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Role of Salt, Oil and Native Acidity in the Preservation of Mango Pickle against Microbial Spoilage

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Mango pickles inoculated with salt tolerant strain of *Aspergillus niger* got spoiled at 10% salt, 40% oil and 4.2% native acidity. Salt concentration of 15% protected the pickles against spoilage by the inoculated organism as well as the native flora of the pickles. Groundnut oil did not have any preservative effect against microbial spoilage.

Pickling is an ancient art of preservation of fruits and vegetables. It is a simple, easy and economical method of preservation and can profitably be used for the benefit of rural population during the season. Sometimes, however, they get spoiled in spite of the presence of common salt added. Preservative properties of common ingredients of pickles have been studied by several workers[1-6] and there are some reports[1,7-14] describing microbial spoilage of pickles. The spoilage could be attributed to inadequate concentration of the preservative – the salt. This paper presents data regarding the optimum concentration of salt that would protect the mango pickle against microbial spoilage.

Materials and Methods

Preparation of mango pickle: Mango pickle containing raw mango pieces and condiments (spice mix containing crushed mustard seeds, chilli powder, turmeric powder and asafoetida) without salt and oil was prepared in the laboratory and distributed in 20 g aliquots in three sets of 30 sterile glass beakers each. The sets represented (i) experimental set with unsterilized samples inoculated with the spoilage organism, (ii) uninoculated, unsterilized control and (iii) uninoculated sterilized control. Common salt and groundnut oil (heated to 120°C and cooled to room temperature) were then added to the samples in different concentrations as detailed in Table 1. The pickles were kept for curing for six days after preparation and before inoculation with the spoilage organism. The experimental set was inoculated with the spoilage organism as detailed below.

Inoculation of the pickle sample with spoilage organism: The organism selected for inoculation was *Aspergillus niger*, the most frequent fungal species reported from spoiled pickles[14] and having salt tolerance up to 26 per cent concentration[15]. The organism was grown on potato dextrose agar for four days and spore suspension was prepared in sterile distilled water. Density of the spore suspension was determined by viable count method using potato dextrose agar and 0.1 ml of the spore suspension with a density of 6.2×10^5/ml was inoculated to each 20 g sample in the experimental set. The beakers in all the three sets covered with aluminium foil were incubated at room temperature (28 ± 2°C) for one year and observed daily for spoilage. The experiment was initiated in June, the usual season for preparation of pickles.

Chemical analysis of the samples: Unsterile and sterilized control samples immediately after preparation and spoiled samples after recording visible spoilage were analyzed for moisture content and total titrable acidity in terms of citric acid according to AOAC methods[16]. pH of the samples was recorded using BDH pH indicator paper (range 2 to 4.5 and 3.5 and 6.0).

Microbiological analysis: All the spoiled samples were microbiologically examined for viable count of fungi by inoculating 0.1 ml aliquot of the appropriate dilution of one per cent w/v suspension of the pickle in citrate buffer of pH 5.4 in Davis yeast extract salt agar[17] of pH 5.4. The plates were incubated at room temperature (28 ± 2°C) for 96 hr. Fungal growth in the spoiled pickle samples was also observed micro-

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scopically using cotton blue stain[18]. The fungi were identified according to Barnett[19] and Kamat[20].

Results and Discussion

The results of the chemical analysis of the uninoculated, unsterile and sterilized samples immediately after preparation are given in Table 1. Initial pH of all the samples was 2.5.

The moisture content of unsterile samples was significantly higher ($p<0.01$) as analysed by paired 't' test[21] than that of sterilised samples. This could be due to loss of moisture during sterilization. On the other hand, sterilization did not affect the acidity of the pickle samples.

All the unsterile samples prepared without salt irrespective of their oil content were spoiled within six days of preparation before inoculation of the samples with *A. niger* (Table 2). The spoilage was visible with heavy, white cottony surface growth with black sporangia. The organisms were identified as *Rhizopus* sp. and appeared to be predominant in the native flora. pH of all the spoiled samples rose from the initial 2.5 to 3.5 and acidity reduced from 4.1 to 2.2. The moisture content did not vary much.

TABLE 1. INITIAL MOISTURE CONTENT AND ACIDITY OF MANGO PICKLE SAMPLES PREPARED WITH DIFFERENT COMBINATIONS OF SALT AND OIL

Combination of salt/oil	Moisture content (g%)		Total titrable acidity (g%)	
	Unsterile	Sterilized	Unsterile	Sterilized
0/0	76.9	71.9	4.1	3.9
0/10	70.9	64.4	2.5	4.8
0/15	69.8	64.9	4.0	2.7
0/20	67.5	61.3	3.2	4.1
0/30	65.3	59.1	2.9	3.9
0/40	60.2	56.3	4.1	3.8
10/0	64.7	65.5	3.8	3.0
10/10	63.5	57.3	3.2	2.3
10/15	61.4	57.7	3.2	2.7
10/20	59.2	55.0	2.5	2.9
10/30	53.2	49.6	2.0	3.5
10/40	56.2	49.2	1.9	2.2
15/0	61.4	63.7	4.2	2.9
15/10	59.2	57.5	2.4	2.1
15/15	56.5	62.4	3.0	3.2
15/20	55.0	46.9	3.0	2.4
15/30	53.4	46.0	2.8	2.4
15/40	50.3	47.1	2.5	2.4
20/0	60.0	53.8	2.3	2.7
20/10	53.3	52.9	3.3	2.2
20/15	51.9	55.9	1.8	2.8
20/20	52.2	50.7	2.3	2.0
20/30	49.4	45.2	2.0	3.5
20/40	41.0	43.6	1.4	1.7
22/0	57.0	57.8	3.1	4.2
22/10	53.5	53.3	2.8	2.5
22/15	51.3	50.6	3.0	1.8
22/20	NE	NE	NE	NE
22/30	"	"	"	"
22/40	"	"	"	"

NE = not estimated

TABLE 2. PHYSICAL, CHEMICAL AND MICROBIOLOGICAL STATUS (FUNGI) OF PICKLE SAMPLES BEFORE AND AFTER INOCULATION WITH *ASPERGILLUS NIGER*

Salt/oil combination	Sample type	Days for spoilage (No.)	Moisture (g%)	Total titrable acidity (g%)	Fungi* (cfu/g)
0/0	US/UI (2 samples)	6	74.0 (76.9)	3.3 (4.1)	2×10^7
0/0	S/UI	85	72.2 (71.9)	0.1 (3.9)	4.5×10^{12}
0/10	US/UI (2 samples)	6	70.0 (70.9)	2.2 (2.5)	4×10^7
0/10	S/UI	–	64.4	4.8	–
0/15	US/UI (2 samples)	6	68.8 (69.8)	2.8 (4.0)	3×10^7
0/15	S/UI	–	64.9	2.7	–
0/20	US/UI (2 samples)	6	66.5 (67.5)	2.8 (3.2)	3×10^7
0/20	S/UI	–	61.3	4.1	–
0/30	US/UI (2 samples)	6	63.2 (65.3)	2.6 (2.9)	2×10^7
0/30	S/UI	–	59.1	3.9	–
0/40	US/UI (2 samples)	6	60.4 (60.2)	2.5 (4.1)	2×10^7
0/40	S/UI	–	56.3	3.8	–
10/0	US/Control	50	63.5	1.9	7×10^7
10/0	S/Control	–	65.5	3.0	–
10/0	US/I with *A. niger*	45	63.4	1.3	4.9×10^{10}
10/10	US/Control	60	62.2	1.8	6×10^7
10/10	S/Control	–	57.3	2.3	–
10/10	US/I with *A. niger*	52	62.3	1.3	3.4×10^{10}
10/15	US/Control	64	60.2	1.8	7×10^7
10/15	S/Control	–	57.7	2.7	–
10/15	US/I with *A. niger*	60	60.1	1.3	3.1×10^{10}
10/20	US/Control	69	58.3	1.8	5×10^7
10/20	S/Control	–	55.0	2.9	–
10/20	US/I with *A. niger*	67	58.4	1.3	3×10^{10}
10/30	US/Control	75	52.1	1.7	3×10^7
10/30	S/Control	–	49.6	3.5	–
10/30	US/I with *A. niger*	72	52.2	1.4	2.3×10^{10}
10/40	US/Control	79	55.2	1.7	2×10^7
10/40	S/Control	–	49.2	2.2	–
10/40	US/I with *A. niger*	75	55.3	1.4	1.7×10^{10}

(Figures in parenthesis are values estimated immediately after preparation)
US – Unsterile; S – Sterilized; UI – Uninoculated; I – Inoculated,
*Total viable count.

All the sterilised control samples irrespective of their salt and oil contents remained healthy throughout the experimental period except for one isolated case which showed spoilage and could be branded as a chance contamination. The fact that the samples without salt but with 10 to 40 per cent oil could not be preserved for as short a period as six days clearly points out that oil did not have any preservative role against microbial spoilage.

Pickle samples prepared with 10 per cent salt and 0 to 40 per cent oil were spoiled within 80 days after inoculation. The viable count of *A. niger* increased from the initial 3.1×10^3 per g of pickle to 4.9×10^{10} per g of spoiled pickle. Moisture content did not change much with spoilage. The total titrable acidity got reduced from initial 3.8 to 1.3 per cent and pH increased from 2.5 to 4.5. These data clearly indicate that the organisms utilized organic acids present in the pickle and grew luxuriantly to form surface growth that affected the aesthetic value of the pickle. It is thus seen that 10 per cent salt irrespective of the oil content was not adequate to preserve the pickles.

Since the pickle samples with 15 per cent salt content did not spoil till the end of one year, it is stated that 15 per cent salt concentration is optimum to preserve the pickle.

As regards the acidity of mango pickle, as much as 4.8 per cent acidity of the samples under study was not enough to protect the pickle. Maximum acidity of 6.2 per cent has been reported[14] for a spoiled pickle sample. Tolerance to 12 per cent citric acid by *A. niger* and 10 per cent by *Penicillium* sp. and *Candida utilis* has also been reported[15]. Fungal spoilage thus would not be arrested by the native acidity of the pickle.

It is thus concluded that only the common salt has preservative role at 15 per cent final concentration in the Indian unfermented mango pickle.

It may be mentioned here that i) maximum concentration of 40 per cent of oil was selected since the observed maximum concentration of the oil was 35 per cent in 21 spoiled samples examined earlier[14]; ii) the oil was heated to 120°C and cooled to room temperature before adding to the pickle since housewives commonly follow this practice. Further, the temperature at which the mustard seeds start crackling in the oil is recorded as 120°C in the laboratory and iii) pickle samples used for inoculation with the spoilage organism were unsterile since it is not a practice to sterilize the pickles. However, sterilized pickles were used as negative control for spoilage.

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Effects of Packaging and In-Package SO_2 Generators on Shelf Life of Perlette Grapes at Ambient and Refrigerated Conditions

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The storage life of 'Perlette' grapes was extended upto 80 days at refrigerated condition whereas for 6 days at ambient condition. The vented polylined corrugated fibre board (CFB) carton (2 kg) equipped with quarter size dual release (DR) SO_2 generator gave excellent results under refrigerated condition. The quick release (QR) type combined with vented polyliner effectively controlled water loss, browning and decay at ambient condition. Newsprint lining resulted in maximum losses. The 2 Kg CFB cartons were handy and provided better aeration to the fruit with the result that overall losses were less in these containers. However, CFB boxes needed more stacking strength. Negligible bleaching was observed in the grapes packed with QR and DR sulphur dioxide generators.

In India, for domestic markets, 'Perlette' grapes are handled at ambient condition. Refrigerated transport is still obscure. In the Northern region, temperature at the time of harvest in June-July is around 35 ± 5° C with relative humidity of 40-60 per cent. This leads to rapid deterioration of grapes due to decay, water loss and browning. Moreover, traditional unvented boxes and rice straw cushioning increase these losses. The normal storage life of 'Perlette' grapes under ambient and refrigerated conditions (0° – 3° C, 85-90 per cent RH) is 2-3 and 35-40 days, respectively[1,2]. There is a pressing need to increase the shelf-life of 'Perlette' grapes at ambient and refrigerated conditions so that fruits can be marketed for longer periods. Suitable decay control systems and packaging need to be tested for this purpose.

In earlier studies, various packaging materials have been tried with varying success. Grapes were stored upto 3 days at ambient condition without any anti-fungal treatment[1]. The polyethylene liners controlled water loss effectively, but decay was more in these packs[3,4]. The incidence of decay was less in bamboo baskets but stacking strength and dimensional stability were needed in these packs[5]. Recently, the performance of corrugated fibre board (CFB) boxes was observed as quite encouraging for storage and transportation of grapes[6].

