of intact hand-dehusked grain was first soaked in a mixture of petroleum ether (60-80°C) and ethanol (1:1 v/v) and gently agitated overnight to remove free lipids. It was then washed twice with water and soaked in distilled water overnight. Sections were made as before and stained with Sudan Black 4 and observed microscopically. Even here the testa layer was coloured indicating that it contained bound lipids while the other layers hardly had any fat.

These results indicate that most of the lipase activity seems to be confined mainly to the testa layer of the grain whereas oil is distributed mostly in the aleurone and in the testa and the bran layers to some extent. Whether the lipase of the testa layer will act on the bound lipids and whether the minor quantities of lipid in the mesocarp layer (bran) is in intimate contact with lipase in the same layer are doubtful and no definite information is available. In any case the bulk of the lipid (aleurone layer) and most of lipase (testa layer) seem to be spatially separated in the intact grain. This explains the higher stability of rice oil in the intact paddy and also the relatively higher stability of the oil in brown rice obtained from rubber roller shellers as compared with disc shellers[2]. During milling in disc shellers, the different layers are damaged and the enzyme and the oil come into contact, leading to quicker deterioration of the oil both in the bran and in the rice grain. A detailed study of the lipid and enzyme content of each layer and of the fatty acid composition also may yield more definitive results.

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APPLICATION OF IN-PACKAGE DESICCATION (IPD) TECHNIQUE FOR ENHANCING THE STORAGE STABILITY OF AFD BEVERAGE POWDER

AFD beverage powder, being extremely hygroscopic, picks up moisture quickly during packaging and thus losses its storage stability under tropical conditions. Use of sea-shell lime as in-package desiccant over comes this problem. It helps in reducing the moisture content to a safer level and also enables to reduce the running cost of the plant by stopping the drying process at a moisture content of about 2% as against less than 1%.

Work has been carried out earlier in this laboratory on the production of fruit juice powders by freeze drying[1,2]. One great advantage of this method over the vacuum puff-drying method[3], viz., superior quality of the product with a very low moisture content is partially lost since the product with low moisture content is extremely hygroscopic and has a tendency to pick up moisture quickly from the sorrounding atmosphere during the process of packing thus losing the storage stability under tropical conditions. The technique of in-package desiccation was tried not only as a solution to this problem but also as a means of cutting down the cost of running the freeze drier as the finished product can be preserved at a higher moisture level.

Freeze dried pineapple jucie powder was prepared as previously described[1] and adjusted to a moisture content of 2.5 per cent. Forty grams of this product was packed in pouches of 60 g paper/0.04 mm foil/150 gauge polyethylene laminate together with a gussetted grease-proof paper bag containing 6g regenerated sea shell

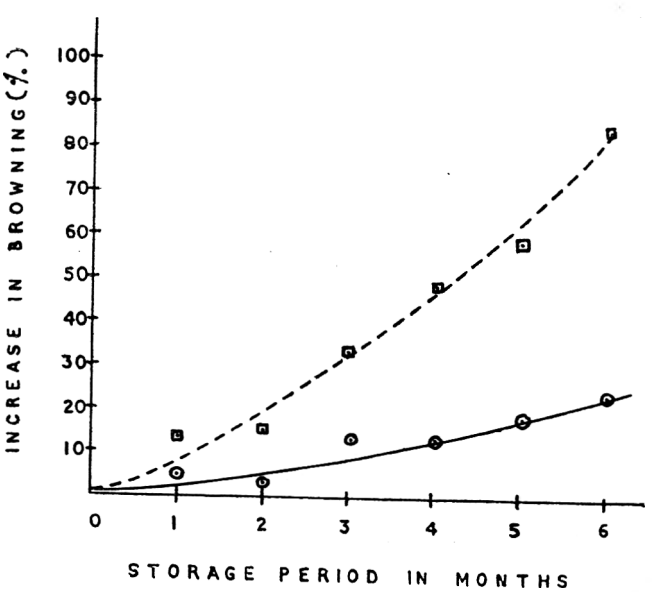

Fig. 1 Effect of inpack desiccant on rate of browning of AFD pineapple beverage powder stored at 37°C.
—, sample with IPD; - - -, sample without IPD.

TABLE 1. STORAGE STUDIES OF AFD PINEAPPLE POWDER WITH OR WITHOUT DESICCANT UNDER ACCELERATED (37°C) AND AMBIENT TEMPERATURE

Storage condition	Storage period (months)	With desiccant			Without desiccant		
		Moisture (%)	Browning (OD at 375 μ)	Organoleptic rating	Moisture (%)	Browning (OD at 375 μ)	Organolepfic rating
Initial		2.49	0.195	Like very much	—	—	—
37°C	1	0.96	0.205	,,	2.94	0.220	Like moderately
Ambient	2	0.76	0.210	,,	3.15	0.210	,,
37°C	2	0.92	0.200	,,	3.69	0.250	Indifferent
37°C	3	0.89	0.220	,,	3.43	0.260	,,
Ambient	4	0.83	0.220	,,	2.65	0.230	,,
37°C	4	0.75	0.220	,,	3.26	0.290	Dislike slightly
37°C	5	0.73	0.230	Like moderately	3.42	0.310	,,
Ambient	6	0.75	0.230	,,	2.96	0.340	Do not like at all
37°C	6	0.58	0.240	,,	3.84	0.360	,,

lime. These packs were stored both under ambient temperature (25°-30°C) and at 37°C. Some control samples of the beverage powder packed as above but without desiccant were also stored similarly. Moisture[4], browning[5], pH and organoleptic rating were determined periodically (Table 1) upto 6 months.

It may be observed from the Table that moisture content is reduced within 1 month from 2.5 to 1 per cent and thereafter the reducion is slower bringing it down to 0.6 per cent in 6 months. Browing is also considerably retarded (Fig. 1). pH did not vary much during the storage period. After 6 months storage at 37°C the product with IPD system remained organcleptically acceptable whereas the samples without the desiccant were disliked even after 4 month's storage either at 37°C or at ambient temperature.

It is necessary that an active form of desiccant should be used as has been previously shown that calcined marine shell is an efficient and convenient form of lime which can be used as desiccant especially for dehydrated foods[6]. It is always advisable to regenerate a lime desiccant by heating in a muffle furnace before use.

In a pilot plant equipment with a load capacity of 10 kg normally the time required to bring fruit juice of 20° Brix to a solid powder of 1.0 per cent moisture is 13 hr. This time can be reduced by $1\frac{1}{2}$ to 2 hr if the moisture content is brought to 2.5 per cent. Hence, the IPD technique in conjunction with freeze drying technique can be used to bring about the triple advantages of (*i*) high quality product, (*ii*) economy, and (*iii*) storage stability under tropical conditions.

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MINIMISING DRY MATTER LOSS IN MALTING OF SORGHUM AND MAIZE

Development of rootlets and acrospires during germination of Maize and Sorghum leads to a loss in the yield of malt. The seeds steeped in 0.3% ammonia showed higher moisture absorption and prevented the formation of rootlets and acrospire and thus would minimise the loss during malting.

As germination progresses, rootlets and coleoptile or acrospire develop which are produced at the expense of material from original seeds[1]. Since rootlets are removed from malt after kilning, the total dry matter loss is approximately 8-10 percent[2]. The earlier observations on regulation of malt root[2] in the brewers barley indicated that gibberellic acid in various combinations with various doses of NaCl, H_2SO_4, CaCl and $KBrO_3$, limited the growth of malt roots but not acrospire growth. But the results of Tahara[3] showed that ammoniacal solution used during malting did inhibit germination and showed normal amylase activity in

TABLE 1. PROXIMATE ANALYSIS OF THE SEEDS

Chemical composition (%)	Maize			Sorghum		
	Contol	Malted Water	Malted Ammonia	Control	Malted Water	Malted Ammonia
Moisture	10.77	4.61	04.82	10.10	6.12	6.83
Crude portein	11.91	9.52	10.88	11.32	9.37	9.54
Crude fibre	01.94	2.23	02.00	01.58	1.93	1.57
Crude fat	03.61	4.11	04.54	02.10	3.15	3.25
Ash	01.42	1.76	01.62	01.44	1.76	1.62
Carbohydrates (By diff.)	70.35	77.77	76.14	73.36	77.67	77.19
Starch	66.10	61.00	65.40	63.44	48.21	52.81
Red. sugars	01.09	04.50	03.68	03.50	07.14	05.65

barley. Hence, in the present work maize and sorghum were treated with ammoniacal solution and allowed to germinate.

The 'Deccan' variety of maize and 'CSH 1' variety of sorghum seeds purchased from the local market were used. A batch of 100 each of the two varieties after washing in running tap water and then in distilled water was steeped in water and the other in 0.3 per cent ammoniacal solution. At intervals of 8 hr the wet grains were surface dried by dry filter paper and weighed and the moisture absorbed calculated. In another experiment the washed wet grains were allowed to germinate at 32±2°C in glass petridishes having moist cotton wool bed, in a closed wooden cup-board. Germination

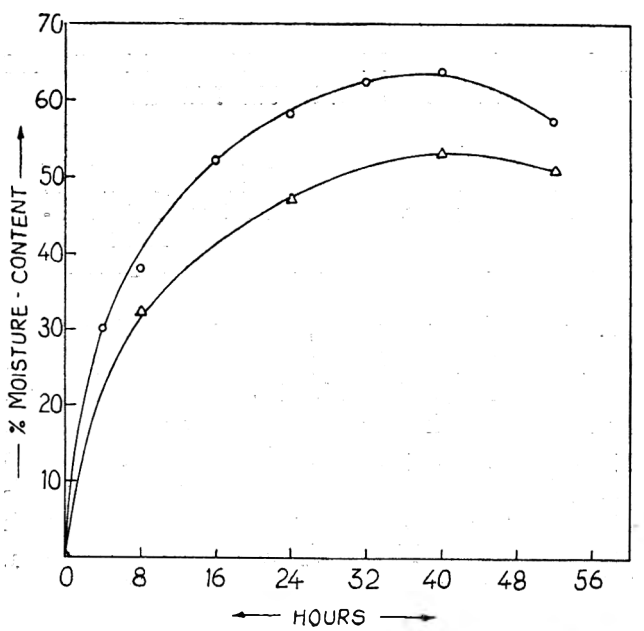

Fig. 1. Moisture absorption curve for maize at 30 ± 2°C
— O —, 0.3% Ammonia; — △ — Water