For in-package fumigation, polyethylene sachets containing potassium metabisulphite with silica were found to be effective in extending shelf-life of 'Perlette' grapes up to 50 days at refrigerated condition[7]. A mixture of $K_2S_2O_5$ and citric acid has also been tried to minimise losses at ambient condition[8]. However, in these systems, the bleaching injury to the fruit was comparatively more due to unregulated release of SO_2. These small sachets were also prone to shifting inside the container during handling. In the recent past, various types of SO_2 generators were reported where the emission of SO_2 was regulated by polymer coating[9,10]. The chances of shifting of these generators inside the containers were very less.

Considering these facts, present storage trials were undertaken at ambient and refrigerated conditions to study the efficacy of quick release (QR) and dual release (DR) SO_2 generators in combination with various packaging systems and determining the effects in-package fumigation on 'Perlette' grapes particularly in terms of bleaching injury and control of browning.

Materials and Methods

During the 1984-85 season, mature 'Perlette' bunches were harvested and trimmed to remove decayed, bruised and immature or shot berries. The bunches were packed and stored as follows.

Packaging material: The 3 ply. un-coated corrugated cartons measuring 25 cm (L) × 20 cm (W) × 10 cm (H) with 2 kg capacity were used. The cartons were punched for ventilation (2 vents of 1.5 cm diameter on 20 cm wide side, each of them being spaced 2.6 cm away from the centre). The wooden boxes measuring 40 cm (L) × 20 cm (W) × 13 cm (H) with 5 kg capacity had lengthwise 1-1.5 cm vide slits on the top and bottom. Conventional wooden boxes have no slits

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have no slits and grapes are packed in them with newsprint lining and rice straw cushioning.

Polyethylene film (0.2 mm thick) and newsprint were used for lining. The vented polyethylene film for 5 and 2 kg capacity box had 40 and 20 holes (each of 5 mm diameter), respectively. Soft paper shreds served as cushioning material.

Storage at ambient condition: The QR SO_2 generators were used for in-package fumigation. This was a single sheet of kraft paper (22 cm × 34 cm) coated with patented $NaHSO_3$ (1.5 g) on one side. After lining the wooden container with polyethylene film, paper shreds were placed and bunches were arranged in a customary manner. The full size QR SO_2 generator was placed on the fruit with paper shreds between the fruit and generator. The liner was folded over the fruit and container was closed. All packs were brought to laboratory from the field and held at ambient condition (34 ± 5°C, 45-55 per cent RH).

Storage at refrigerated condition: The DR SO_2 generators (40 cm × 20 cm) had a quick release sheet attached to white pouched section. The quick white pouched section were coated with patented polymer. Full size DR SO_2 generator contained 5.5 of $NaHSO_3$. The mode of packing was similar to what was mentioned above. The full and quarter size DR generators were used for 5 and 2 kg grapes, respectively. All packs were held at refrigerated condition (1 ± 1°C, 85-90 per cent RH) and evaluated at 40 and 80 days of storage.

Grapes were evaluated for per cent weight loss, berry shatter, decay, internal browning, bleaching and berry cracking. The internal browning was perceptible in intact berries also and hence such berries were weighed to estimate per cent browning. The location of internal browning and its development was studied by taking longitudinal sections of the normal, internally brown and bruised berries. Bleaching was characterised by fading of chlorophyll and change of berry colour to white. Berries with slight (quarter to one fifth area near pedicel) to heavy (more than two third berry surface) bleaching were taken into account while calculating per cent bleaching.

Three containers from each of the five treatments were taken out at each interval for evaluation. The analysis of variance of the data was carried out and the Duncan's multiple range test of means was followed to find out treatment significance.

Results and Discussion

Under ambient condition, grapes in the newsprint lined cartons lost the maximum weight (4 per cent) up to 6 days (Table 1). The loss from the unvented polylined packs was 1 per cent followed by 2 per cent in vented polylined containers. In newsprint lined wooden and corrugated cartons, rachis and pedicels were dry and brown although berries were turgid. This excessive water loss can be attributed to the very high temperatures and low relative humidity (40-50 per cent) prevailing during summer. Under refrigerated conditions, the vented polylined cartons and wooden containers with in-package SO_2 generators proved very effective in controlling weight loss upto 80 days. The reduction in water loss in SO_2 treated fruits can be attributed to the supression of respiration rate of grapes. Moreover, the lower temperature coupled with high relative humidity must have resulted in decreased vapour pressure of water in the fruit and consequently weight loss was less. The weight loss was slightly less in CFB cartons as compared to wooden boxes and this was probably due to more air circulation and cooling of grapes leading to reduced respiration and transpiration rate.

The remarkable effect of liners on berry shatter is clearly evident from the data shown in Tables 1 and 2. Maximum berries shattered from the grapes packed in newsprint lined containers whereas grapes packed in polylined CFB cartons with SO_2 generators recorded least shatter losses. The berry shatter was mainly due

TABLE 1. EFFECT OF PACKAGING CONTAINERS, LINERS AND SO_2 TREATMENT ON CHARACTERISTICS OF PERLETTE GRAPES HELD UNDER AMBIENT CONDITION UP TO 6 DAYS*

Treatment	Wt loss (%)	Berry shatter (%)	Decay (%)	Browning (%)	Cracking (%)	Bleaching (%)
1. Wooden box, newsprint liner without SO_2 generator	4.0^{a}	5.0^{c}	4.5^{c}	6.0^{b}	0^{a}	–
2. Wooden box, vented polyliner with QR generator	2.0^{a}	1.0^{ab}	0.0^{a}	2.5^{a}	3.5^{a}	2.6
3. CFB carton, newsprint liner without SO_2 generator	3.5^{a}	3.0^{bc}	4.0^{c}	4.0^{ab}	0.5^{a}	–
4. CFB carton, vented polyliner without SO_2 generator	2.0^{a}	0.75^{ab}	2.5^{b}	4.25^{ab}	3.0^{b}	–
5. CFB carton, unvented polyliner without SO_2 generator	1.0^{a}	0.5^{a}	5.0^{a}	3.0^{a}	5.5^{c}	–

*Figures followed by different superscripts under each column differ significantly at 5% level.

TABLE 2. EFFECT OF PACKAGING CONTAINERS, LINERS AND SO_2 TREATMENT ON CHARACTERISTICS OF PERLETTE GRAPES HELD UNDER REFRIGERATED CONDITION FOR DIFFERENT DAYS(d)

	Wt loss (%)		Berry shatter (%)		Decay (%)		Browning (%)		Cracking (%)		Bleaching (%)	
	40 d	80 d	40 d	80 d	40 d	80 d	40 d	80 d	40 d	80 d	40 d	80 d
1. Wooden box, newsprint liner without SO_2	4.5^{b}	8.0^{b}	5.0^{c}	9.9^{b}	5.5^{c}	13.5^{c}	5.5^{b}	10.0	0^{a}	0^{a}	–	–
2. Wooden box, vented polyliner with DR generator	1.75^{a}	3.0^{ab}	2.0^{ab}	3.8^{a}	0^{a}	0^{a}	2.5^{abc}	5.0^{a}	4.0^{c}	6.0^{b}	3.5	5.6
3. CFB carton, newsprint liner without SO_2	3.5^{ab}	7.2^{ab}	3.55^{bc}	4.2^{a}	3.5^{b}	5.0^{b}	4.0^{ab}	5.0^{a}	0^{a}	0^{a}	–	–
4. CFB carton, vented polyliner without SO_2 generator	3.0^{ab}	4.0^{ab}	2.25^{ab}	3.5^{a}	4.0^{bc}	7.5^{b}	5.0^{ab}	7.5^{ab}	2.5^{b}	6.0^{b}	–	–
5. CFB carton, vented polyliner with DR generator	1.5^{a}	2.5^{a}	1.0^{a}	1.85^{a}	0^{a}	0^{a}	2.0^{a}	5.0^{a}	3.5^{bc}	5.0^{b}	2.25	3.00

*Figures followed by different superscripts under each column differ significantly at 5% level.

to drying of pedicels and laterals particularly at their junction. During handling, the flexing of cuticle and crushing of tissue resulted in water loss from these areas and this led to shatter in the form of single berry or clumps of 2 or more berries. The newsprint lining presently being used in India for packing grapes proved unsuitable at ambient and refrigerated conditions. In polylined boxes equipped with SO_2 generators, polyliners preserved natural freshness of stem structures and berries while SO_2 helped in retaining natural colour by minimizing browning.

The decay completely eliminated in the wooden as well as CFB packs equipped with SO_2 generators (Tables 1 and 2). Under refrigerated conditions, the maximum decay was observed in newsprint lined wooden container followed by vented polylined and newsprint lined CFB cartons. At ambient conditions, decay was more in unvented polylined cartons and this can be attributed to higher temperature and humidity conditions in these packs. In newsprint liners, decay spread slowly. Under refrigerated conditions, the decay level was higher in wooden boxes compared to CFB boxes and this was probably due to slow cooling in the former. The major fungi responsible for decay were *Aspergillus niger, A. flavus, Penicillium* sp. and *Rhizopus* sp.

The development of internal browning was highest in the fruits packed in newsprint lined wooden containers (Tables 1 and 2). The internal browning was chiefly due to enzymatic oxidation of phenolic compounds and it was evidenced by reduction in polyphenol oxidase activity in SO_2 treated grapes.[12] Under ambient as well as refrigerated conditions, the internal browning was minimized considerably due to SO_2 treatment and bunch appearance was fairly good.

Internal browning spread centripetally as well as from basal end. At advanced stage, the discolouration was observed throughout the pulp. In earlier studies, the internal browning was reported as initiated at the vascular tissues or at rudimentary seed area in 'Thompson Seedless' grapes and internal O_2 supply, was suggested as a possible cause[13]. The centripetal browning was started probably due to bruising, pressing and handling impact on skin tissue resulting in structural disintegration and distruption of substrate – enzyme compartmentation. The internally brown berries were firm and had flat in taste. Internal browning increased with protracted storage. Temperature had appreciable control on overall phenomenon as the browning increased after removal of containers from cool chamber. A direct correlation between browning and temperature has been observed by Nelson[14].

In the SO_2 generator equipped containers, some bleached berries were observed. (Tables 1 and 2). The bleaching injury (light yellow colour at basel end of the berry) in top bunches can be attributed to the direct contact of generators with grapes due to displacement of paper shreds. Therefore, proper placement of paper shreds in between fruit and SO_2 generator is necessary. Under refrigerated conditions, where DR SO_2 generators are used, it is necessary to cool the containers immediately. Otherwise, excessive release of SO_2 may take place from the white pouched section. Slightly more bleaching of grapes in wooden containers (Table 2) as compared to CFB cartons can be attributed to slow cooling of grapes in those boxes. However, excess bleaching was avoided due to venting of the polyliners leading to purging out of SO_2. The venting of polyliners, proper placement of SO_2 generators, quick cooling of containers, elimination of temperature fluctuations and timely inspection of fruit can be suggested as some of the steps to be taken to minimise bleaching during large scale commercial

storage of grapes.

Berry cracking was much more in unvented polylined packs as compared to newsprint lined containers (Tables 1 and 2). The grapes packed in vented polyliners with or without SO_2 generator recorded lower cracking. The incidence increased with the extension of storage period. The cracking can develop due to physical stress i.e. by external force of handling or by internal force of hydrostatic/turgor pressure. The high incidence of cracking in unvented packs may be attributed to the high relative humidity and possibly condensation of water leading to high turgor pressure of berries. The cracking due to physical stress of lid on top berries shows the importance of stacking strength in CFB boxes and warrants further study.

Findings of this study indicate that decay and browning can be controlled up to 6 days at ambient condition if grapes are packed with full size QR SO_2 generator in 5 kg boxes. The results would be equally good with half the SO_2 generator in 2 kg packs. Bleaching, weight loss and cracking problem were less in vented polylined cartons. This system of packing grapes has significant potential in India where produce has to be marketed for 4-5 days after harvest at ambient condition. Grape growers can take their produce to large markets (big cities) in the country where extra cost of packaging and transportation can be recovered. Long term storage (upto 80 days) is also possible with DR SO_2 generator and it will be highly economical if fruit is stored at distribution point and released according to demand.

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Acceptability of Packaging Systems for Processed Cheese – a Sensory Analysis*

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Application of mean sensory scores for processed cheese packaged in polystyrene cup, low density polyethylene tub and small lacquered tins indicated the acceptability of processed cheese packaged in tins when stored at 30°C/60°% R.H. and 7-8°C/80°% R.H., respectively. Under the latter conditions, acceptability was upto 90 days. These scores were based on numerical scoring test for the appearance, body and texture and aroma and flavour.

The importance of processed cheese as a food is very well known. In 1982, the estimated total cheese production comprising Cheddar, Gouda and processed types in India was 2,000 tonnes[1]. Singh and Kanawjia[2] recommended that the cheese should become a part of regular diet of our explorers in Antartica and soldiers in Siachin.

The processed cheese in foreign countries is generally being wrapped in sliced form in polypropylene (PP) film or co-extruded PP, and the wrapped portions placed in a polyvinylidiene chloride (PVDC) film[3]. In India, the processed cheese usually in 25 g quantities are packed in aluminium (Al) foil, which in turn, are placed in a carton. A few organizations are also packaging processed cheese in small lacquered tins. The wrapping of processed cheese in PP required high speed machine, while the use of Al foil plus cartons or tins is expensive. Hence, the present studies were undertaken to find out the keeping quality of processed cheese packaged in indigenously manufactured, low cost, hygienic and attractive plastic containers, in comparison with tins.

Materials and Methods

Selection of packaging materials: Polystyrene (PS) cups with lids and low density polyethylene (LDPE) tubs with lids were procured from the leading manufacturers of the country. The capacity of each containers was 150 g. The PS cups (P1) and their lids were opaque and white in colour, while the LDPE tubs (P2) and their lids were transluscent. The height and top diameter of the PS cup were 7.1 and 6.2 cm respectively. The thickness of the cup sheet was 0.20 mm. The height and top diameter of the LDPE tub were 3.4 and 7.9 cm respectively and the thickness of the tub sheet was 0.60 mm. Lacquered tins (P3) used in the investigation were obtained from reputed Indian firms and had a capacity of 240 g. The height and diameter of the tins were 7.8 and 5.9 cm, respectively.

Sterilization of packaging materials: The plastic containers and their lids were cleaned by using 'teepol' and tap water. They were then chemically sterilized by keeping the chlorine solution (200 ppm) for 3 min[4] Thereafter, the packages were air dried. The sterilization of the packages was done immediately before use as far as possible under aseptic conditions. The tin cans and their lids already cleaned with hot detergent solution and tap water were sterilized in a hot air oven at 165-170°C for 2 hr, immediately before use[4].

Preparation, packaging and storage of processed cheese: The methods suggested by Kosikowski[5] were followed for the preparation of processed cheese by using medium ripened (upto 6 months old) Cheddar cheese collected from the Experimental Dairy of the institute and prepared from cow's milk. During the manufacture of processed cheese, when the cheese mass became velvety, smooth and attained a temperature of 70°C, it was directly filled into the three types of pre-sterilized packages (P1, P2 and P3). Immediately after filling, the plastic containers were covered with their respective lids, which in turn, were further sealed by using cellotape of 2.5 cm width. The tins were sealed off. The sealed packages after 1 hr were stored at 30°C and 60 per cent R.H. (condition A), and 7-8°C and 80 per cent R.H. (condition B).

Sensory evaluation: The samples of processed cheese packaged in the 3 different types of containers and stored under conditions A and B were evaluated

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organoleptically by a panel of 5 experienced judges for appearance, body and texture, and aroma and flavour. The packaged samples of processed cheese stored under condition A were examined for sensory attributes on 0, 10, 20 and 30 days, while the samples stored under condition B were evaluated on 0, 30, 60 and 90 days. The stored test samples were tempered at 10-15.5°C for 3 hr before they were presented under code numbers to each judge. The product was evaluated by the judges using the following Numerical Scoring Guidelines:

Excellent : 19-20 for appearance, 38-40 for body and texture, 38-40 for aroma and flavour;

Good : 17-18 for appearance, 32-37 for body and texture, 32-37 for aroma and flavour;

Fair : 14-16 for appearance, 27-31 for body and texture, 27-31 for aroma and flavour;

Poor : 13 or below for appearance, 26 or below for body and texture, 26 or below for aroma and flavour.

Statistical analysis: The data obtained during the present study were subjected to statistical analysis following the methods and techniques of Snedecor and Cochran[6].

Results and Discussion

The acceptance data for appearance, body and texture, and aroma and flavour as mean sensory scores based on numerical scoring test of the processed cheese samples stored in the 3 different types of packages (P1, P2 and P3) for different periods under conditions A and B are depicted in Tables 1 and 2 respectively.

Storage at 30°C/60 per cent R.H.: The initial score for appearance of the processed cheese samples decreased in all the three types of packages after 30 days of storage (Table 1). The samples packed in P3 showed minimum change for appearance, followed by P2 and P1 in ascending order. However, the samples in all the 3 types of packages from the viewpoint of appearance remained acceptable upto 30 days and they ranged from good to fair in grading. The statistical analysis of the data revealed that the differences due to the intervals of storage, the types of packages, and the various judges, each individually, contributed significantly ($P<0.01$) towards the changes in appearance of processed cheese samples. Also, the interaction intervals × packages significantly ($P<0.05$) influenced the appearance of the processed cheese samples, whereas interactions judges × intervals; judges × packages; and intervals × packages × judges were found to be not significant.

As expected, the body and texture of the cheese

TABLE 1. MEAN SENSORY SCORES (BASED ON NUMERICAL SCORING TEST) FOR PROCESSED CHEESE STORED IN DIFFERENT PACKAGES AT 30°C AND 60 PER CENT R.H.

	0 – day storage			10 – day storage			20 – day storage			30 – day storage		
	Poly-styrene	LDPE	Tin	Poly-styrene	LDPE	Tin	Poly-styrene	LDPE	Tin	Poly-styrene	LDPE	Tin
						Appearance						
Trial I	16.8	16.8	16.8	14.8	16.0	16.8	15.8	16.8	17.4	14.8	16.0	16.8
Trial II	17.4	17.4	17.4	16.4	15.4	16.6	15.8	16.8	17.4	14.2	15.2	15.8
Trial III	18.2	18.2	18.2	16.4	17.0	16.8	16.4	15.8	15.8	14.4	15.2	15.8
Mean	17.5	17.5	17.5	15.9	16.1	16.7	16.0	16.5	16.8	14.5	15.5	16.1
						Body and Texture						
Trial I	33.2	33.2	33.2	31.8	33.0	34.2	30.0	31.0	32.6	28.2	29.8	30.8
Trial II	34.4	34.4	34.4	31.2	32.6	31.8	30.0	32.0	31.4	28.4	29.6	30.8
Trial III	37.6	37.6	37.6	32.0	33.2	34.2	29.8	31.8	32.4	28.4	30.6	31.2
Mean	35.1	35.1	35.1	31.7	32.9	33.4	29.9	31.6	32.1	28.3	30.0	30.9
						Aroma and Flavour						
Trial I	32.4	32.4	32.4	30.2	31.4	32.4	29.4	32.2	32.2	24.4	24.7	31.0
Trial II	33.2	33.2	33.2	27.6	30.2	31.4	28.0	29.2	30.8	23.7	24.8	30.0
Trial III	36.8	36.8	36.8	30.0	29.8	32.0	28.0	31.0	31.0	23.3	24.3	29.6
Mean	34.1	34.1	34.1	29.3	30.5	31.9	28.5	30.8	31.3	23.8	24.6	30.2

Mean of 5 judges

samples were observed to be least affected when packed and stored in lacquered tins, as these tins are impermeable to moisture vapours. The differences in sensory scores for body and texture of the samples were possibly due to the different water vapour permeabilities of the plastic containers; P1 being made of PS in all possibilities might have permitted the escape of more moisture resulting in lower scores as compared to P2, which was made of LDPE[7,8]. The results are in agreement with the findings of Clotet Ballus *et al.*,[9] who also reported lower scores for the samples of processed cheese packed in plastic containers having more water vapour permeabilities. Statistically, the changes in the sensory scores for body and texture of the processed cheese samples due to the 3 types of packages, the durations of storage, and the judges were highly significant ($P<0.01$). Interaction judges × intervals also significantly ($P<0.01$) effected changes in the scores for body and texture of the processed cheese samples.

The initial score for aroma and flavour of the processed cheese decreased most in samples packed in P1 followed by P2 and P3 in ascending order after 30 days of storage. The samples packaged in tins, which are impermeable to odours and oxygen, showed highest scores for aroma and flavour (Table 1). Since, oxygen transmission rate of PS is more than that of LDPE[7,8], this might have resulted in lower scoring for aroma and flavour of the samples packed in P1. The analysis of variance of the data established significant ($P<0.01$) effect on aroma and flavour scores of the processed cheese samples due to the types of packages and the periods of storage. Interactions between packages and intervals, and between intervals and judges were observed to be significant ($P<0.01$), while the interactions judges × packages, and intervals × packages × judges were not significant for effecting differences in scores for aroma and flavour of the processed cheese samples.

Storage at 7-8°C/80 per cent R.H.: The highest scores for appearance were awarded to the processed cheese samples packed in tin containers P3 followed by the packages P2 and P1 in descending order after 30,60 and 90 days of storage (Table 2). The appearance of the stored cheese was acceptable upto 90 days in all the three types of packages. The differences due to the types of packages, intervals of storage, and the various judges, each individually, contributed more significantly ($P<0.001$) towards the changes in appearance than the interaction intervals × packages ($P<0.05$), while the effect of interactions, packages × judges; intervals × judges; and intervals × packages × judges was found to be not significant.

The numerical scores assigned by the panelists for body and texture to the processed cheese samples stored in different packages for various time intervals

TABLE 2. MEAN SENSORY SCORES (BASED ON NUMERICAL SCORING TEST) FOR PROCESSED CHEESE STORED IN DIFFERENT PACKAGES AT 7-8°C AND 80 PER CENT R.H.

	0 – day storage			30 – day storage			60 – day storage			90 – day storage		
	Poly-styrene	LDPE	Tin	Poly-styrene	LDPE	Tin	Poly-styrene	LDPE	Tin	Poly-styrene	LDPE	Tin
Appearance												
Trial I	17.0	17.0	17.0	16.0	16.6	16.8	15.4	16.2	17.2	15.6	16.2	17.2
Trial II	17.2	17.2	17.2	16.0	16.2	16.2	15.0	15.6	16.0	14.2	14.8	15.4
Trial III	18.2	18.2	18.2	16.2	16.4	17.4	16.2	16.6	17.0	15.2	15.8	16.4
Mean	17.5	17.5	17.5	16.1	16.4	16.8	15.5	16.1	16.7	15.0	15.6	16.3
Body and Texture												
Trial I	35.2	35.2	35.2	32.0	34.2	34.0	29.4	32.0	32.4	28.2	28.8	30.2
Trial II	34.2	34.2	34.2	34.4	36.0	33.4	30.2	33.8	33.2	28.6	30.0	30.6
Trial III	33.4	33.4	33.4	29.4	31.6	33.0	29.4	31.8	32.8	28.2	30.2	30.2
Mean	34.3	34.3	34.3	31.9	33.9	33.5	29.6	32.5	32.8	28.3	29.7	30.3
Aroma and Flavour												
Trial I	32.8	32.8	32.8	30.8	31.4	32.6	28.4	30.2	32.4	26.4	28.4	29.8
Trial II	37.2	37.2	37.2	33.8	35.8	36.6	29.2	31.6	34.4	27.8	29.8	30.8
Trial III	37.2	37.2	37.2	32.2	32.2	33.8	31.8	32.0	33.6	26.8	29.6	30.4
Mean	35.7	35.7	35.7	32.3	33.1	34.3	29.8	31.3	33.5	27.0	29.3	30.3

Mean of 5 judges

reveal that the scores were maximum for the product packed in P3, followed by P2 and P1 respectively. It appears that the differences in the water vapour permeabilities of the various packages used for the packaging and storage of the cheese samples might have played a significant role in affecting the body and textural characteristics of the samples, as the package P3 made of tin was completely impermeable to water vapours, and the P1 made of polystyrene was more permeable to water vapours than P2 which was made of LDPE[7,8], and this most probably resulted in minimum scores for body and texture in case of the samples packaged in P1. Similar observations have been reported by Clotet Ballus *et al*[9]., who also recorded lower scores for the processed cheese samples packaged in plastic containers with more water vapour permeabilities. The analysis of variance of the data revealed that the variations in the sensory scores for body and texture of the processed cheese samples were significant ($P<0.01$) due to the 3 types of packages, durations of storage and the judges. Interaction between intervals of storage and the judges was also significant ($P<0.01$), while interactions packages × judges, and intervals × packages × judges were found to be not significant.

It is evident from Table 2 that the samples of processed cheese packed in P3 were rated highest also for aroma and flavour followed by the samples packaged in P2 and P1 in descending order. This was so probably because the package P3 being tin containers was completely impermeable to odours and oxygen, and P1 being PS was more permeable to oxygen than P2, which was made of LDPE[7,8]. The types of packages and the durations of storage had more significant ($P<0.01$) effect than the judges ($P<0.05$) on the aroma and flavour scores of the processed cheese samples. Interaction between intervals and packages was also highly significant ($P<0.01$), while interactions judges × intervals, judges × packages, and intervals × packages × judges were found to be not significantly affecting the aroma and flavour scores for the processed cheese samples.