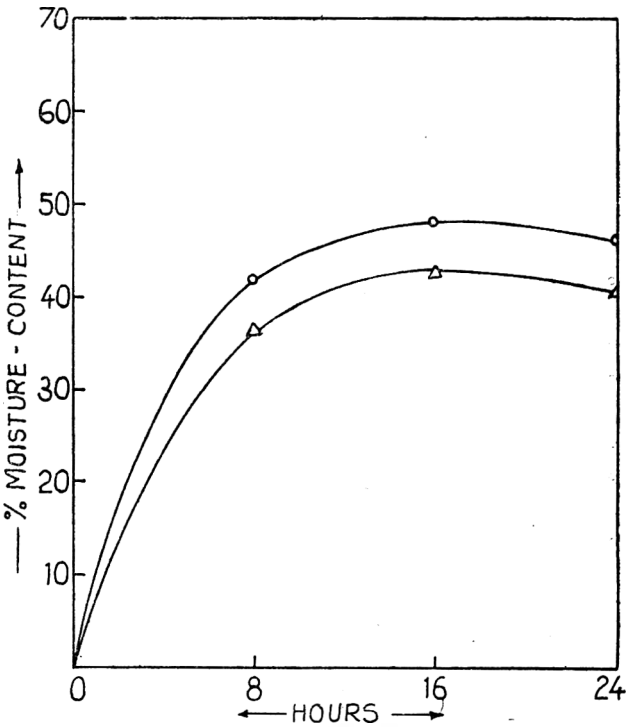

FIG.2: MOISTURE ABSORPTION CURVE FOR SORGHUM AT 30±2°C
—o—, 0.3% AMMONIA; —Δ— WATER

was continued for varying periods lasting upto 96 hr. After the specified period of germination the seeds were thoroughly dried in an oven at 50°C for 8 hrs and ground in a domestic grinder cum mixer. The powdered samples were analysed for starch and reducing sugars,[4] protein and moisture according to the AOAC methods[5].

The moisture absorption characteristics of sorghum and maize are presented in the Fig. 1 and 2. The rate of moisture absorption was more in the initial period of steeping. The maximum moisture gain was observed at 40 hr in maize and at 16 hr in sorghum. However, the moisture retention was higher in the seeds steeped in 0.3 per cent ammoniacal solution. The effect may be due to the ammonia present acting as scalding agent. Hence 40 hr. for maize and 16 hr for sorghum were selected as the steeping periods. Control samples indicate the development of acrospires and rootlets but the ammonia treated do not show these during the four days of germination. The percentage of rootlets and acrospire formed seeds in the ammonia treated samples was less than 5 per cent even on the seventh day of germination.

The results of the proximate analysis of malted and unmalted seeds are presented in Table 1. The decrease in the quantity of protein and starch but increase in reducing sugars in the malted sample indicate the formation and development of the malt enzymes even in the

ammonia treated sample. This means that the ammonia treatment, has no effect on the formation of malt enzymes but prevents the formation of rootlets and acrospire which minimises the dry matter loss and increases the malt yield.

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LABORATORY TRIALS ON BREWING USING BARLEY GRAIN, DEGERMED MAIZE AND INDUSTRIAL ENZYMES

The present studies show that barley malt can be replaced by barley grain upto 50 per cent and a brew with acceptable characters can be obtained by making use of commercial microbial enzymes such as bacterial proteinases and alpha amylases. Conditions have been standardised in the laboratory to obtain a wort with desirable characters that could be fermented smoothly. Alpha amino nitrogen content of the microbial enzyme treated wort was more and the quality of wort resembled in colour and flavour that of all-malt wort. The per cent original extract was also more in the former than in the latter.

The cost difference between barley grain and barley malt being very wide, technologists all over the world are considering alternate ways of producing beer using minimum quantity of malt and a larger quantity of adjuncts. Any deviation made from the traditional practice of brewing should not, however, affect the quality of wort or beer. Though considerable progress has been made in determining the composition of wort, a complete picture of it still remains obscure. However, some of the chemical constitutents of wort which form nutrients to yeast have been identified and attempts have been made to find these in wort derived by unconventional methods.

A large quantity of adjuncts like barley grain, maize, etc. are used in the unconventional method of brewing for producing wort. Mashing is, done by microbial enzymes like proteinasses and amylases[1-8]. Depending upon the nature and character of the enzymes used, suitable modifications of mashing process have to be made to obtain wort of desirable characters. Rao and Narasimham[9] have brought out the importance of enzymes in brewing. Wieg[7] obtained an acceptable quality brew using 75 per cent adjuncts (60 per cent barley and 15 per cent corn or 75 per cent barley) with 25 per cent barley malt employing wet-milling and using different enzymes for wort production. Hansen[5] successfully employed 55.7 per cent adjuncts (35.6 per cent barley and 20.1 per cent maize) and 40.4 per cent malt for the production of wort of desirable characters using bacterial proteinases and amylases. Pfenniger, *et al*[3]. adopted wet-milling technique to mash 75 per cent barley and 25 per cent malt along with the use of Brew-N-Zyme (a trade enzyme). Wort derived either by infusion or by decoction process could be fermented smoothly. By using microbial proteases and amylases, Crisp and East[4] obtained wort resembling normal wort in most characters. Bley, *et al*[1] conducted full scale brew trials using barley grain (upto 65 per cent) along with proteinases and amylases. By suitable modification of equipments, particularly mash tun and by using special malts to control aroma and colour, they stated that high proportions of barley grain could be used in brewing.

Studies conducted on the quality of wort and of fermented brew derived from mixtures of (*i*) barley grain and barley malt and (*ii*) degermed maize barley grain and barley malt with the aid of microbial enzymes like proteases and alpha amylases have been reported.

Materials and Methods

Mashing: Barley grain and barley malt in different proportions were mashed by infusion process according to the method of Enevoldsen[10] with suitable modifications.

To a mixture of milled barely grain and barley malt was added four times its volume of water along with proteases and maintained at 30°C for 30 min. The temperature was gradually raised to 52°C at the rate of 1°C per min and alpha amylases was added. After 30 min, the temperature was again raised to 63°C at the same rate and after allowing it for 60 min at 63°C, the temperature was further raised to 75°C and maintained there for 10 min and then boiled for 10 min. Commercially available (Indian proprietory brands) fungal protease (activity: 672 μg tyrosine released by 1 mg proteases acting upon 1 per cent casein solution at 37°C/30 min at pH 7.0) and bacterial α-amylases activity: 666.7 mg of pure soluble starch digested by 1 mg of the bacterial α-amylases at 70°C/60 min at pH 6.8) at 100 and 200 mg per cent level respectively

were used as reported[11] earlier. Barley malt, barley grain and degermed maize (30:20:50) were mashed in the following ways:

Method 1. Gelatinisation of starch in ground barley grain and degermed maize was carried out at 80°C. After cooling to 70°C, ∝-amylase was added. It was then cooled to 30°C after the reaction, and, malt grist and proteases (50 mg per cent) were added. Proteolysis and saccharification were completed in the normal way.

Method 2. Gelatinisation of starch in ground degermed maize alone was completed at 80°C and then cooled to 70°C. The temperature of pretreated ground barley grain-barley malt mixture with protease (50 mg per cent) at 42°C was gradually raised to 70°C and added to gelatinised degermed maize. ∝-amylase was added at this stage and saccharification was achieved in the normal way.

Method 3. Mashing of barley malt, barley grain and degermed maize (50:30:20) was done as follows:

Mixture of powdered grains with water and proteases (200 mg per cent) was allowed at 30°C for 30 min and the temperature was raised to 42° and maintained for 30 min before raising to 52°C. ∝-amylase was added at 52°C and allowed for 30 min and again the temperature was raised to 62°C and after allowing for 30 min raised to 70°C at which temperature it was maintained for 70 min. This was followed by boiling for 10 min.

Specific gravity, pH, total carbohydrates and ∝-amino nitrogen were determined as reported[11] earlier.

Apparent extract, real extract, original extract and ethyl alcohol were determined according to standard methods[12,13].

Results and Discussion

During germination of barley, various enzymes are formed and liberated, chief amongst them being proteinsases, amylases, β-glucanases, cellulases and pentosanases. Some of these enzymes are partially inactivated during kilning but are completely inactivated during wort boiling. The presence of β-glucanases would be advantageous to a brewer when larger percentages of adjuncts like barely grain are mashed, as otherwise lautering would be difficult. With a view to utilise these enzymes, green malt was wet ground to get an extract and was used at 20 per cent level during mashing of raw barley. The resulting wort had low ∝-amino nitrogen perhaps due to lack of enzymes; the wort had raw and husky flavour.

Experiments were, therefore, conducted to mash barley grain and barely malt mixture using fungal proteases and bacterial amylases. The characters of wort derived are given in Table 1. Controls in the different combinations of barley grain and barley malt mixtures did not receive extra microbial enzymes. Wort characters of all-malt were also included as a standard for comparison.

It is seen from the Table that the volume of the extract was generally more in the enzyme treated mixture than in the control. However, a reduction in the volume of the extract was noticed with the increase in content of barley grain. With the increased level of barley grain, β-glucan content also increases and this may be responsible for lesser yield as it is known to affect easy filtration. The colour of the wort compared well with the standard wort except in the case of wort derived by using 60 per cent barley grain. The specific gravity and the pH were practically same whether barley-malt mixture was treated with or without added enzymes. The mixture treated with microbial enzyme gave a wort with greater amount of ∝-amino nitrogen, indicating the usefulness of added proteases. Wort had normal flavour except in the mixture of barley-malt (60:40) which had slightly raw flavour. The maximum amount of barley grain that could replace barley malt was found to be 50 per cent and the wort resembled all-malt wort in colour and flavour.