At 30°C and 60 per cent R.H., the processed cheese samples packaged in tins (P3) were acceptable upto 30 days, while the processed cheese samples packed in PS cups (P1) and LDPE tubs (P2) were acceptable to the panelists only upto 20 days. The processed cheese samples stored at 7-8°C and 80 per cent R.H. were found to be of acceptable quality in all the 3 packages (P1, P2 and P3) for 90 days. Snegireva *et al.*[10] also observed that the shelf life of processed cheese in PS cups was 3 months at -4 ± 1°C and 85-90 per cent R.H. Interestingly, in the evaluation of the packages, Table 1 and 2 show that consistently P3>P2>P1 for storage of processed cheese at both the conditions. The above results also confirm the earlier findings of Snegireva *et al.*[10] and Komissarova[11] that the shelf life of processed cheese declined with the increase of storage temperature.

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Studies on Ready-Mix for *Kheer*

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A ready-mix for *Kheer* (*Payasam*) based on 30% wheat *soji* (semolina) roasted to an optimum temperature of 145°C, in a grain roaster, 30% powdered sugar and 40% whole milk powder (WMP) has been developed. The ready-mix could be processed within 5 min into *Kheer* with desired taste, aroma and consistency. Inclusion of 5% each of preprocessed cashewnut and raisins, 0.7% of cardamom powder and 25 ppm of edible 'Sunset' yellow colour in the ready-mix enhanced the appearance as well as overall acceptability of the *Kheer*. The product evaluation studies have shown that the *Kheer* from ready-mixes based on white *soji* (from aestivum wheat) and WMP or skim milk powder (SMP) with 5% added fat had better acceptability than that of the *Kheer* from similar ready-mixes based on *Bansi soji* (from durum wheat), costing almost twice the white *soji*. Amylograph and Brookfield viscometer studies indicated that *Bansi soji* in dispersion had lower viscosity than the white *soji*, suggesting thereby, that the white *soji* was better suited for the *Kheer* ready-mix.

Whether based on cereals, millets or pulses, traditional foods[1,2] occupy a very important place in the Indian dietary. Indian consumer naturally prefers *Chapati, paratha, roti, nan, batura* or *puri* to bread. Likewise, he opts for *idli, dosa, vada, upma,* pickles, *papads, chutney, murabba, kesari bath (shira), Kheer* or *payasam*, etc. in preference to Western products like macaroni, noodles, cake, pastries, sauces, sauerkraut, etc. However, while bakery products are being manufactured and marketed commercially, traditional food items for breakfast or meals are amenable to only household preparation, for meeting economically the requirements of a family.

Though wheat and rice form the basic ingredients for several regional traditional foods, in recent years some items like *dosa, idli, vada, upma, nan*, have gained increasing popularity to attain national status. *Kheer* (as known in North India) or *payasam* (as known in South India) is one such item, which is highly popular with all households, irrespective of the economic status.

In the newly emerging era of fast and convenience foods, ready-mixes or instant foods are becoming increasingly popular among Indian households, in view of "Kitchen" convenience as well as for meeting the urgent and exigency situations of offering hospitality to unexpected guests. With this background, studies were undertaken to develop a ready-mix for *Kheer*, suitable for any occasion. The results are presented here.

Materials and Methods

Commercial samples (coarse: –24, +32 mesh; medium: –32, +45 mesh; and fine: –45, +60 mesh) of white semolina (*soji*) milled from aestivum wheat and *Bansi* semolina from durum wheat, 'Nespray' whole milk powder (WMP) and 'Anikspray' skim milk powder (SMP), fine cane sugar powder passing through 80 mesh sieve, raisins, cashewnut, cardamom, edible 'Sunset' yellow colour and hydrogenated vegetable fat ('Dalda') were procured from local market for developing the ready-mix for *Kheer*. The process is described below.

Soji samples were roasted with 5 per cent fat as per the house-hold practice and also without fat in an iron pan heated by cooking gas at temperatures ranging from 110 to 125°C. Similar roasting trials were carried out in a laboratory electrical grain roaster of 4 kg capacity at temperatures of 125-155°C. The ranges of temperatures for trials were chosen, keeping in view the influencing factors of roasting time, heat source and charring during household as well as commercial roasting operations.

Cardamom seeds were ground in a coffee grinder using fine sugar to minimize the loss of flavour. Cashewnuts were cleaned and broken into small pieces 5 mm) and roasted in fat at 150°C for about 30 sec. till the pieces attained light brown colour. Likewise, the cleaned raisins were also fried lightly in fat at 120°C for about 10 sec.

Different mixes of main ingredients, namely optimally roasted white and *Bansi soji*, finely powdered sugar and whole or skim milk powder, included at levels ranging from 25 to 40 per cent were tried to arrive at the desired formulation. Pre-processed cashewnut pieces (2.5-10), raisins (2.5-10),

'Sunset' yellow colour (20-50 ppm) and powdered cardamom (0.2-1.0) were also tried to arrive at optimum percentages. Various acceptability parameters such as, consistency of *Kheer* at desired dilution ratio of 1:5, (arrived at, after preliminary trials with dilution ratios ranging from 1:4 to 1:8), flavour, sweetness, adequacy of milk taste, settling of cooked *soji* particles etc. and cost were considered as deciding factors. Cashewnut and raisins were added to individual unit pouches to ensure their uniform distribution in *Kheer* ready-mix.

Proximate composition of *soji* samples and *Kheer* ready-mix formulation was determined in duplicate according to AACC procedures[3]. Amylogram characteristics of *soji* and ready-mixes were studied using 100 g samples dispersed in 450 ml water on Brabender amylograph according to AACC procedure[3]. Apparent viscosity studies of *soji* and ready-mixes were carried out using Brookfield LVT 4 viscometer. Weighed samples were added to boiling water and boiling was continued gently for 4-5 min. Apparent viscosity was measured at 50°C and 34°C.

For preparation of *Kheer* from ready-mix formulations, 100 g samples were dispersed in 500 ml boiling water and boiling was continued for 4-5 min with minimum evaporation losses. *Kheer* thus prepared was cooled to 50-60°C and offered to judges for sensory evaluation.

Sensory evaluation[4] of product was carried out by a panel of 10 judges on different formulations using numerical scoring test on a scale of 5 marks for different quality ratings: excellent-5, very good-4, good-3, fair-2, poor-1. Duncan's multiple range test[5] was applied to arrive at significant differences among samples.

Results and Discussion

Proximate composition of soji and Kheer *ready-mix:* The data on proximate composition presented in Table 1 show that *Bansi soji* from durum wheat had a higher protein content than that of white *soji* from aestivum wheat. Besides having high fat content of 11.5 per cent, *Kheer* ready-mix has excellent nutritive value, as is evident from its 13.3 per cent protein, nearly three-fourths of which is contributed by milk proteins of high biological value.

Formulations of Kheer *ready-mix:* On the basis of preliminary trials, roasting at 115-120°C in iron pan and at 145°C in grain roaster yielded optimally roasted *soji* with desired aroma and no charred particles.

Based on the evaluation by the panel of judges, it was inferred that the formulation based on 30 per cent roasted *soji*, 30 per cent fine sugar and 40 per cent whole milk powder (WMP) gives *Kheer* with desired consistency, taste and aroma (Table 2), *Kheer* based on only 25 per cent roasted *soji* had a somewhat thin consistency, with settling of cooked *soji* at the bottom. On the other hand, *Kheer* with 35 per cent roasted *soji* and 25-30 per cent sugar was lacking in taste of milk and sweetness. Use of 25 and 35 per cent sugar in ready-mix resulted in products of insufficient and more sweetness respectively. *Kheer* from ready-mix containing 30 and 35 per cent WMP had a somewhat "watery" taste. Further, keeping in view both the cost and taste factors, 5 per cent each of pre-processed cashewnut and raisins and 0.7 per cent of cardamom

TABLE 1. PROXIMATE COMPOSITION OF *SOJI* AND *KHEER* READY-MIX

Constituents	*Bansi soji*[a] (%)	White *soji*[b] (%)	*Kheer* ready-mix[c] (%)
Moisture	10.00	11.00	4.20
Protein	11.00[c]	9.50[c]	13.30[d]
Ether extractives	1.00	0.80	11.50
Total ash	0.85	0.65	3.01
Carbohydrates (by diff.)	77.20	78.10	68.00

[a]from extra hard durum wheat. [b]from medium hard *aestivum* wheat. [c]N × 5.7, [d]N × 6.25. [e]based on white *soji*

TABLE 2. FORMULATIONS TRIED FOR *KHEER* READY-MIX

Roasted *soji*[1] (%)	Powdered sugar (%)	Whole milk powder (%)
25	35	40
30	35	35
30	30	40
35	25	40
35	30	35
35	35	30

[1]White and *Bansi soji* used were processed from *Triticum aestivum* and durum wheats respectively.

TABLE 3. *KHEER* READY-MIX FORMULATIONS - SENSORY EVALUATION USING NUMERICAL SCORING AND DUNCAN'S MULTIPLE RANGE TESTS

Formulations*	Constituents	Mean panel scores+
A	*Bansi soji* + WMP	3.1 a
B	White *soji* + WMP	4.0 b
C	White *soji* + SMP	2.7 a
D	White *soji* + SMP + 5% fat**	3.5 ab
SE_m (27 d.f.)		±0.29

*All *Kheer* ready-mixes contained equal quantity of sugar.
**Added to simulate fat content contributed by WMP
+Means followed by different letters (a,b) differ significantly according to Duncan's multiple range test ($P<0.05$).

powder were considered as optimum. Likewise, 25 ppm of edible 'Sunset' yellow yielded *Kheer* with improved appearance.

Acceptability of Kheer: Sensory evaluation studies (Table 3) indicated no significant differences in acceptability between *Kheer* based on white *soji* + WMP and that based on white *soji* + SMP + 5 per cent fat (added to simulate fat contributed by WMP). The better acceptability of *Kheer* containing WMP as compared to that with SMP may be attributed to the fat content of WMP, which contributes towards better flavour, taste and consistency of product.

The *Kheer* based on white *soji* + WMP had better acceptability than that based on either *Bansi soji* + WMP or white *soji* + SMP. Possibly, extra hard granules of *Bansi soji* from durum wheat retained their shape even on cooking for 4-5 min and settled at the bottom of the container giving *Kheer* of non-uniform consistency. On the other hand, the relatively softer granules of white *soji* from aestivum wheat burst open easily on cooking and yielded a homogeneous product of uniform consistency. Further, white *soji* is more economical, since it costs about half as much as *Bansi soji*.

Amylograph characteristics: Amylograph data indicated that roasting of white or *Bansi soji* with or without 5 per cent fat did not show any difference in their peak viscosities (Table 4). As such, 5 per cent fat can be used for obtaining roasted *soji* of better aroma with lesser risk of charring and without affecting its consistency. The peak viscosity of unroasted *Bansi soji* was less than that of unroasted white *soji*, possibly due to extra hard nature of *Bansi* wheat containing more protein and lesser starch than aestivum wheat used for processing white *soji*. The results possibly indicate that the lower consistency of *Kheer* based on *Bansi soji* may be contributing to its lesser acceptability than the product based on white *soji*.

Amylograph studies on the effect of temperature (135-155°C) of roasting of white *soji* showed no change in the gelatinization temperature of 61°C. Peak viscosity of *soji* roasted at 145 or 155°C was the same (2138 BU) and was higher than that (1618 BU) of *soji* roasted at 135°C, as possibly, alpha-amylase activity is destroyed completely at the dry heat of 145°C, but not at 135°C. Based upon these observations, 145°C was considered as the optimum temperature for roasting of *soji* in a grain roaster.

TABLE 4. EFFECT OF FAT ON AMYLOGRAM CHARACTERISTICS OF ROASTED *SOJI*[1]

Sample	Gelatinization temp. °C		Peak viscosity[2] (B.U.)	
	White *soji*	*Bansi soji*	White *soji*	*Bansi soji*
Unroasted (control)	63	63	1342	828
Roasted				
with 5% fat	61	61	2138	1562
without fat	60	60	2138	1562

[1]roasting temperature 145°C
[2]measured in duplicate.

TABLE 5. APPARENT VISCOSITY[1] OF PROCESSED *SOJI* AND *KHEER* READY-MIX

Treatment of *soji*	Dilution ratio[3]	Apparent viscosity (poise) at	
		34°C	50°C
Soji (unroasted)			
White	1:10	77	34
Bansi	1:10	36	9
Soji (roasted)[2]			
White	1:10	86	48
Bansi	1:10	46	22
Kheer ready-mix			
White *soji* + WMP	1:5	5.0	3.2
White *soji* + SMP with 5% fat	1:5	4.5	3.0
White *soji* + SMP	1:5	3.4	2.6
Bansi soji + WMP	1:5	2.0	1.0

[1]Measured in triplicate using Brookfield LVT 4 viscometer with spindle number 3, speed used: 30 rpm.
[2]Roasted with 5% fat
[3]Used for dispersion of *soji* in water and reconstitution of ready-mix into *Kheer*.
WMP: whole milk powder; SMP: skim milk powder.