The qualities of wort derived by the first two methods in the case of barley malt-barley grain-degermed maize mixture indicated no significant differences. It was, however, observed that the amount of ∝-amino nitrogen

Table 1. PHYSICO-CHEMICAL CHARACTERS OF WORT

Ratio of barley to malt	Sp gr	pH	∝-amino N (mg/lit.)	Total CHO (g %)	Volume (ml)	Colour	Flavour
20:80 with enzyme	1.047	5.5	250	8.33	600	Brownish Y	Normal
No „	1.046	5.5	185	8.30	540	„	„
30:70 with enzyme	1.043	5.5	230	8.33	600	„	„
No „	1.046	5.5	160	8.40	540	„	„
40:60 with enzyme	1.044	5.5	210	8.33	600	„	„
No „	1.047	5.5	140	8.40	510	„	„
50:50 with enzyme	1.041	5.5	175	8.33	500	„	„
No „	1.044	5.5	120	8.40	465	„	„
60:40 with enzyme	1.047	5.7	150	10.10	450	Yellowish brown	Slightly raw
No „	1.047	5.8	100	10.01	400	„	„
All malt	1.047	5.5	185	11.11	570	Brownish yellow	Normal

TABLE 2. PHYSICO-CHEMICAL CHARACTERS OF WORT

Raw material	Protease conc. (mg%)	Sp. gr	pH	Total CHO (g%)	∝-amino N (mg/lit)	Vol. (ml)	Colour	Flavour
Malt : Barley : Maize 30: 20 : 50	100	1.045	5.5	9.44	110	600	Pale yellow	Raw
	150	1.046	5.6	10.00	125	600	,,	,,
	200	1.045	5.7	9.44	150	600	,,	,,
All malt	—	1.047	5.7	9.20	151	570	Brownish yellow	Normal
Malt : Barley : Maize All 50: 30 : 20	200	1.044	6.0	11.60	175	550	,,	,,

was inadequate (90 mg/lit.) to meet the nutritional requirement of yeast during subsequent fermentation. It was possible that the amount of enzyme added was inadequate to act upon protein or it was likely that the combined enzymes (added proteases and the one present in barley malt) were not potent enough to act upon proteins of barley grain, barley malt and of degermed maize. The level of added protease was, therefore, increased and the qualities of wort derived are indicated in the Table 2.

It is evident that the desired level of ∝ -amino nitrogen was achieved by adding 200 mg of fungal proteases during mashing of barley malt-barley grain-maize combination.

Clear unhopped worts derived from (*i*) barley grain and barley malt (50:50) mixture and (*ii*) barley malt, barley grain and degermed maize (50:30:20) mixture were fermented with a strain of bottom yeast (*Saccharomyces carlsbergensis*) at 10°C and the qualities of the fermented brew are given in Table 3.

TABLE 3. ANALYSIS OF THE FERMENTED BREW

Physico-chemical characters	Raw Material: Barley grain Barley malt (50:50)	All-Malt	Barley malt Barley grain degermed maize (50:30:20)
Apparent extract	4.5	2.5	3.8
Real extract, %	4.1	4.3	4.8
Ethyl alcohol, wt. %	4.4	3.5	3.0
Original extract, %	12.6	11.1	10.65
Apparent degree of fermentation, %	64.2	97.5	64.5
Real degree of fermentation, %	67.4	61.3	55.1
pH	5.0	4.2	4.9
Colour	Normal	Normal	Normal
Saccharification	Normal	Normal	Normal

The apparent extract was more in the experimental brews than in the standard brew, contrary to the results obtained by Olesen[2]. The real extract was practically same in all the cases similar to the findings of Olesen[2]. Apparent degree of fermentation was less than that of the standard brew. Perhaps this could be corrected by incorporating suitable enzymes during fermentation which are capable of splitting non-fermentable sugars. Alcohol content was slightly more in the experimental barley grain-barley malt brew and slightly less in the one brewed along with the degermed maize as compared to the standard brew. This may be attributed to the higher original extract in the first experimental brew. The colour and flavour of the brews were practically similar. These preliminary laboratory studies indicate that cheaper raw materials could be used in brewing there by reducing the production cost as well as capital outlay. However, pilot plant trials are necessary to arrive at a definite conclusion.

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RAPID METHOD FOR DETECTION OF ADULTERATION OF TOMATO KETCHUP WITH RED PUMPKIN

The rapid thin layer chromatographic method for the detection of adulteration of tomato ketchup with red pumpkin is based on the difference in their carotenoid composition. Red pumpkin contains a pigment, tentatively identified as an esterified xanthophyll, which is absent in tomato.

Tomato ketchup is occasionally adulterated with cheaper raw materials like pumpkin, ashgourd, papaya, apple, sweet potato, etc.[1] While sweet potato in ketchup could be specifically detected by the presence of matose[2], for the other adulterants, a quantitative estimation of lycopene has been suggested[3]. The present study proposes a rapid method for detection of adulteration of tomato ketchup by red pumpkin based on the difference in their carotenoid composition.

Ripe tomato (*Lycopersicon esculentum*) and red pumpkin (*Cucurbita maxima*) brought from local market were used.

Tomato and pumpkin carotenoids were extracted by the method of Tomes.[4] The extract was filtered, dried over anhydrous sodium sulphate, evaporated to dryness in vacuum and redissolved in hexane. This was applied as a thin band on. 5 mm thick, activated silica gel G plates. The plates were developed with 5 per cent acetone in hexane solvent at 25°C. The colour and Rf of the separated bands were recorded.

Tomato ketchup was prepared by concentrating the fresh tomato juice in an open jacketted kettle followed by addition of sugar, salt and vinegar so as to adjust the total soluble solids to approximately 32-35 per cent.

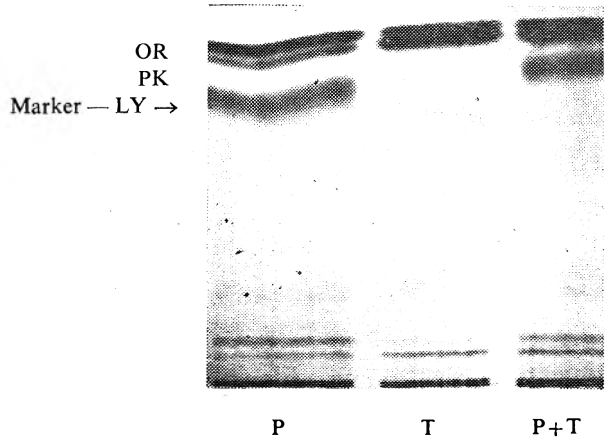

Fig. 1. Thin layer chromatographic separation profile of carotenoids extracted from fresh pumpkin and fresh tomatoes. P = Pumpkin carotenoids; T = tomato carotenoids; P + T = Pumpkin and tomato carotenoids; OR = Orange colour; PK = Pink colour; LY = Lemon yellow colour.

For the preparation of adulterated ketchups, red pumpkin pulp was made by macerating the pieces with 10 per cent water in a waring blender. This pulp was boiled for 10 min to inactivate the enzymes and reduce the flavour and then mixed with the tomato ketchup in concentrations of 10, 40, 50 and 60 per cent of the weight of the final product. Clove, onion, garlic, pepper and cinnamon were added to the control and adulterated samples to impart desirable flavour. 500 ppm of sodium benzoate was added as preservative, filled hot into 250 ml bottles, corked, dipped in molten wax and cooled. The bottles were stored at ambient temperature. It was observed that homogenised red pumpkin pulp could be added upto a level of 40 per cent of the weight of the ketchup without appreciably affecting the colour. Red pumpkin, besides contributing to the colour also improves the viscosity of the ketchup. The mild flavour of the pumpkin is masked by the vinegar and spices added to the ketchup.

Preliminary studies on the carotenoid composition of fresh tomato and red pumpkin by TLC indicated to differ with respect to atleast on pigment of pumpkin which was absent in tomato. This qualitative difference in the composition of the carotenoids has been explored to detect the possible adulteration of tomato ketchup with red pumpkin.

The prepared samples of control and adulterated ketchups were stored for 0, 1, 2 and 3 months and analysed for carotenoid profile by TLC. In the case of stored samples, considerably more pigment loading was

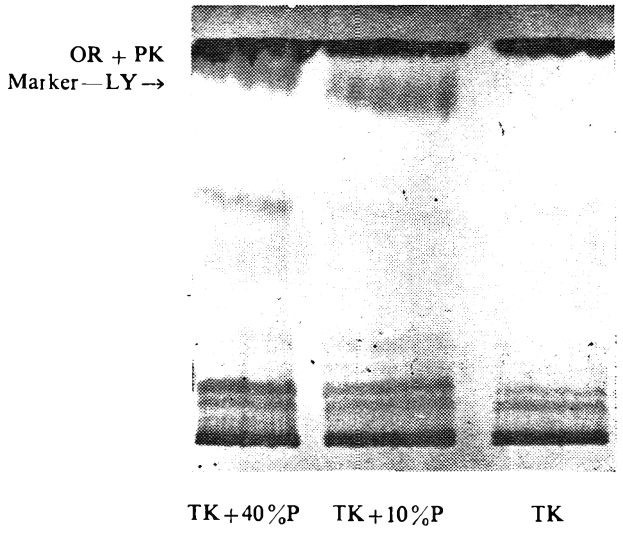

Fig. 2. Thin layer chromatographic separation profile of carotenoids extracts from three month old sample of pure and adulterated tomato ketchups.
TK = tomato ketchup; TK+10% P = Tomato ketchup adulterated with 10% pumpkin; TK+40% P = Tomato ketchup adulterated with 40% pumpkin; OR + PK = Orange + pink colour.

necessary for the separation of marker probably due to partial loss of pigments. The carotenoid marker of pumpkin was visible even after 3 months of storage and separates as a band in the vicinity of Rf=0.78-0.80 (Fig. 2). The appearance of this band on silica gel TLC is a clear indication of the adulteration of tomato ketchup with red pumpkin. This marker has a distinct lemon yellow colour and differs from the nearest bands of carotenes (orange) and lycopene (pink red) which appear as merged or individual bands between a Rf of 0.95 and 0.99 (Fig 1 & 2). The presence of lycopene and carotenes have already been well established in tomatoes[5].

This marker carotenoid band was scrapped off from TLC plates, eluted with acetone: hexane (5:95), evaporated under vacuum, and redissolved in hexane. The unsaponified pigment was completely epiphasic in hexane and had a decreased affinity on silica gel TLC plates. These characteristics indicated the pigment to be esterified a xanthophyll[6,7]. After saponification, partitioning of the pigment in different concentrations of methanol was:

H:95 per cent Me-18:82; H:85 per cent Me-45:55; H:80 per cent Me-70:30; H:75 per cent Me-95:5; H:70 per cent Me-100:0 (H:Hexane, Me:Methanol). These results indicate the esterified nature of the pigment. The pigment had absorption maxima at 423, 451 and 475 nm. From a comparison of the partition coefficient data[8] and spectral properties, the pigment appeared to be an esterified xanthophyll containing one OH group.

The TLC method described above could be adopted to detect the adulteration of the tomato ketchup by red pumpkin. The procedure is simple, convenient and the time required is 2 to 3 hr.

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EFFECT OF DANSYL CHLORIDE ON THE RENNET SUSCEPTIBILITY OF THE CASEIN MICELLES

Casein micelles isolated from buffalo and cow milk on treatment with DNS-Cl (at 2% level) exhibited reduced rennet susceptibility. Results indicate that DNS-Cl treatment had more drastic effect on the bovine micelles. The rate of release of sialic acid, NPN and GMP by rennet was less compared to the buffalo micelles. The turbidity formation by rennet was also considerably diminished in the bovine micelles due to dansylation. However, DNS-Cl treatment did not alter the electrophoretic patterns of the casein micelles. Dansylated micelles in acetone medium on chromatography through Sephadex G-100 gel, resolved into two prominent protein peaks whereas in isopropanol media, micelles eluted as a single peak.

Ashoor[1] has investigated the internal structure of the casein micelles by treatment with dansyl chloride (1-dimethylamino-naphthalene-5-sulphonyl chloride). Treatment of micelles with dansyl chloride (DNS-Cl) led to a rapid labelling of all three main casein fractions, suggesting their uniform distribution throughout the micelles.[1,2] In addition, DNS-Cl has been found to be a very effective reagent for functional group analysis of casein micelles and casein fractions. Ashoor *etal.*[3] have indicated that dansylation of the functional groups of *k*-casein has drastic effect on its stabilizing power towards α s-and β-casein. However, Hill and Cracker[4] have reported that stabilizing ability of *k*-casein decreased only 15 per cent after treatment with 6 per cent DNS-Cl. Hill and Cracker[4], and Ribadeau-Dumas and Garnier[2] have also observed that dansylating 2-3 lysyl residues in *k*-casein prevented clot formation after the action of rennin. Such observations are, however, lacking with respect to the micellar form of casein. Hence, in the present investigation an attempt has been made to examine the effects of dansylation on the casein micelles from cow and buffalo milk, in relation to its susceptibility to rennet action.

Composite milk samples were collected from *Murrah* breed of buffalo and three breeds of cow, namely, *Sahiwal*, *Red Sindhi* and *Tharparkar* maintained at the Institute's herd. Milk was skimmed at $1000 \times g$ in a Alfa-Laval cream separator. The skimmed milk thus obtained was used for the isolation of casein micelles

by centrifuging at 105,00 $\times g$ in a Beckman Model L preparative ultracentrifuge following the procedure of Gupta and Ganguli.[5]

The casein micelles were treated with DNS-Cl in the following manner: 250 mg dry casein micelles were dispersed in 10.0 ml $NaHCO_3$ solution (0.1 M). 5.0 ml acetone containing 5.0 mg DNS-Cl was added. The control sample contained all components as the experimental sample but without DNS-Cl. All the samples were incubated for one hour at room temperature (30°C) with frequent stirring. After one hour, a little quantity of (3 to 4 ml) 0.1 N NaOH was added to solubilize the micelle suspension. In case of cow micelles, the NaOH addition was not required since the addition of acetone to the micelle suspension in $NaHCO_3$ brought it to an almost clear solution, a characteristic observation in contrast to the buffalo micelles. The pH of the casein micelle solution was brought to 6.5 with 1N HCl and the final volume was made up to 25.0 ml with maleate buffer (0.2 M, pH 6.5) in each case. Rennet action was assessed by evaluating the release of sialic acid, non-protein nitrogen, and glycomacropeptides and also by the turbidity formation, according the procedures described earlier.[6]

For starch gel electrophoresis, the DNS-Cl treated casein micelle solution was dissolved in veronal buffer pH 8.6, containing 7M urea. The Petri-dish technique for starch gel electrophoresis devised by Ganguli and Majumder[7] was used. For the assessment of gel filtration pattern, the DNS-Cl treated casein micelle solution was first dialysed against 0.2M KCl solution for 36 hr to remove excess DNS-Cl and reaction product (DNS-OH). In some of the experiments, isopropanol was used instead of acetone to serve as the vehicle for

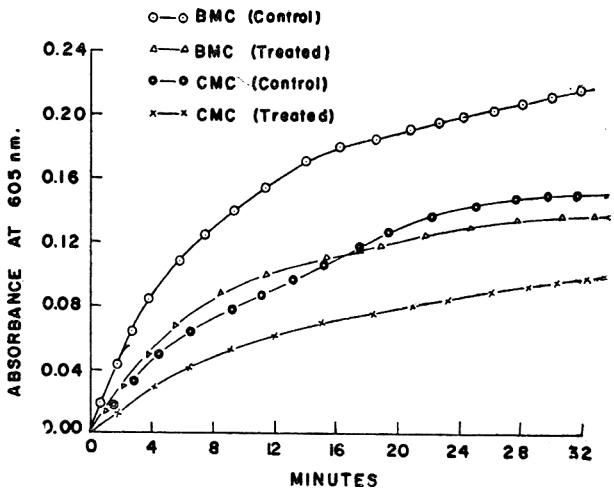

Fig. 1. Rate of turbidity development in casein micelles by rennet before and after DNS-Cl treatment.

DNS-Cl. These were then passed through the Sephadex G-100 (Pharmacia) gel column (45 × 2.6 cm) according to the method of Yaguchi and Tarassuk[8] using 0.02 M sodium phosphate buffer (pH 8.6) containing 6M urea. The effluents were continuously monitored by means of a Lkb-Uvicord at 254 nm.

The effect of dansylation of the casein micelles towards rennet susceptibility was studied by treating the casein micelles with DNS-Cl at 2 per cent level. The results shown in Table 1 on the release of sialic acid, non-protein nitrogen (NPN) and glycomacropeptides (GMP) by rennet from the DNS-Cl treated casein micelles of buffalo and cow milk clearly indicate that the rate of release of these components by rennet was significantly diminished ($P < 0.01$) in both buffalo and cow casein micelles as a result of DNS-Cl treatment. The average

TABLE 1. RENNET SUSCEPTIBILITY OF CASEIN MICELLES AFTER TREATMENT WITH DANSYL CHLORIDE

Source of casein micelles	Treatment	Release of components from micelles*		
		Sialic acid (%)	Non-protein N (% of total N_2	Glycomacropeptide (Klett reading)
Buffalo Milk	Control	68.59 (66.86–70.02)	0.926 (0.880–0.970)	32 (30–35)
	DNS-Cl**	62.27 (60.78–66.00)	0.751 (0.705–0.783	25 (24–27)
Cow Milk	Control	76.00 (75.00–77.00)	1.540 (1 362–1.801	133 (124–138)
	DNS-Cl**	67.76 (65.00–70.00)	1.200 (1.022–1.460	122 (112–127)

Figures in parentheses indicate range of variation

*The number of samples analysed in each case was five

**The samples were treated at 2% level of DNS-Gl.

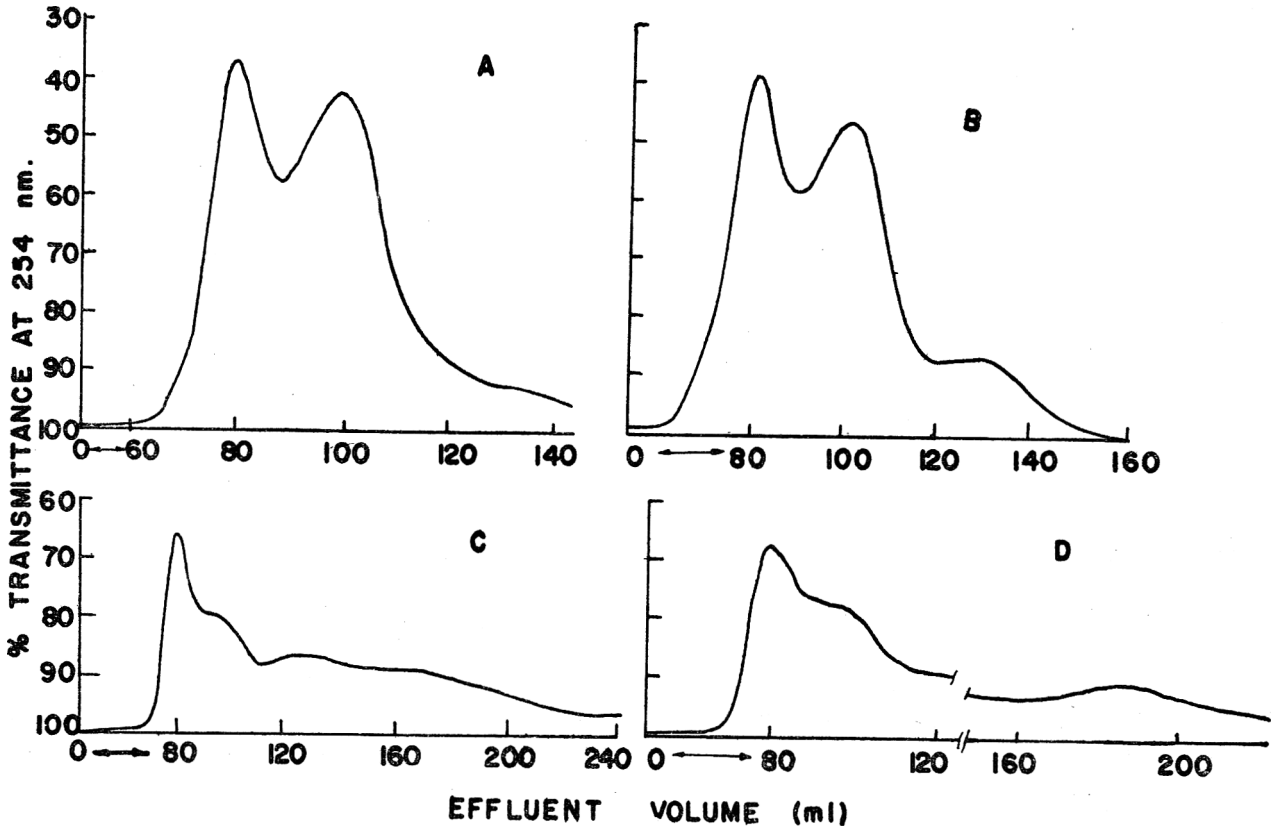

Fig. 2. Effect of DNS-Cl treatment on the gel filtration pattern of the casein micelles (acetone medium) A, B – DNS-Cl treated buffalo and cow micelles; C, D – control buffalo and cow micelles.