Viscosity studies: Apparent viscosity observed both at 34 and 50°C (Table 5) for *Kheer* based on white *soji* + WMP was higher than that of *Kheer* based on white *soji* + SMP + 5 per cent fat, possibly because, fat in WMP is in an emulsion form and contributes more towards increase in apparent viscosity than the fat added to SMP. The apparent viscosities observed at 50°C were lesser than those at 34°C for all the *Kheer* samples, as during cooling of *Kheer*, the jellying of starch at 34°C was comparatively more than at 50°C. *Bansi soji* exhibited lower apparent viscosity than white *soji*, because of the extra hard nature of granules of *Bansi soji*.

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Functional Role of Linseed (*Linum usitatissimum* L.) Polysaccharide in Steamed Pudding (*idli*)

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The seed coat of linseed (*Linum usitatissimum* L.) contains a mucilaginous polysaccharide which renders an aqueous suspension of the seeds highly viscous. Roasting the seeds before extraction of the polysaccharide or heat treatment of the polysaccharide after isolation reduces the viscosity by 50-60% in aqueous dispersions. Polysaccharide obtained from raw or roasted seeds were tested along with rice semolina and defatted peanut or sesame flours as sources of surface active proteins in steamed pudding (*idli*) type preparations. The porous texture of the puddings was stabilized satisfactorily by the native polysaccharide. A larger quantity of the polysaccharide from roasted seeds was required to stabilize the porous texture. Increasing the polysaccharide concentration above the optimal levels in the *idli* mix resulted in puddings with increased bulk density.

Among the fermented foods of India, *idli*, a popular breakfast food made by steaming blackgram flour and rice semolina after autofermenation of a batter, is relished for its special taste and texture qualities[1]. In recent years, attempts have been made to obtain *idli*-like products by substituting the cereal and legume components either separately or simultaneously[2-5]. Proteins from oilseed flours such as soybean, sesame and peanut have been shown to possess high surface activity and could replace the surface active proteins of blackgram in imparting a soft and porous texture to the steamed puddings[6]. However, they lacked the requisite adhesion or binding properties as carbohydrates of peanut[7] or sesame[8] are not sources of viscous polysaccharide. Addition of guar gum imparted the desirable adhesion or binding to the steamed puddings. Linseed contains 38-40 per cent fat, 23-25 per cent proteins and 15-20 per cent carbohydrates. The functional and physico-chemical properties[9-14] of the proteins have been studied. The structural aspects of the polysaccharide[15,16] present in the husk have been studied after fractionation and purification by ion-exchange chromatography[17]. Information available on the functional properties of this polysaccharide is rather scanty. In an earlier publication[18], the isolation and characterization of this polysaccharide and its ability to stabilise the foam formed by surface active proteins against thermal disruption has been described. Dev and Quensel[14] have observed that the presence of mucilaginous polysaccharide in linseed protein isolate increased its water absorption and emulsifying properties. In the present investigation, the effects of heat treatment of the seeds and of the isolated polysaccharide on the viscosity of aqueous dispersions and of the batters have been studied. The possibility of substituting blackgram polysaccharide by polysaccharide preparations from raw and roasted linseeds in the preparation *idli* type of steamed puddings has been tested and the results are described in this paper.

Materials and Methods

Linseed (*Linum usitatissimum* L.) 'Khategaon' was purchased from M/s. Flour and Foods Ltd., Indore, India. Rice, blackgram, peanut and sesame were purchased from the local market, cleaned and stored in sealed containers at 5-7°C until use. D-glucono-δ-lactone was from Pfizers, Chemical Division, New York. Rice semolina (30 mesh, BSS sieve) blackgram flour (80 mesh), trichloroacetic acid extracted and acetone-precipitated blackgram polysaccharide and defatted peanut and sesame flours were prepared according to the method of Susheelamma and Rao[1,6].

Estimations: Nitrogen was determined according to micro-kjeldahl procedure[19], protein according to Lowry *et al.*[20] and total sugar by phenol-sulphuric acid method of Dubois[21]. Surface activity was measured in a Stiepel type foam meter according to Susheelamma and Rao[1].

Preparation of the polysaccharide: Three lots of linseed (100 g each) were washed with water and soaked in one l of water at 5-7°C for 8, 16 and 24 hr respectively. The suspensions containing the swollen seeds were passed through a nylon cloth. The mucilaginous polysaccharide from the viscous fluid (750 ml) was precipitated by adding 2.25 l of ethanol

or acetone. The precipitated polysaccharide was redispersed in water, dialysed and lyophilised. Aliquots of dialysed polysaccharide extracts (250 ml) were dried at 110°C for 2-3 hr and redispersed in water for further work. The yields of polysaccharides from 8, 16 and 24 hr soaked seeds were about 5 per cent. They contained 93-95 per cent total sugar and 0.3-0.4 per cent micro-Kjeldahl nitrogen.

Heat treatment: One hundred and fifty gram batches of linseed were either roasted in an open pan on fire for about 5 min with continuous stirring or in an electrical roaster at 135°C for 2 min. The seeds were transferred to a cup, the bulk temperature (~133°C) measured and cooled to room temperature after spreading as a thin layer. Pan or roaster heated seeds (100 g) were soaked in water for 8 hr and the polysaccharide isolated as described above. Yield of the polysaccharide was about 5 per cent from either pan or roaster heated seeds. All analyses were done in triplicates from pan and roaster heated seeds.

Viscosity: Specific viscosity measurements were made at 27 ± 0.1°C in an Ostwald viscometer having a flow time of 30 sec with distilled water. Polysaccharide solutions (0.2-1 per cent) were prepared in distilled water and flow time recorded to ± 0.2 sec with a stop watch.

Aqueous suspensions of rice semolina, blackgram, peanut or sesame flour (5-35 per cent) or their batters containing rice semolina with blackgram, peanut or sesame flour (15-60 per cent) in the mix were prepared. Polysaccharides from blackgram or raw or roasted linseed were added to batters (0.1-0.5 per cent concentration). Viscosity measurements on these were made in triplicate in a Brookfield viscometer, model LVT, with spindle No. 4 at 25 ± 1°C.

Preparation of steamed puddings and bulk density determination: Dry mixes containing rice semolina along with blackgram, peanut or sesame flour, 12.5 mg of $NaHCO_3$, 25 mg of D-glucono-δ-lactone, 1.6 ml of water per g of dry mix were prepared. These batters were steamed for 10-12 min at 96-97°C in a closed vessel. Bulk densities of steamed puddings were determined according to Susheelamma and Rao[1]. Averages of bulk densities from three idependent experiments were taken in all cases. Photographs of cross sections were taken from the central portion of the steamed puddings.

Results and Discussion

The soft and spongy texture of *idli* has been associated with the presence of a highly surface active protein and viscous polysaccharide from black gram[1,22]. Attempts at substitution of black gram polysaccharide and protein by other sources will therefore have to be evaluated by determination of surface activity and viscosity, which are important parameters governing the texture of such products.

TABLE 1. PROTEIN CONTENT AND SURFACE ACTIVITY OF BLACKGRAM, PEANUT AND SESAME FLOURS

	Protein			Surface activity units	
	Total N×6.25 (%)	Q.01N NaOH soluble (%)	5% NaCl soluble (%)	10^{-3}/g	mg/Protein
Peanut	55	56.4	44	7.5	17
Sesame	35	37.8	34	8.0	23
Blackgram	24	23.5	18	5.0	27

Values are means of three independent determinations

Results shown in Table 1 indicate that peanut, sesame and black gram flours contained about 55, 39 and 24 per cent proteins (N × 6.25) respectively. Protein contents of alkaline extracts from these as determined by Folin phenol reagent were 56.4, 37.8 and 23.5 per cent respectively. The surface activities of peanut and sesame flours were comparable. Both had surface activities higher than blackgram flour, but specific activities (surface activity/mg protein) were lower. The major portion of surface active constituents could be extracted with 5 per cent NaCl and precipitated with ammonium sulphate between 40 and 75 per cent (w/v) concentration with a four – fold increase in specific (surface) activity.

Linseed polysaccharide was isolated after soaking the seeds in water. The effect of soaking time on viscosity (Table 2) indicates that specific viscosity of the polysaccharide did not change until 16 hr, but shows a marginal decrease of 10 per cent after soaking for 24 hr.

Heat treatment and viscosity: The effect of heat treatment on viscosity of the polysaccharide is shown in Fig. 1. Native linseed polysaccharide is less viscous than blackgram polysaccharide. Polysaccharide

TABLE 2. EFFECT OF SOAKING TIME ON VISCOSITY OF LINSEED POLYSACCHARIDE

Time (hr)	Concn of polysaccharide (%)	Specific viscosity (cps)
8	0.90	103.2
8	0.96	108.5
16	0.91	104.5
24	0.91	95.0

Values are means of three independent determinations

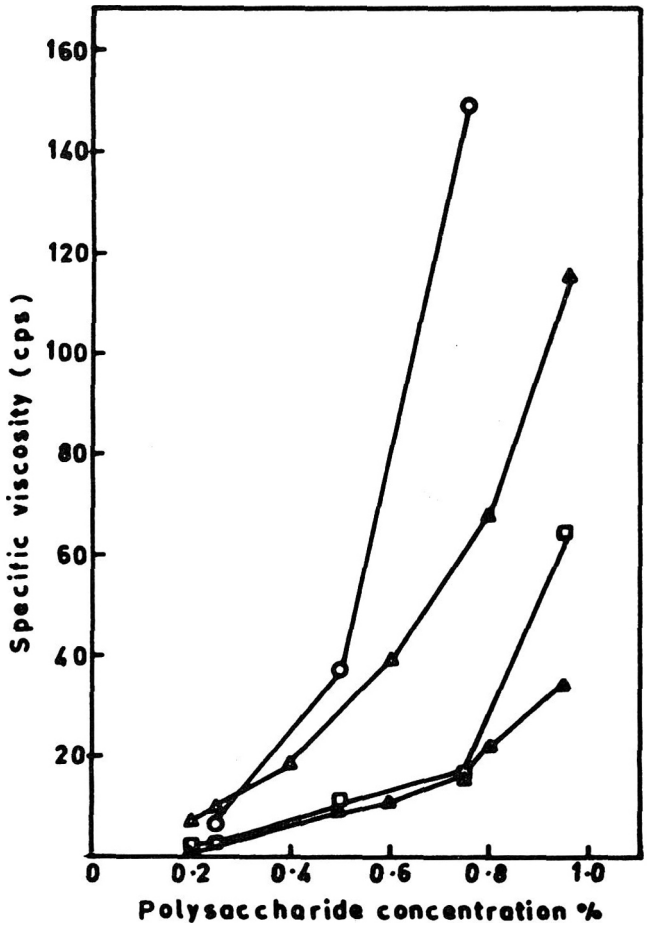

Fig. 1. Viscosity of linseed polysaccharide before and after heat treatment.
TCA polysaccharide from blackgram o———o; linseed polysaccharide from raw seeds △———△; and after drying at 110°C for 2-3 hr▲———▲; polysaccharide from roasted linseed □———□.

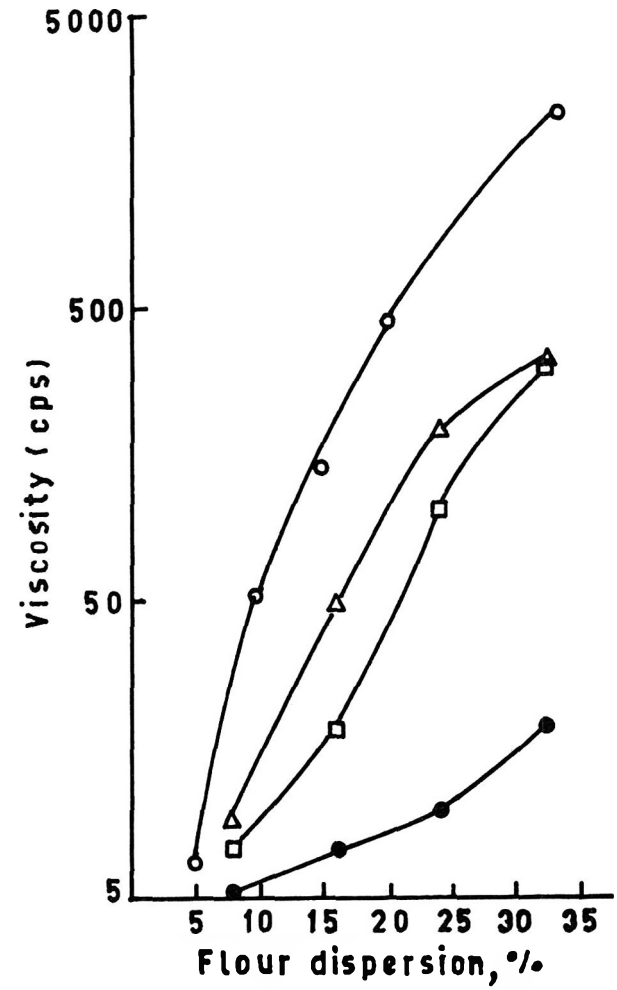

Fig. 2. Viscosity of flour dispersions.
Rice semolina ●———●; blackgram flour o———o; sesame flour △———△ and peanut flour □———□.

obtained from roasted seeds or native polysaccharide dried by heating at 110°C for 2-3 hr and redispersed in water showed about 50-60 per cent decrease in viscosity over 0.25-0.75 per cent concentration. However, it has been reported earlier that heating aqueous dispersion of native polysaccharide at 95°C for 30 min and cooling did not bring about any change in viscosity[18].