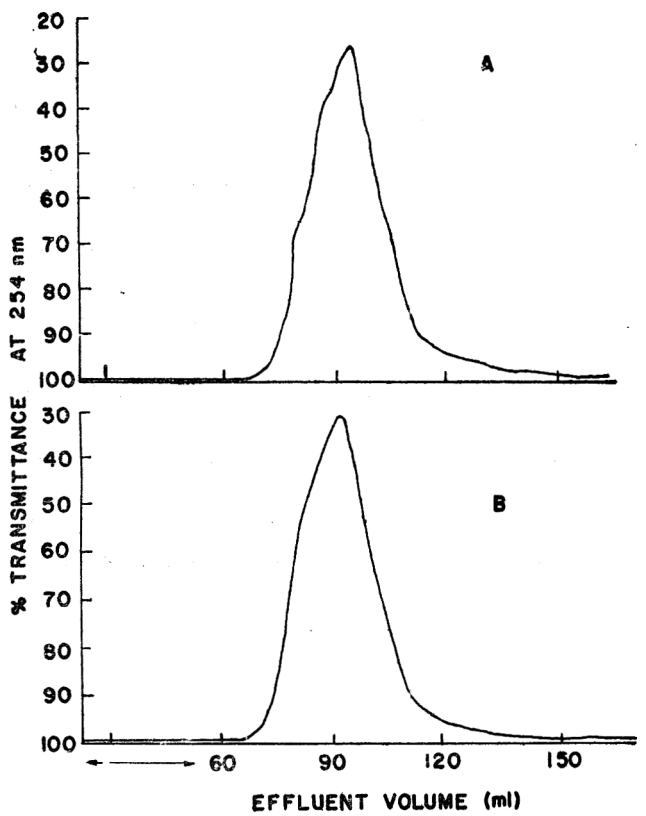

Fig. 3. Same as in fig. 2 in isopropanal medium. A & B refer to DNS-Cl treated buffalo and cow micelles, respectively.

release of sialic acid from DNS-Cl treated buffalo casein micelles was 62.27 per cent as against 68.59 per cent from the untreated (control) buffalo micelles. In bovine micelles, these values were 67.76 and 76.00 per cent from treated and control samples, respectively. The average values for released NPN (as per cent of total N) were 0.751 and 1.200 as against 0.926 and 1.540 from treated and untreated buffalo and cow casein micelles, respectively. The average values (Klett readings) for released GMP from DNS-Cl treated samples were 25 and 122 as against 32 and 133 from untreated samples of buffalo and cow casein micelles. Statistical analysis indicates that the DNS-Cl treatment has significantly greater ($P < 0.01$) drastic effect on the cow casein micelles than the buffalo casein micelles. The rate of turbidity formation by rennet was significantly lowered in both buffalo and cow casein micelles, as a result of DNS-Cl treatment (Fig. 1.) The cow casein however, exhibited much lower rate of turbidity formation than the buffalo casein micelles. Electrophoretic patterns of the casein micelles treated with DNS-Cl at 2 per cent did not reveal any change. Electrophoretic patterns as well as electrophoretic mobilities of the treated casein micelles were the same as controls.

Gel filtration of DNS-Cl treated casein micelles gave certain interesting results. When acetone was used as carrier for DNS-Cl, considerable interference was

recorded in the elution pattern, since DNS-Cl as well as acetone absorb strong U.V. light in the same regions as those of proteins. Hence, treated samples were dialysed exhaustively against 0.1 M KCl solution to remove excess DNS-Cl and its reaction product (DNS-OH) before passing through the gel column. The molecular sieve chromatograms thus obtained (Fig. 2A and B) illustrate that both buffalo and cow casein micelles resolved into two prominent protein peaks. An additional small peak was also observed towards the terminating edge of the second peak in case of cow micelles. Since the casein micelles after treatment with DNS-Cl in acetone were dialysed against 0.1 M KCl solution, there was possibility that some of the calcium was released from the micelles and thus diffused out of the dialysis tubing. Hence, for comparative assessment, the casein micelles were treated in similar manner, but without any DNS-Cl and dialysed against 0.1 M KCl and passed through the gel column exactly in the same manner as for the treated micelles. The molecular sieve chromatograms (Fig. 2C and D) for both buffalo and cow casein micelles, exhibited one main protein peak and a shoulder appearing with a small peak and considerable trailing of the terminal edge. It, therefore, seems likely that the reason for the emergence of the second peak in DNS-Cl treated micelles may be partly due to the release of calcium as a result of dialysis.

In another experiment, where isopropanol was used as a vehicle for DNS-Cl instead of acetone, the casein micelles eluted as a single peak in both buffalo and cow casein micelles (Fig. 3). However, it was interesting to note that a considerable heightening of the peak resulted in this case. The dialysis was omitted in this experiment, since isopropanol does not absorb U.V. light and DNS-Cl eluted out much later.

The drastic effect of dansylation of functional groups of k-casein on its stability and stabilizing power as reported by other workers[2,3,4] may be responsible for the decrease in the rate of release of sialic acid, NPN and GMP as observed in the present investigation, since all these components originate from the k-casein of the casein micelles. A significant reduction in the rate of turbidity formation in dansylated casein micelles by rennet indicates further that the secondary phase of rennet action, which is purely a physical phenomenon, is also adversely affected.

Abolishing the charge on the ϵ-amino groups of a limited of number lysine residues by dansylation[3,9] apparently was not sufficient to affect the electrophoretic mobility of the casein components. It has also been shown by starch gel electrophoresis[2] that all the caseins bind DNS-Cl even at low concentration of the reagent. There was no preferential binding to any of the three main casein sub-units.

It has been reported that DNS-Cl increases the hydrophobicity of the casein fractions[3,10] which may cause aggregation of the micelles. The erratic gel filtration behaviour of the casein micelles obtained in the two different assay systems may be due to differences in the effects exerted by these two systems, since the DNS-Cl in acetone has been found to be more effective than in isopropanol.[4]

The authors express their sincere thanks to Dr. D. Sundaresan, Director, National Dairy Research Institute, Karnal, for his continued interest in this study. One of us (MPG) is also grateful to the Indian Council of Agricultural Research, New Delhi, for the award of Senior Research Fellowship during the tenure of the present investigation.

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BOOK REVIEWS

Radiation Chemistry of Major Food Components: Eds. P. S. Elias and A. J. Cohen, Elsevier Publishing Co., Amsterdam, 1977, pp. xii+220.

The effects of radiation at various energy levels on individual chemicals occurring in foods as well as on the major food components and eventually on combinations of foods have been studied fro many years. Many factors apart from the nature of the food, such as irradiation temperature, atmosphere, medium, dose and dose rate, post-irradiation conditions and methods of analysis, will influence the type and extent of chemical changes which may occur as a result of the irradiation of food. The collection of detailed quantitative data for each situation would be impractical and is also unnecessary, since an understanding of the general mechanisms by which these changes may arise, as gained from studies with individual food components and information on the influence of different parameters on these changes, should suffice in the extrapolation of results from one situation to another.

When ionising radiation is absorbed by a product irradiated, a certain number, depending on the radiation energy deposited, of primary reactive species—Solvated electrons, hydrogen and hydroxyl ions, etc.—are generated. Upon reaction with matter, these primary radicals give rise to secondary radicals which are eventually stabilized in the form of reaction products. Reactions involving macromolecules are detectable at very low dose levels while reactions involving smaller molecules require substantially higher dose levels in order to be detected. The combination of the important role of DNA and its size account for the lethal effects of low doses of radiation on living organisms; this fortunate circumstance therefore enables the destruction of undesirable spoilage micro-organisms in an almost selective manner and without any great changes in the food. Thus, the absorption of energy in the irradiation process for foods is approximately 11 Kcals/g for sterilization or radappertization dose and the amount of radiolytic products formed is also correspondingly small; e.g., about 700 ppb. in beef radappertized at -40°C, 4.5 Mard dose. It is frequently not realized that the nature of the decomposition products formed during irradiation and by heat treatment are mostly similar, although, however, far more of these have been identified in thermally processed foods than in irradiated foods.

This book presents a critical review of the radiation chemistry of carbohydrates, lipids, proteins and vitamins, with emphasis on the nature and concentration of the radiolytic products formed. In general, each review compares the response of the food component following irradiation in the dry state, in aqueous solution, in model systems, in composite mixtures and in whole food products. The various reviews provide evidence of the great similarity in radiolytic products in related foods treated with radiation doses up to the megarad range. The reactions of the food constituents to radiation display a remarkable uniformity. Most of the radiolytic products identified in irradiated foods are also those generated in foods by other conventional processing techniques. Their concentrations are confined to ppm ranges with radiation doses normally employed and these concentrations fall off considerably as the dose ranges are reduced. The available data on the chemical structure of the radiolytic products in foods and the very low concentrations in which they have been detected suggest that they pose negligible health hazards.

Radiation preservation of foods has been extensively studied for over two decades now and there exists a voluminous amount of literature on the wholesomeness of irradiated foods from the nutritional, microbiological and toxicological angles. On the basis of all available information, various national and international agencies have cleared a large number of irradiated foods for human consumption. Notwithstanding the efforts that have been expended on the development of this technology, practical applications have been extremely limited to date. The concern about the hazards of radiation generally is appraently reflected in the prevailing apprehensions over irradiated foods. Insistance in some quarters on additional methods of safety evaluation which are obiously both unrealistic and unsound especially in the context of present-day environmental problems has also to some extent been hampering progress. Reassurances of the kind presented in these reviews as to the safety of the process of food irradiation from the point of view of the radiolytic products formed should help to achieve general acceptance of the process. Major hurdles that need to be convincingly cleared relate to demonstration of techno-economic feasibility, intra–and inter-country legislative aspects and, importantly, consumer attitudes and resistance.

A. SREENIVASAN
BOMBAY

Sweetners and Enhancers 1977: by Nicholas D. Pintauro, Published by Noyes Data Corporation, Park Ridge, New Jersey 07656: pp. 390; Price: $ 39.

The Noyes Data Corporation publishes almost every year patent data filed in U.S.A., in specialised areas of food technology. Current controversies regarding the use of cyclamates and saccharin as sweetners in various foods have particularly stimulated search for new natural and synthetic sweetners. The present book on "Sweetners and Enhancers", which deals with several hundred U.S. Patents is timely. The diversity of compounds is illustrated as proteins like Monellin, Miraculin, various aspartyl peptides, dihydrochalcones, tryptophan derivatives, substituted tetrazoles etc., are covered. Methods of preparation of many of these compounds and their use in specific formulations are also given in these patents. In addition almost forty pages are devoted to various saccharin combinations and special formulations. Processes for increasing the bulk of mixes containing sweetners, preparation of drinks, jellies, desserts, baked goods, with artificial sweetners are also given. A very extensive field is covered in these patents.

The usefulness of patent information either in the actual manufacturing process or as a starting point for further investigation is debatable and it is interesting to note what the different view points are in this area (P. Sperber in Chem. Tech., I. 225 (1977)).

The present book on U.S. Patents dealing with sweetners and enhancers will be mostly useful to R & D Organisations in food industries and research institutions involved in food science and technology.

D. RAJAGOPAL RAO
C.F.T.R.I., MYSORE

Recipe and Process Development for Canners: by Maxwell Philip, Food Trade Press, England, 1976, pp. 163; Price £ 9.

The book is covered in four sections, namely (*i*) developing the product, (*ii*) developing the process, (*iii*) quality control of the process and (*iv*) cost control of the production process. Brief summaries are given at the end of each section.

The first section dealing with the development of a product lays stress on formulating a correct recipe. A product containing various ingredients, like thickeners, meat and vegetables is more complex and requires careful control. With this in view, typical examples are given for the recipe, preparation and processing of products, such as pork luncheon meat, spaghetti in cheese and tomato sauce, tomato soup, etc. Containers (cans) required for such products and choice of enamels for cans are briefly covered. The section also deals with the essentials of Food laws of United Kingdom which the canner should be aware of, particularly those relating to labelling, preservatives, colouring matter, canned meat regulations, emusifiers and stabilizers, limits of mineral contaminants, etc. Thermal process evaluation, which is a must for canners, is covered briefly.

The second section on developing the process relates to the transfer from laboratory scale to the factory scale, which involves several unit operations to be performed in food canning, like ingredient preparation and inspection, blanching, mixing and batching of ingredients, emulsification, heat transfer, pumping, filling, can seaming, product sterilization, assessment of quality standards, yield assessment, hygiene of the plant and avoidance of waste, etc. Emphasis is laid on technical principles underlying some typical operations rather than on surveying various types of equipment used in food canning.

The third section deals with quality control of the process. In the control of raw material quality of filled weight, testing and inspection of the finished product and product hygiene, the statistical methods given will certainly be of help irrespective of the size of the factory.

The last section deals with cost control of the production process. Budgetary control and standard costing systems, though recognised as being aids to economy in manufacturing process, may not be applicable in small canneries. The subject matter is dealt under production budget, production personnel budget, standard costing systems, etc.

On page 80, the percentage overlap for a good seam is mentioned as 45 per cent (minimum) which is according to the recommendation of the Metal Box Company. However, Hersom and Hulland (Ref. *Canned Foods*, 6th Edition (1969), p. 295) point out that it is more advantageous to set the minimum overlap as 55 per cent. This aspect may be given some thought, since the recipe products indicated are of the low acid type. Formula for calculating the per cent overlap and a table giving common seam defects and their diagonsis should have also been included.

The get up of the book is good and it contains a lot of information for the canner of the type of products indicated. However, the price of the book is on the high side.

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

Theileriosis: report of workshop held in Nairobi, Kenya, 7-9 December 1976, by Henson J. B. and Compbell, M., Ottawa, IDRC, 1977, pp. 112.

"Theileriosis" is a compilation of papers presented in a workshop held at Nairobi, Kenya during 7th to 9th Dec. 1976 on the topic. Dr. J. B. Henson, Director, International Laboratory for Research on Animal Diseases has taken great pains to edit the title in collaboration with Marilyn Compbell, senior Technical Editor, International Development Research Centre, to arrange the contents in a manner so that the attention of the readers is directed towards the importance of the problem.

Theileriosis, a protozoan disease caused by several members of the genus Theileria such as *T. parva, T. lawrencei, T. annulata* and *T. mutans* spread through ticks. The disease has assumed international importance and is prevalent in several countries. At persent, it has gained foot hold in India, fifteen African and seven Middle east countries affecting their cattle population. Recent studies have shown that wild-life and buffaloes act as a reservoir for this disease and unless an effective control is planned to prevent the malady it might bring about a set back to the livestock production programme envisaged by most of the developing countries. Therefore, it is quite appropriate to have a compilation on this subject at this critical juncture.

The contents of the title are divided into reports presented by various workers about the status of the problem in different countries, views of specialists and Institutions on the issue and recommendations from four different committees with specific guidelines on the subject.

It is a nice attempt to focus global attention on this important disease complex, to bring in more international coordination in handling research programmes, relating to the area.

P. C. PANDA
HARYANA AGRICULTURAL UNIVERSITY, HISSAR

1975 *Evaluations of Some Pesticide Residues in Food:* WHO Pesticide Review Series No. 5, World Health Organisation, Geneva· 1976, pp. viii+409.

This volume is a report of the joint meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residue held in Geneva in Nov-Dec. 1975. It includes monographs on thirty-seven pesticides, out of which four are new. Only those information that have become available in recent years are included on conventional pesticides, but reference is made to earlier publications which could be consulted by readers who wish to obtain a complete evaluation of the compound.

Comprehensive treatment is given on newer pesticides including their chemical and physical properties, use pattern, acute and chronic toxicity, methods of residue analysis and levels of residues in agricultural and food products. Information is also presented on the fate of residues in experimental animals and in food products. Based on the information available to the committee, some of which were unpublished, recommendations have been made for the tolerance limit of residues in different types of foods. Specific recommendations have also been made on the type of additional data that are required and desirable in evaluating these compounds for extensive use.

Amongst the new compounds, Bioresmethrin, a synthetic pyrethroid is one of the most effective broad spectrum contact insecticide currently available. Because of its long (8 to 20 week) biological-half life under storage conditions it shows good promise of becoming a major insecticide for use in the control of stored product pests. It is not only 16 times more effective than pyrethrins, but also effective against several insect species resistant to other insecticides No tolerance limits have yet been established and the recommended rate of application to raw cereals is 1 to 4 mg/kg

Sec-butylamine has been shown to have good fungicidal and fungistatic properties, which make it suitable for several fruits including those of citrus family. Maximum residue limits vary from 50 mg/kg for dried citrus pulp to 0.5 mg/kg in citrus juice.

The remaining two new insecticides are chlorpyrifos-methyl and Cynofenophos and both are organophosphorus compounds with broad spectrum activity. Dosage rates of Chloropyrifos-methyl vary from 0.1 to 2.0 mg/kg under field conditions and from 2.5 to 10 mg/kg in grain storage, and its maximum residue limits vary from 20 mg/kg on bran to as low as 0.01 mg/kg in milk.

Cynofenophos, is reported to be moderately persistent and therefore, offers a possible alternative to persistent organochlorine compounds. Temporary maximum residue limits vary from 2 mg/kg on cabbage to 0.95 mg/kg on commodities like cucumber, ginger and onions.

Annexure I includes the recommendations concerning acceptable daily intakes and residue limits of all the pesticides reported in the volume. These recommendations are additional, or ammendements to those recorded in earlier reports and thus provide a ready source of reference.

Except for a few typographical errors and repeated

mention of various committees, I feel that WHO Pesticide Series No 5 is valuable addition to the library.

PREM LATA BHATNAGAR-THOMAS
B.A.R.C., BOMBAY

Rheology and Texture in Food Quality: Ed. by J. M. DeMan, P. W. Voisey, V. F. Rasper and D. W. Stanley; The AVI Publishing Company, Inc., Westport, Conn; 1976; pp. 588.

The decade of 1960 saw the understanding of texture as a comprehensive primary food property and the decade of 1970 is seeing the development of defining the subjective and objective aspects of food texture. As a consequence a distinct area of scientific endeavour with its own journal, symposia-seminars, and committee efforts at a standardisation of definitions and conditions of measurements, have emerged. Rapid developments in a number of concerned areas as basics of rheology, instrumental measurement of mechanical properties, psychophysical, measurements of sensory textural properties make discussion and review of the developments very important. Though not expressly stated the book under review appears to have been developed out of the presentations at the University of Guelph symposium on 'Advances in Food Texture' in 1974. It is a welcome addition to the recent books on texture and rheology of foods.

The chapters, on the rational development of instruments to measure individual or simpler combinations of effects in the complex changes perceived as food texture, developments of total texture test systems and choice. These will be much appreciated by the students and technologists for whom the book is intended. A chapter on 'Interpretation of force curves from instrumental texture measurements', is particularly welcome for emphasis on first establishing correct conditions of obtaining force-time/distance curves and then an exposition of the complexity of the curves, obtained with different foods. The importance of visual observations of the foods under stress, to determine the changes occurring to validly interpret the different parts of the texture profile curves and study of varied interpretations for correlation with sensorily perceived texture is emphasised. Two general chapters on basic concepts on mechanical behaviour of foods and relation between microstructure and texture of foods are written crisply and clearly for giving confidence to the student and novice researcher.