When similar experiments were carried out on blackgram seeds[23], it was found that roasting the seeds to 110°C and 155°C reduced the viscosity of the isolated polysaccharide by 80 and 98 per cent respectively. Native polysaccharide after drying at 155°C for 20 min and redispersion in water showed about 97-98 per cent reduction in viscosity. Heating aqueous dispersion of native polysaccharide at 95°C for 30 min and cooling caused a reduction of 20 per cent viscosity. These results indicate that blackgram polysaccharide has higher viscosity and also higher thermal sensitivity than linseed polysaccharide.

Viscosity of aqueous dispersions and batter: Data shown in Fig. 2 and Fig. 3 indicate that viscosity of peanut and sesame flour dispersions and their corresponding batters was much less than that of blackgram. Viscosities of batters were greater than those of flour dispersions, probably due to the presence of rice semolina which may exhibit some dilatancy, and give rise to an apparent increase in viscosity. This effect is more pronounced in blackgram batters due to the presence of a highly viscous polysaccharide. At about 0.2 per cent concentration of polysaccharide from raw linseeds and at 0.4 per cent of polysaccharide from roasted seeds (isoviscous levels) viscosities of batters were comparable to that containing 0.1 per cent polysaccharide from blackgram (Fig. 4).

Bulk density: Data presented in Table 3 indicate that rice semolina alone (control) had a bulk density of 0.89; addition of blackgram flour at an optimum level of cereal to pulse of 2:1, reduced it to 0.55.

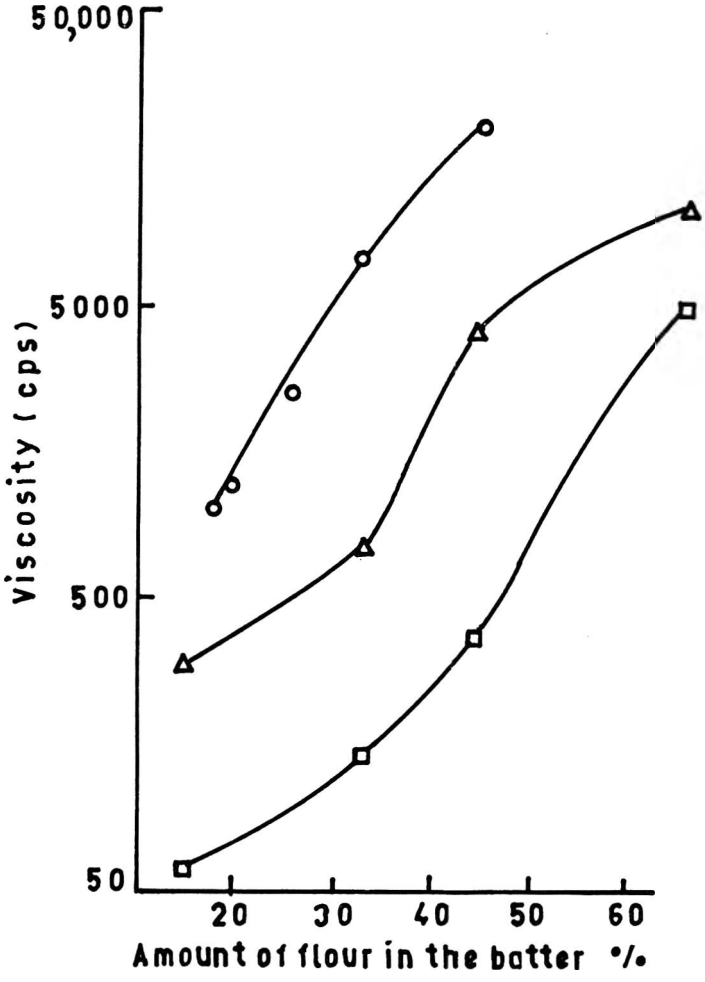

Fig. 3. Viscosity of batters containing rice semolina along with blackgram flour o———o; sesame flour △———△ and peanut flour □———□.

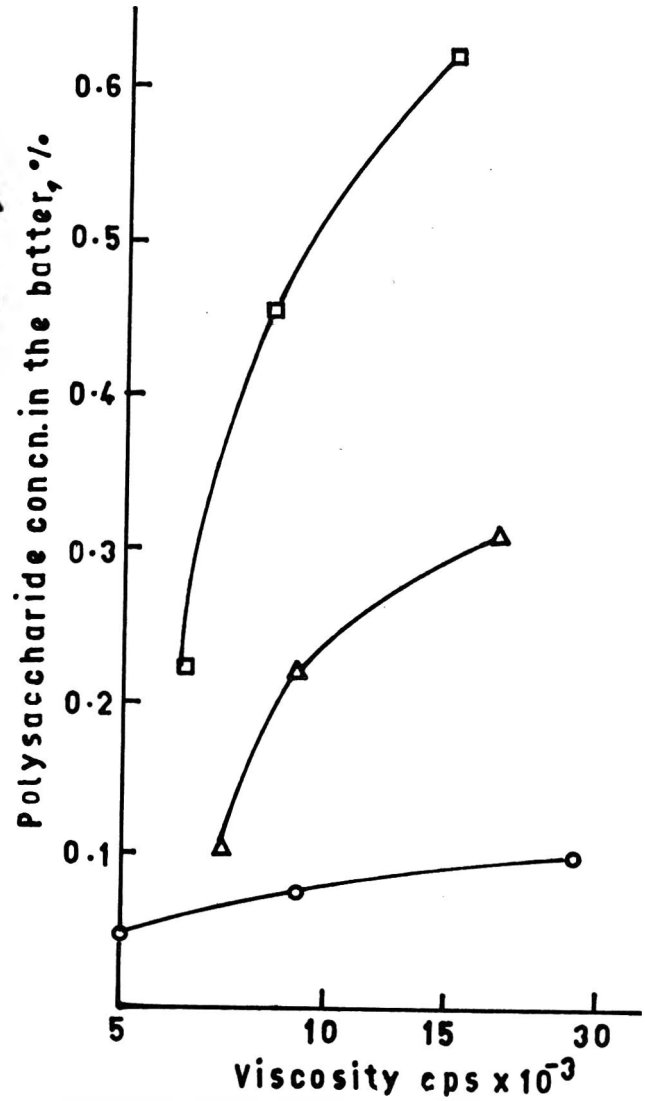

Fig. 4. Viscosity of batters containing rice semolina along with blackgram polysaccharide o———o; sesame flour (20 per cent) and linseed polysaccharide from raw seeds △———△ and from roasted seeds □———□.

Steamed puddings containing 25 per cent peanut flour or 20 per cent sesame flour in the mix but without any polysaccharide or up to 0.1 per cent concentration of polysaccharide could be expected to give lower (<0.55) values of bulk density (as observed by the volume of batter after steaming), but could not be handled for bulk density determination as they were very fluffy and floury. Addition of 0.2 and 0.4 per cent polysaccharide from raw and roasted linseed to the above batters reduced the bulk density values comparable to that having 33 per cent blackgram flour in the batter. Puddings containing sesame flour had slightly lower bulk density compared to those containing peanut flour. As the polysaccharide concentration from raw or roasted seeds was increased in the batter, bulk densities of the puddings increased with either peanut or sesame flour in the mix. These results are similar to those obtained with blackgram flour[22].

Cross sections of the steamed puddings are shown in Fig. 5. It is seen that as the amount of polysaccharide in the batter increases, the porosity decreases. At higher concentrations of the polysaccharide, the full expansion of the porous texture imparted by the surface active protein was prevented due to increased viscosity of the batter. This may lead to reduced evaporation of moisture from the porous matrix giving rise to denser products.

Similar trend was observed during stabilization of foam (formed by the surface active proteins) by the polysaccharide preparations. Lower foam volumes were obtained at higher concentrations[18,22]. At functionally optimal concentration of the polysaccharide and protein in the batter, the bulk densities of the puddings will be lower and the texture will be satisfactory. (with adequate binding in the product).

TABLE 3. BULK DENSITY OF STEAMED PUDDING CONTAINING LINSEED POLYSACCHARIDE

Sample	Polysaccharide concn. (%)	Bulk density (g/ml)
Rice semolina (RS)	–	0.89
RS + Blackgram flour (33%)	–	0.55
RS + Sesame flour (20%) + LP	0.20	0.58
	0.25	0.71
	0.30	0.83
RS + Sesame flour (20%) + LPH	0.40	0.65
	0.51	0.72
	0.60	0.86
RS + Peanut flour (25%) + LP	0.20	0.60
	0.25	0.73
	0.30	0.85
RS + Peanut flour (25%) + LPH	0.40	0.67
	0.51	0.76
	0.60	0.88

Values are means of three independent determinations
Total weight of mix was 15g in all cases.
LP = Linseed polysaccharide from raw seeds. LPH = Linseed polysaccharide from roasted seeds.

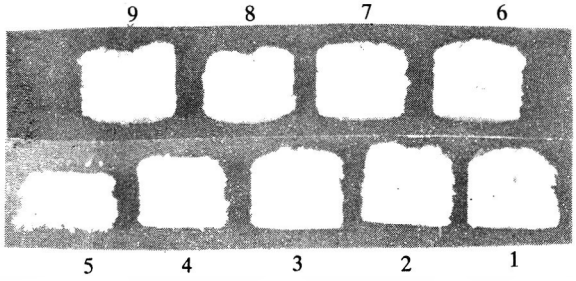

Fig. 5. Cross sections of steamed puddings containing rice semolina along with sesame flour (20 per cent) and different concentrations of linseed polysaccharide in the mix. 1,2,3,4, contained 0.20, 0.40, 0.50, and 0.60 per cent of polysaccharide from roasted linseeds in the mix.
5. Rice semilina control.
6,7,8,9, contained 0.10, 0.20, 0.25 and 0.30 per cent of polysaccharide (from raw linseeds) in the mix.

These investigations indicate that polysaccharide from linseeds could satisfactorily replace the polysaccharide from blackgram in *idli* type of puddings. After heat treatment of the seeds (as used in some condiment type of foods) for a short time, the isolated polysaccharide looses 50 per cent of its viscosity, but in greater quantities could still find use in steam pudding (*idli*) type of preparations.

Acknowledgement

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Microflora Associated with Indian Punjabi *Warri* Fermentation

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Microbiological analysis of all the fermented *warri* dough samples collected from the local market and those prepared in the laboratory revealed the occurrence of bacteria in the range of 10^9 to 10^{12}/g belonging to six species. Half of the samples also exhibited ten yeasts upto 10^7/g. The development and prevalence of microorganisms was affected by season; summers being more favourable for bacteria and winters for yeasts. Laboratory studies indicated that both the microbial types, whenever present tended to increase significantly with the progress in fermentation. *Leuconostoc mesenteroides, Lactobacillus fermentum* and *Streptococcus faecalis* were the principal bacteria in *warri* dough fermentation causing acidification and leavening. Yeast flora, whenever present comprised mainly *Saccharomyces cerevisiae, Pichia membranaefaciens* and *Trichosporon beigelii.*

Fermented foods derived from pulses form an important part of the human diet in developing countries including India. Since the traditional oriental food fermentations involve the natural microflora coming from staples and surroundings, the study of biological agents actually associated with fermented foodstuffs has attracted considerable attention over the years. A review of the published reports shows that the microorganisms involved in fermentations include filamentous fungi, yeasts and bacteria occurring in succession or in combination[1-5].

Warries, somewhat like japanese *miso*, are spicy, hollow, brittle, friable balls 5-8 cm in diameter, very popular in Punjab. These are prepared from fermented black gram (*Phaseolus mungo*) paste supplemented with several spices. Although some reports have appeared on the distribution of microorganisms in fermented *warri* doughs[1,6,7], there is no comprehensive study on the organisms actually playing a role in fermentation.