Other chapters that follow deal with different classes of foods such as fruits and vegetables; dough, pasta and baked products; fats and fat products; dairy products, meat and chocolate. Pleasing features of these chapters are the comprehensive treatments from the structural, physiological changes on storage and processing and their effect on texture, appropriate instruments extensively used for the individual types of foods and good electron micrographs to illustrate microstructure and textural changes. The behaviour of natural and modified starches and emulsions as food additives are discussed in three chapters expertly, with very good illustrations.

A chapter briefly outlining the spinning and extrusion process for 'Engineering' for food texture, appears odd in this collection. Two rather brief chapters deal with sensory measutement of food texture and texture profile which do not give guidance on how to choose the best and efficient methods or the difficulties of conducting the profile method. The last chapter is an excellent research paper on psychophysical relations in texture which recommends the newer applications of magnitude estimation and multidimensional analysis.

Each chapter has an excellent selection of bibliography for further reading and the book has a good subject index. The book as is usual with AVI, is excellently produced.

The reviewer congratulates the editors on assembling an expert group of reviewers to cover the different aspects of this complex new field. He would, however, like to point out that adequate justice has not been done on the word 'quality' in the title of the book. This could be done only through a more extensive coverage of sensory texture evaluation of different foods and their correlation to the objective measurements in the different chapters dealing with individual foods. This would have emphasised the great need for much more intense work on defining denotative terms, mathematical procedures for relating the rheological measurements and the sensory texture of the stimuli as shown in the thematic beginning chapter, 'How far can studies of food texture be scientific'.

A few points for consideration of the editors in future editions are: While on page 187 the efficacy of Armour tenderometer in predicting cooked meat tenderness is reported not very good, on page 420 it is reported to correlate well. Figs 6.8 and 18.1 and 6.11 and 18.2 are unnecessarily repeated and could be omitted in chapter 18. Is there a semantic problem in the titles of the chapters 'Starch Texture', 'Rheology of chocolate', 'Textural characteristics of Food Emulsions'?

V. S. GOVINDARAJAN
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Evaluation of Proteins for Humans: Ed. C. E. Boodwell; AVI Publishing Company Inc., Westport, Connecticut USA.

The proceedings of a symposium on "Evaluation of proteins for humans" sponsored by the nutrition division of IFT are published with a view to update the current information on proteins in human feeding.

In the introductory section Scrimshaw and Young succintly highlight the need for expanding food and protein production, the importance of nutritional evaluation and the lacuna in the present knowledge on the precise human energy and protein requirements. They have emphasised that there is a special need for studies on determination of protein requirements in various age groups in populations and under conditions relevant to developing countries. The effects of amino acid disproportions on modifying the utilization of dietary protein must be taken into consideration in relation to evaluation of protein quality. The urgent need to explore and devise *functional* measures of protein nutritional status in long term metabolic studies in human subjects is rightly emphasised (page 48).

In Section II the methodological aspects of protein quality evaluation have been elaborated. These include the animal bioassays,. *in vitro* tests, nitrogen balance studies in humans and other proposed biochemical indices. *In vitro* indices such as chemical score based on amino acid composition, available lysine test by carpenter's method or modification microbiological methods using and dye binding techniques etc. at best provide an approximate idea of the nutritional quality of protein but are subject to several limitations such as the unreliability of such *in vitro* tests to reveal differences in biological availability of amino acids and the lack of a single chemical procedure for predicting protein quality for all proteins specially in processed foods. A critical review of the conventional animal assay procedures such as PER, B and NPR is given by Simmonds and Hegsted. They have proposed a modified slope assay ratio (relative protein value) for proteins and stress that the proposed RPV tests protein showed less inter laboratory variation unlike in other methods. Miller and Lachance however in their review of techniques in rat bioassays indicate that the RPV method is relatively new and more complete and specific protocols seem necessary to improve its reproducibility (Chapter 7, page 156). Bresoani (Chapter 5) has compared the relationship between human and animal protein quality assay and points out that the agreement between human and rat assays can be good especially when the assay technique used is similar. Several biochemical indices for protein quality are listed and reviewed by Bodwell. Studies on changes in postprandial plasma urea nitrogen as a possible index of protein quality appears to have potential as a short term and less expensive test.

Jansen has briefly reviewed the conflicting literature on possible beneficial effects on fortification of cereals with lysine and notes (page 182) that the practical value of lysine fortification in human nutrition has not yet been conclusively demonstrated.

Breasanni (Chapter 10, p. 230) notes that for developing countries where the availability of protein sources (such as animal proteins) is relatively low and socio-economical conditions and food distribution are far from ideal, better nutrition may be accomplished by promoting *planned* production of legumes and cereals. Proper mixtures of the cereals and pulses could provide the required protein nutritive.

Chemical changes in food proteins processing and possibilities of chemical modification of protein for producing texturized proteins, effects of different types of processing on the bioavailability of the limiting and the non-limiting amino acids in a protein, have also been discussed (Chapter 11 and 12). In particular the nephrotoxic effects of lysinoalanine (LAL) has been discussed at length and the authors based on their data conclude that free LAL and not protein bound LAL impart Nephrotoxic properties. Further, the possibility that peptide linked LAL in small protein fragments may be nephrotoxic and that such renal changes may be species specific need to be further investigated.

A brief and succint review by Liener on protease inhibitors and hemagglutinins in legumes is followed by a chapter (Martine) on other antinutritional factors such as gossypol (cotton seed) glucosinolates (in seeds of mustard family) and phytates in cereals etc. Further research on the effect of phytates and phytic acid on trace mineral availability and development of appropriate methods of extraction and assay of phytates are suggested.

In essence this volume provides an excellent summary of human protein nutrition aspects. This book is a welcome addition to the library of nutritionists/ food technologists.

P. B. RAMA RAO
C.F.T.R.I., MYSORE

Sausage Products Technology: by Endel Karmas, Noyes Data Corporation Park Ridge, New Jersey, USA, 1977, pp 316, price 39 U.S. $.

The book is a collection of U.S.Patents on the topic interspaced with discussions. The volume is divided

into two main parts—I. Emulsion Ingredients, II. Production methods. The first part includes emulsion additives from the view point of colour and flavour development and stability of emulsion and ingredient control. The second part deals with all aspects of sausage processing. The chapter on emulsion ingredients has been dealt with under four sub-headings—accelerated development of colour and its stability, flavouring and colouring additives, prevention of rancid flavour and fermented flavours. Discussion of patents covering the addition of soluble calcium salts, organic acids, nitric oxide gas, cell free bacterial cultures, coated turmeric acid, treatment of casings with ascorbic acid, sorbitol and many more. Particular mention could be made of use of nicotinic acid derivatives to reduce the level of NO_2 in the emulsion. Use of cured blood pigment, oleoresins from spices, whole mustard....is also catalogued.

Use of antioxidants to prevent rancidity and production of fermented sausages are also discussed.

The second section deals with improving the texture, flavour and stability of sausages. Texturizing agents like citrus peel meal, casein calcium phosphate gels, calcium casine modified skim milk powder, alkali treated milk, milk powder and enzymes, methyl cellulose, heat treated mustard powder. The use of mustard powder reduces the incidence of Jelley pockets and also the mucilagenous principles of mustard facilitate peeling operation. Bovine blood plasma concentrate and salt soluble protiens are also discussed. Beneficial effects of magnesium ions, polymeric phosphates, mixture of phosphates, carbonates, organic acids, alkali, and trivalent metal phosphates are reported.

The chapter on proteinaceous ingredients deals with separation of lean meat and fat, low temperature rendering of fat and separation of meat from bones.

The chapter on product control methods emphasises the importance of quality control of ingredients and the final product. To achieve this end, composition control, testing the emulsion stability, rapid method of estimation of fat, moisture and protein, colorimetic separation of lean fat, the use of tracers like maltol, EDTA, souble tin salts have been discussed.

In Part II Chapters on emulsion processing; stuffing, linking and forming methods; cooking and related methods; casing removal methods and various modified products are dealt with. The Chapter on stuffiing, linking, forming methods deals with many patents on machinery to improve the production of uniform quality product and uniform average wt. per link.

In the Chapter on cooking and related methods the functions of cooking have been described and patents concerning different methods of cooking are given.

Next Chapter is on casing removal methods. The two aspects-casing releasing agents—casing removal and related machinery are dealt with separately.

The last Chapter on modified products deals with collagen encased fresh sausages, cooked sausages which could be reheated in 5-8 min, prior to consumption. Manufacture of annualar sausages, ribbon sausages filled frankfurters and multicoloured sausages have also been covered.

Company index, inventor index, U. S. Patent number index provided at the end facilitate the selection of the specific topic.

It is a useful book to those engaged in manufacture of comminuted meat products.

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

ASSOCIATION NEWS

Southern Regional Branch

Proceedings

The Annual General Body meeting of the Southern Region, was held on 17th December '77 in Madras.

The president welcomed Dr. Balagopal Raju, invitees and all members to the Second Annual General Body Meeting and requested Dr. Balagopal Raju to address the members.

1.1 Dr. Balagopal Raju, Former Director, Institute of Child Health and Hospital for Children, Madras-8, then addressed the members on nutritional aspects of child health and the avenues where Food Scientists could help the paediatricians by making available safe and nutritious food products. He dealt with the various types of cheap nutritious foods developed by different organisations all over the country.

The Annual Report for 1977 was adopted after duly proposed by Shri K. L. Radhakrishnan and seconded by Shri K. S. Kannan.

The audited statement of accounts was presented by the Treasurer Shri S. Mylvaganan. After clarifying some points raised by the members the statement of accounts was adopted.

The General Body with the recommendation of the Executive Committee adopted the following office bearers for 1978:

Shri S. Subrahmanyan	..	*President*
Shri A. Govindan	..	*Secretary*
Shri K. S. Kannan	..	*Vice-president*
Shri H. S. Jayadevaiah	..	*Jt. Secretary*
Shri M. Sreekrishna	..	*Treasurer*
Shri S. Rajagopalan	..	*Editor-Newsletter*
Shri S. T. Chari	..	*Member*
Shri V. Koteeswara Rao	..	*Member*
Shri S. P. Malik	..	*Member*
Shri R. N. Ramani	..	*Member*
Dr. T. S. Santhanakrishnan	..	*Member*
Shri T. V. Subramanyan	..	*Member*
Mrs. E. Sunderrajen	..	*Member*
Shri T. C. Sunder Rajen	..	*Member*

The President-elect Shri S. Subrahmanyan thanked the members for having reposed their confidence in him and assured that the activities of AFST(SRB) will be kept up for which he sought their cooperation.