The present study was aimed at finding out the different types of microorganisms in fermented *warri* dough samples collected from the local market and those prepared in the laboratory, the effect of seasonal variations on their prevalence and the sequential development and role of various predominant microbial types during the fermentation.

Materials and Methods

The traditional method in practice for the preparation of punjabi *warri* is as follows. Dehulled black gram (*Phaseolus mungo*) is washed and soaked overnight in water and ground to a paste. The paste is added, with asafoetida (*Ferula foetida*, 0.5-1.0 per cent), caraway (*Carum carvi*, 0.5-1.0 per cent), cardamom (*Elettaria cardamomum*, 1.0 per cent), cloves (*Syzygium aromaticum*, 0.4-0.5 per cent), fenugreek (*Trigonella faenumgraecum* 1.0 per cent), ginger (*Zingiber officinale*, 8.0 per cent) and red pepper (*Capsicum annum*, 1.0 per cent) and the resulting dough moulded into small balls which assume the form of hollow, spicy and brittle friable balls 3-5 cm in diameter after undergoing simultaneous fermentation and drying in open air for 4-8 days.

Market samples: *Warri* dough samples at different stages of fermentation were collected from different places of Amritsar city (India) during summers and winters.

Laboratory fermented samples: *Warries* were also prepared in the laboratory by fermenting dehulled black gram employing the procedure as mentioned earlier and the samples were drawn successively at 24 hr intervals for 5 days.

Analysis of samples: All the market and laboratory samples along with the raw materials were analysed immediately for microbial load, its predominant types and the moisture content.

The microbial load was analysed by taking 1 g (wet weight) sample in a small weighing bottle and making serial dilutions in sterilised distilled water. Appropriate dilutions were spread on nutrient agar, APT agar (HI-Media, Bombay, India), GYP-$CaCO_3$ agar[8] and yeast extract-malt extract agar plates for the enumeration of total bacteria, *Leuconostoc* and

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lactobacilli, acid producing bacteria and total yeasts, respectively. The plates for bacteria and yeasts were incubated at 37° and 28°C respectively and the colonies appearing after 48 hr were picked up and purified by streaking on agar plates.

Detailed taxonomic study of bacteria was carried out according to the Bergey's manual of determinative bacteriology[9] while yeasts were identified according to the criteria laid down by Barnett *et al.*[10] and Kreger van Rij[11]. Moisture content in the doughs was determined by drying 1 g samples at 95-100°C to constant weight.

Results and Discussion

Occurrence and prevalence of microflora in fermented warri doughs: Table 1 gives the complete microbiological analysis of fermented *warri* doughs collected from the market and those prepared in the laboratory. The microflora consisted of bacteria alone or bacteria with yeasts. All the 40 samples contained

TABLE 1. OCCURRENCE OF BACTERIA AND YEASTS IN FERMENTED *WARRI* DOUGHS

Source of dough	Season	Atmospheric temp (°C)	Samples studied (No.)*	Bacteria			Yeasts			
				Total count/g D.M.	No.of isolates		+ve Samples (No.)	Total count/g.D.M.	No.of isolates	
					Genera	Species			Genera	Species
Local market	Summer	35–42	10	10^{10}–10^{12}	4	4	3	0–10^{4}	4	4
	Winter	15–25	10	10^{9}–10^{10}	3	3	8	0–10^{7}	6	7
Laboratory	Summer	35–42	10	10^{11}–10^{12}	6	6	2	0–10^{3}	6	6
	Winter	15–25	10	10^{10}–10^{11}	5	5	7	0–10^{6}	8	10

*All the samples were positive for bacteria
D.M.: Dry matter

TABLE 2. PREVALENCE OF DIFFERENT BACTERIA AND YEASTS IN FERMENTED *WARRI* DOUGHS

Bacteria	Positive samples		Yeasts	Positive samples	
	(No.)	(%)		(No.)	(%)
Market Samples*					
Leuconostoc mesenteroides (SW)	20	100	*Saccharomyces cerevisiae* (SW)	11	55
Streptococcus faecalis (SW)	19	95	*Pichia membranaefaciens* SW)	11	55
Lactobacillus fermentum (SW)	18	90	*Candida vartiovaarai* (W)	8	40
Bacillus subtilis (S)	12	60	*Kluyveromyces marxianus* (W)	8	40
			Trichosporon beigelii (SW)	8	40
			Candida krusei (W)	6	30
			Hansenula anomala (SW)	6	30
Laboratory Samples*					
Leuconostoc mesenteroides (SW)	18	90	*Kluyeromyces marxianus* (SW)	9	45
Lactobacillus fermentum (SW)	17	85	*Candida aquatica* (W)	9	45
Streptococcus faecalis (SW)	16	80	*Candida krusei* (SW)	9	45
Bacillus subtilis (SW)	15	75	*Pichia membranae faciens* (SW)	9	45
Flavobacter sp. (SW)	15	75	*Saccharomyces cerevisiae* (SW)	8	40
Enterobacter sp. (S)	12	60	*Trichosporon beigelii* (SW)	7	35
			Candida vartiovaarai (W)	4	20
			Hansenula anomala (SW)	4	20
			Cryptococcus humicolus (W)	2	10
			Geotrichum candidum (W)	2	10

(S) Present in summer samples
(W) Present in winter samples
(SW) Present during both the seasons
*Expressed on the basis of a total of 20 samples each

bacteria while only half of the samples had yeasts. The market samples contained bacteria ranging from 10^9 to 10^{12}/g dry matter and belonged to *Leuconostoc mesenteroides, Streptococcus faecalis, Lactobacillus fermentum* and *Bacillus subtilis* (Table 2). Yeasts ranged from 0 to 10^7/g dry matter and were present in 55 per cent samples. They included *Saccharomyces cerevisiae* and *Pichia membranaefaciens*, the most predominant species followed by *Candida vartiovaarai, Kluyveromyces marxianus* and *Trichosporon beigelii* and others as indicated in Table 2.

The laboratory fermented samples contained higher bacterial load and belonged to 6 genera and 6 species (Table 1). Yeasts were present in 45 per cent samples and ranged up to 10^6/g yielding 10 species belonging to 8 genera. The predominance of the microorganisms belonging to bacteria and yeasts was similar to that observed in the market samples (Table 2).

The common occurrence of *Leuconostoc mesenteroides, Lactobacillus fermentum, Streptococcus faecalis* and less prevalence of yeasts in the market and laboratory fermented samples indicate the direct involvement of the former in the traditional *warri* fermentation process. Moreover, since the source of inoculum in the fermentation is not apparent, it is likely that the black gram and the spices mixture used in the preparation of doughs, workers handling the preparations and the aerial environment are contributing to the initial inoculation for the fermentation. Microbiological analysis of the dry grains of the ingredients and spices used exhibited the association of *Leuconostoc* and Lactobacilli. Yeasts were however negligible in market and laboratory samples. Some earlier findings have also suggested the role of natural microbial load of ingredients and the environment in the initiation of traditional fermentations. Mukherjee *et al.*[12] and Ramakrishnan[13] reported that dehulled black gram harbours *Leuconostoc mesenteroides* and other lactic acid bacteria in large numbers which play a major role in black gram fermentation. Presoaking of the ingredients releases the free sugars and non-protein nitrogen which support the growth of lactic acid bacteria in traditional fermentations[14,15]. Sandhu and Waraich[16] isolated 7 yeasts including *Saccharomyces cerevisiae* from air and suggested that the aerial contamination of doughs by some of these yeasts cannot, therefore, be ruled out[7].

The lower frequency of occurrence of yeasts in the fermentations carried out by natural microflora is probably due to their less prevalence on the dry ingredients and/or due to low moisture content of the latter. Since the black gram grains are presoaked in water and then ground to a paste, yeast species probably develop only after the softening of these, presumably, because yeasts require higher moisture content for their survival than bacteria. Spices have also been found to stimulate the development of lactic acid bacteria[17] and some of these contain manganese as a factor responsible for the stimulatory action[18].

Seasonal variations: Market samples collected in summer exhibited a higher bacterial load yielding 4 isolates than the samples collected during winter which yielded 3 different species. Yeasts were cultured from 30 per cent summer samples (0 to 10^4/g, 4 isolates) and 80 per cent winter samples (0 to 10^7/g; Table 1). Prevalence of various bacterial and yeast species during summers and winters has been mentioned in Table 2. Samples fermented in the laboratory during the two seasons also exhibited a similar pattern.

The effect of seasonal variations on the development and prevalence of microbes could be attributed to the appreciable difference in atmospheric temperature during the two seasons. Temperature of 37 to 42°C during summers probably favoured the rapid multiplication of bacteria and was unfavourable for the yeasts which show optimum growth at 28°C. Since the number of yeasts capable of growing at 37-42°C are only a few, less prevalence of these during summers can be justified. The occurrence of *Saccharomyces cerevisiae, Pichia membranaefaciens, Trichosporon beigelii* and *Hansenula anomala* in some summer samples is probably due to their ability to withstand the temperature upto 42°C, as indicated by their physiological studies (Table 4). On the other hand, high prevalence of yeasts during winters can be justified as due to prevailing favourable temperature for their propagation.

Successive changes in microbial populations during warri *dough fermentation:* Table 3 shows the prevalence of microbial populations and their predominant types during 5 day fermentation. Both bacteria and yeasts increased significantly with the progress in fermentation. The increase in microbial load was followed by a decrease in pH and increase in dough volume. The profile of bacterial and yeast genera is shown in Table 3.

The initial appearance of *Leuconostoc* and lactobacilli, as indicated by the successional study on *warri* fermentation, is presumably due to their association with the dry black gram grains and spices mixture, the main ingredients for fermentation. The biochemical and physiological characterisation of the predominant microorganisms (Table 4) indicated that both *Leuconostoc* and lactobacilli perhaps produce acid and gas with the progress in fermentation causing acidification and leavening, leading to the fall in pH and rise in volume, thus making the environment unfit

TABLE 3. SUCCESSIVE CHANGES IN MICROFLORA DURING *WARRI* FERMENTATION

Incubation period (days)	pH	Volume (ml)	Bacteria		Yeasts+	
			Count/g	Predominant types*	Count/g	Predominant types*
0	5.65	200	1.3×10^{10}	*Leuconostoc mesenteroides* *Lactobacillus delbrueckii* *Lactobacillus fermentum* *Bacillus subtilis* *Flavobacter* sp.	$0–8.0\times10^{4}$	*Trichosporon beigelii* *Saccharomyces cerevisiae* *Candida krusei* *Pichia membranaefaciens* *Hansenula anomala*
1	4.70	420	2.1×10^{12}	*Leuconostoc mesenteroides* *Lactobacillus fermentum* *Streptococcus faecalis* *Bacillus subtilis*	$0–1.7\times10^{6}$	*Trichosporon beigelii* *Saccharomyces cerevisiae* *Candida krusei* *Pichia membranaefaciens*
2	3.90	420	3.0×10^{12}	*Leuconostoc mesenteroides* *Lactobacillus fermentum* *Streptococcus faecalis*	$0–2.1\times10^{6}$	*Trichosporon beigelii* *Saccharomyces cerevisiae* *Candida krusei*
3	*3.50*	*420*	4.1×10^{12}	*Leuconostoc mesenteroides* *Lactobacillus fermentum* *Streptococcus faecalis*	$0–9.1\times10^{6}$	*Trichosporon beigelii* *Saccharomyces cerevisiae*
4	3.25	420	4.7×10^{12}	*Leuconostoc mesenteroides* *Lactobacillus fermentum*	$0–6.9\times10^{7}$	*Saccharomyces cerevisiae* *Trichosporon beigelii*
5	3.20	420	6.5×10^{12}	*Leuconostoc mesenteroides* *Lactobacillus fermentum*	$0–9.6\times10^{6}$	*Saccharomyces cerevisiae* *Trichosporon beigelii*

*Arranged in the decreasing order of frequency
+Yeasts were observed in 9 batches only
The data represent the average values of 20 batches of laboratory fermentations

TABLE 4. SOME BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERISTICS OF PREDOMINANT MICROORGANISMS INVOLVED IN PUNJABI *WARRI* FERMENTATION

Organism	Production			Assimilation		Growth at indicated temp & pH		
	Glucose	Maltose	Sucrose	Maltose	Starch	37°C	42°C	pH 4.0
Lactobacillus fermentum	+	+	+a	–	–	+	+	+
Leuconostoc mesenteroides	+	+a	+a	–	+	+	+	+
Streptococcus faecalis	+	+	–	–	+	+	+	+
Candida krusei	+	–	–	–	–	+	–	+
Pichia membranaefaciens	–	–	–	–	–	+,–	–	+
Saccharomyces cerevisiae	+	+,–	+,–	+	+,–	+,–	+,–	+
Trichosporon beigelii	–	–	–	+	+	+,–	+,–	+

a Production of acid but not gas
+,– Variable in different isolates

for other contaminants. *Streptococcus faecalis* probably comes from air in later stages and produces more acid causing further acidification. Yeasts perhaps help in the degradation of starch into maltose and glucose by producing extracellular amylolytic enzymes (Table 4). Some strains of *Saccharomyces cerevisiae* also produce acid and gas from starch and may thus add to the leavening during fermentation. The major role of *Leuconostoc mesenteroides* has also been reported earlier in black gram fermentations by Batra[1] and Sandhu *et al.*[6] who also isolated *Lactobacillus fermentum* and *Saccharomyces cerevisiae* from *warri* paste.