The Secretary proposed a hearty vote of thanks and the meeting adjourned for dinner.

Eastern Regional Branch

Dr. S. Pedlington, Unilever Research Laboratory, England, on 29th September 1977, delivered a talk on "Some aspects of Tea Research".

Dr. P. Chattopadhyay, of the Department of Food Technology and Biochemical Engineering, Jadavpur University, gave a talk on "Cryogenic Freezing of Food" on 23rd December 1977.

Ordinary Members

Dr. Kamalakar Vishwanath Shankhapal, Lecturer, Department of Biochemistry and Microbiology, Laxminarayan Institute of Technology Premises, Nagpur-10

Mr. K. T. Vergheese, Modern Bakeries (India) Ltd. Adyar, Madras-600 020.

Mr. G. Sundararajan, T. N. Agro Industries Corporation Ltd, Agro House, Madras-32.

Mr. M. V. Pimple, C/o Jain Lottery Centre, Bus stand, Raipur (M.P)

Dr. R. Seshadri, PP & FT Discipline, CFTRI, Mysore-570 013.

Mr. Narendrapal Jain, 34/1 Santnagar, Civil Lines, Ludhiana.

Mr. C. S. Bhullar, Chief Agricultral Officer, Ludhiana.

Mr. N. K. Garg, Indian Grain Storage Institute, Punjab Agricultural University Campus, Ludhiana-141 004.

Mr. Jagdish Singh Parmer, Indian Grain Storage Institute, Punjab Agricultural University Campus, Ludhiana-141 004.

Mr. Ramesh Chandra, Indian Grain Storage Institute Punjab Agricultural University Campus. Ludhiana-141 004.

Mr. Surendrapal Singh, Indian Grain Storage Institute, Punjab Agricultural University Campus, Ludhiana-141 004.

Mr. V. M. Venkatachalam, Assistant Quality Inspector, T.N.C.S.C. Ltd., Karamadai-641 104.

Mr. Satyendranath Tripathi, Indian Grain Storage Institute, Punjab Agricultural University Campus, Ludhiana-141 004

Mr. Ravindranath Singh, Indian Grain Storage Institute, Punjab Agricultural University Campus, Ludhiana-141 004.

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Mr. Tejinder Singh, Department of Food Science and Technology, Punjab Agicultural University, Ludhiana-141 004.

Mr. Anand Vaidya, Nepal Kisan Fruit Preservation, Murli, Birganj (Nepal).

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Mr. Manohar Dev, Rashtriya Confectionery Works, 48 Fort Industrial Estate, Indore-452 002.

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Mr. K. Gopalakrishnan, 3 Vishnupuri, Indore-452 001.

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Dr. M. A. Rao, Department of Food Science, P.O. Box. No. 462, Geneva N. Y. 14456 (USA).

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Mr. Hukum Singh Kaintura, 21/2 Labour Colony, Vishnupuri, Nawabganj, Kanpur-208 002.

Mr. M. R. Adhia, 160, Town Area, Mithapur, Jamnagar District, (Gujarat).

Mr. Shamim Ahmed, Final Year—Catering Technology (Fd. Tech.), H. B. Technological Institute, Kanpur-208 002.

Mr. Vipin Kumar Gupta, C/o Sri S. S. Gupta, 7/82 Tilaknagar, Kanpur-208 002.

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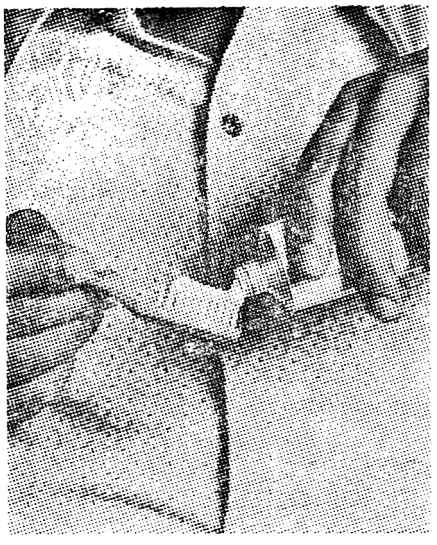

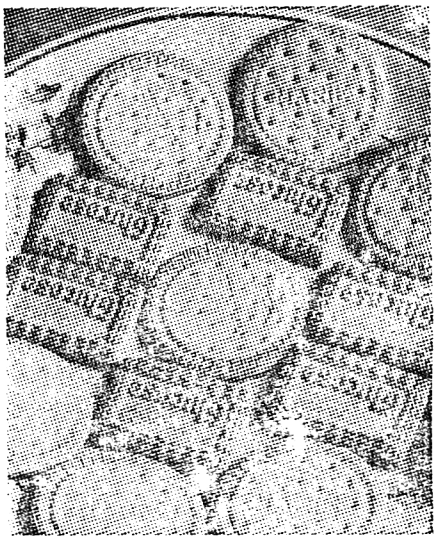

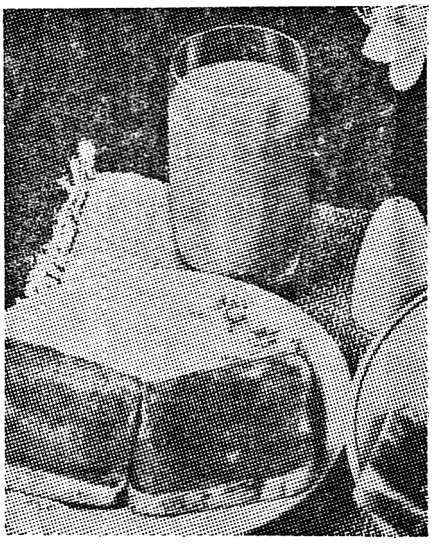

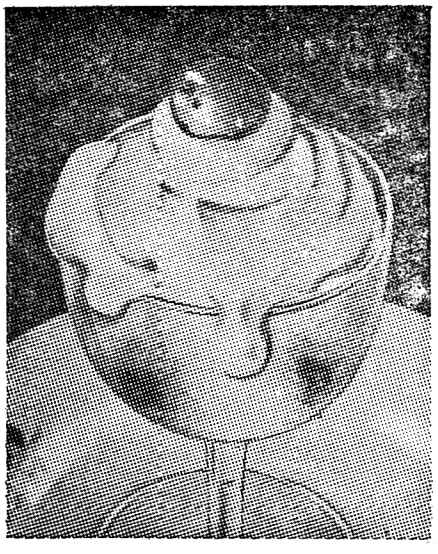

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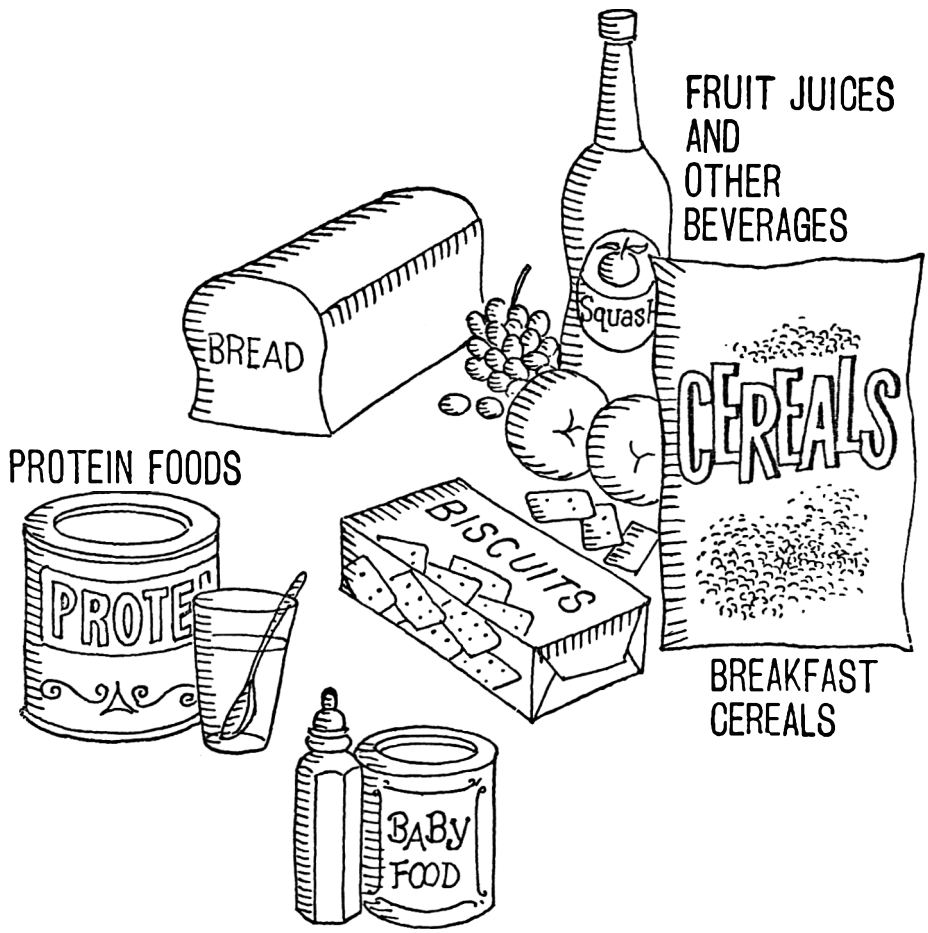

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3. Names of chemical compounds and not their formulae should be used in the text. Superscript and subscripts should be legibly and carefully placed. Foot notes should be avoided as far as possible.

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.

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   (*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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ON MEASUREMENT OF COLOUR OF MAIZE AND ITS PRODUCTS
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BREAD, BISCUIT AND CHAPATI MAKING QUALITY OF INDIAN TRITICALES
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PROXIMATE COMPOSITION AND NUTRITIVE VALUE OF *PHASEOLUS MUNGOREOUS* A CROSS BETWEEN *PHASEOLUS MUNGO* AND *PHASEOLUS AUREUS*
Kaushalya Gupta and D. S. Wagle

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