The increase in total acidity as indicated by the fall in pH as a result of fermentation probably helps in enhancing the shelf-life of *warris* and prevents the growth and transmission of various pathogenic micro-organisms. These assumptions justify the earlier reports of van Veen and Schoefer[19], who stated that fermentations prolong the shelf-life particularly of the foods containing salt and spices.

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Surface Heat Transfer Coefficient of Rice Puffed with Sand

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An unsteady state heat transfer analysis was made on rice grain while it was being manually agitated inside heated sand. The analysis considered rice grain as long circular cylinder and heat transfer took place along the radial direction only. 'Kukri' variety of rice at 10% moisture when puffed at sand temperatures of 230 to 250°C showed that the average puffing temperature was 156.6°C. Based on estimated values of thermal properties of rice grain, the value of heat transfer coefficient on the grain surface was found to be 72 W/m^{20}C.

Traditionally puffing of grains is done by agitating them with sand in a vessel while supplying heat by fire from underneath the vessel. The sand acts as a convenient medium of heat transfer. The separation of sand from puffed grain is done through a sieve wherein the puffed material is retained and the sand passes through the sieve. For making expanded rice, parboiled rice is normally puffed. Prior to puffing, the parboiled rice is conditioned by mixing with salt and water followed by subsequent heating so that it attains a desirable moisture. In a study by Chinnaswamy and Bhattacharya[1] the maximum expansion of 'Intan', 'Asm 44' and 'Chianan 8' varieties of parboiled rice was obtained at moisture content 10.5-11 per cent with 15 times its weight of fine sand at 250°C for 10-11 sec. They also observed that particle size of sand (60 to 22 mesh) did not have any significant effect on the expansion of rice. For puffing of paddy, Murugesan and Bhattacharya[2] found the optimum sand temperature to be 190-210°C and the duration of puffing was 40-45 sec. The process of preparation of parboiled rice considerably affects the quality of puffed product[3]. Other than sand, hot air or oil can also be used[4].

A knowledge of the heat transfer characteristics of heated sand is important in order to bring about any improvement in the indigenous process. Heated sand, apart from being used as a convenient heating medium for the puffing of rice, is also used for making parched rice and beaten rice. Design of a continuous sand roaster requires that the grain should reside inside the roaster for the requisite duration so that the grain attains an average temperature sufficient to puff at the discharge end of the roaster. It is expected that at this temperature, the vapour pressure of water within the grain develops so high that the grain can no longer hold it in vapour form. It then comes out with an explosion. Conditioning and subsequent heating help the grain to retain moisture to a pressure higher than the atmospheric pressure. These two steps probably bring some change in the surface starch and block surface pores resulting in the retention of moisture inside the grain to a higher pressure. As the sand is agitated during the puffing process, the temperature at the surface of the grain will not be equal to the temperature of the sand and a convective resistance to heat transfer will be present. The present investigation was aimed at finding the average temperature of puffing of rice and the convective heat transfer coefficient on the surface of rice grain inside an agitated sand bed which is normally used for puffing rice.

Materials and Methods

Preconditioning and puffing of rice: 'Kukri' variety of double parboiled commercial rice was used. The rice was hand picked to remove foreign material and brokens. It was washed with water for a few seconds which increased the moisture content to about 20 per cent. Two per cent salt was then sprinkled and mixed well for 20-30 min. The salted rice was dried in a tray drier at 80°C to bring down the moisture content to 10 per cent.

Puffing of the rice was done a few days after the conditioning process in an open aluminium pan with a grain to sand (passed through 36 mesh and retained on 30 mesh) ratio of 1:10. The heat was obtained from a liquified petroleum gas burner. Sand in the pan was manually agitated with a stainless steel ladle. The temperature was measured by a chromel alumel thermocouple attached on the flat surface of the ladle. The lead wires from the thermocouple were connected to a digital temperature indicator (Masibus, Ahmedabad) which had a least count of 1°C. When

the sand attained the desired temperature the gas burner was put off and the rice (100 g) was poured into it. The rice was vigorously agitated and a stop watch having a least count of 0.1 sec was used to measure the time required for the start and finish of the puffing. For the analysis of heat transfer, the average value of these two times was used. After completion of puffing, the rice-sand mixture was poured on a sieve and the puffed product was sieved off. The size of the sieve was large enough to drain out the sand almost instantaneously. The puffed rice was quickly collected at one corner of the sieve and the tip of the thermocouple (already removed from the ladle) was inserted into the centre of the heaped mass of rice. The thermocouple tip was slightly moved around the centre and the maximum indicated temperature was recorded. This temperature was considered as the temperature at which the puffing of rice took place.

Thermal properties of rice grain: Thermal conductivity and thermal diffusivity of rice grain are the properties required for the analysis of heat transfer during puffing. The value of thermal conductivity, k, and thermal diffusivity, α were estimated based on weighted average of the respective values for moisture and dry solids present in the grain[5].

$$k = 0.15\,(1 - X_w) + k_w X_w \quad .. (1)$$

$$\alpha = 0.0885 \times 10^{-6}\,(1 - X_w) + \alpha\, \varpi\, X_w \quad .. (2)$$

where k and k_w are the thermal conductivity of rice and water respectively, W/m°C, α and α_w are the thermal diffusivity of rice and water respectively, m^2/sec, and X_w is the fraction of water present in the grain. The value of k_w and α_w were obtained from the properties of liquid water at the average of initial and final temperatures of grain[6].

Heat conduction in rice grain: The length of a rice grain is normally much greater than its other two dimensions. The major amount of heat transfer would, therefore, occur along the two short dimensions which generally form an ellipse[7]. Since parboiling, drying and milling processes distort the elliptic cross-section, we shall consider the grain to be circular having diameter as the average of its two short dimensions.

Since puffing is completed within a few seconds, the temperature of sand during the process may be assumed to remain constant. For the case of unsteady state, heat conduction through a long cylindrical grain having diameter 2a, the average temperature, T of the grain after a time θ measured from the time the grain is put into the hot sand is given by[8],

$$\frac{T' - \bar{T}}{T' - T_0} = 4\left(\frac{ha}{k}\right) \frac{J_1(A_1)}{A_1\left[A_1^2 + \left(\frac{ha}{k}\right)^2\right] J_0(A_1)}\, e^{-A_1^2 \frac{\alpha\theta}{a^2}} \quad .. (3)$$

Subject to the condition that $\alpha\theta/a^2 > 0.2$. T_0 is the initial temperature (°C) of the grain and this is considered to be uniform and the same as that of the ambient temperature. T′ is the temperature of the hot sand at the time of puffing, °C h is the heat transfer coefficient on the surface of the grain, W/m^2 °C and a is the equivalent radius of the grain given by,

$$a = \frac{(l_1 + l_2)}{4} \quad .. (4)$$

where l_1 and l_2 are the two short dimensions of the grain in meters.

The value of A_1 in Eqn. (3) is the first root of the transcendental equation,

$$A\, J_1(A) = \left(\frac{ha}{k}\right) J_0(A) \quad .. (5)$$

where J_0 and J_1 are the Bessel function of order 0 and 1 respectively. For various values of (ha/k) the first root A_1 of Eqn. (5) is available in mathematical tables[8].

Assuming that no moisture loss takes place from the grain until it puffs, the values of k and α of the grain would remain constant during puffing. If our hypothesis that a rice grain would puff at a constant average temperatures $\bar{T}$ irrespective of the temperature of hot sand is true, then for a series of values of sand temperature, T′ and the corresponding puffing time, θ a plot of log $[(T' - \bar{T})/(T' - T_0)]$ against θ would yield a straight line, the slope of which will be $A_2^1\, \alpha/(2.303\, a^2)$ and from the known values of α and a of the grain the values of A_1 will be known. Corresponding to this value of A_1, the value of (ha/k) would then be obtained from mathematical tables. The value of h can be calculated from the known value of (ha/k).

Results and Discussion

Sand temperature and time of puffing: The time required for the rice to start and finish puffing at various sand temperatures are shown in Table 1.

TABLE 1. SAND TEMPERATURE, TIME OF PUFFING AND TEMPERATURE OF PUFFED RICE

Sand temp. T′ (°C)	Time required for		Av duration of puffing, θ	Temp. of puffed rice, $\bar{T}$
	Start of puffing (sec.)	Finish puffing (sec.)	(sec.)	(°C)
230	8.3	18.0	13.2	157
235	8.0	17.0	12.5	158
240	8.0	15.0	11.5	158
245	7.0	14.5	10.8	155
250	6.0	14.0	10.0	155

Initial temp. of rice (T_0) is 31 °C

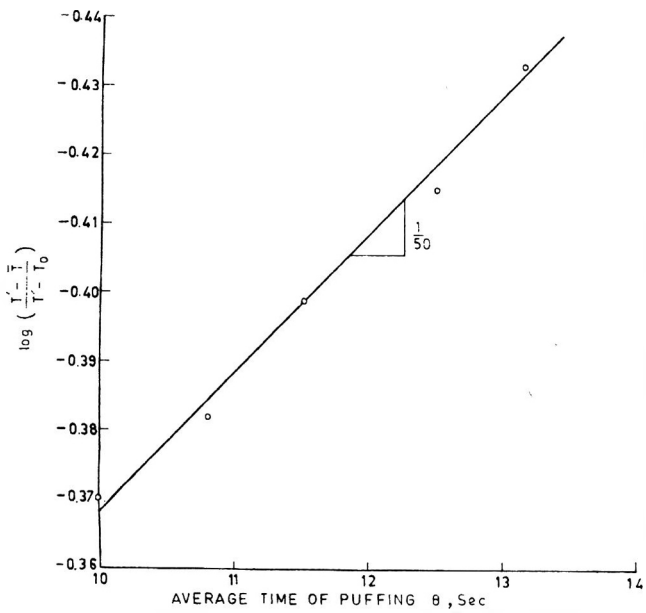

Fig. 1. Relationship between log $[(T - \bar{T})/(T' - T_0)]$ and θ

From Table 1 it is observed that the 'Kukri' variety of rice has puffed at nearly a constant temperature irrespective of the varying sand temperatures. This temperature is the average of the five observed T values, i.e., 156.6 ± 1.4°C.

Physical properties of rice: The average size of the 'Kukri' variety of parboiled rice were: length, 5.7 ± 0.56 mm, width, 2.6 ± 0.12 mm and thickness, 1.35 ± 0.16 mm. The length of the grain is thus much greater than its width and thickness. The cross section of the grain was such that it could be assumed to be nearly circular, average radius, α of this circle being 0.25 (2.69 + 1.85) i.e., 1.135 mm.

The thermal conductivity, k_w of water (Eqn.1) was estimated at the mean of initial grain temperature T_0 and the average temperature, $\bar{T}$ of the product. Since T_0 = 31°C and $\bar{T}$ =156.6°C, the value of k_w was obtained at the average of these two temperatures, i.e., 94°C and this was 0.6806 W/m°C. At this temperature, the density and specific heat of water are 962.556 kg/m^3 and 4212.4 J/kg°C respectively[6]. The value of α_w in Eq. (2) is, therefore, 0.6806/ (962.556 × 4212.4), i.e., 1.68×10^{-7} m^2/sec.

Using the values of k_w and α_w the values of k and α of the grain at 10 per cent moisture (X_w = 0.1) were estimated as 0.2031 W/m° C and 8.133×10^{-8} m^2/sec respectively.

For the minimum puffing time, θ = 10.9 sec the value of $\alpha\theta/a^2 = (8.133 \times 10^{-8})\ (10.9)/\ (1.135 \times 10^{-3})^2$ =0.688. Since this is greater than 0.2, Eqn. (3) can be applied to the rice grain.

Fig.1 shows the plot of log $[(T' - \bar{T})/(T' - T_0)]$ and time, θ of puffing. The slope of the resulting straight line is 1/50. Equating this value to $\hat{A}_1\ \alpha/\ (2.303\ a^2)$ we get A_1 = 0.8842 and from Eqn. (5) the value of ha/k = 0.403. Since the thermal conductivity, k of rice grain is 0.2031 W/m.°C, the value of the heat transfer coefficient, h on the surface of rice grain, is 72.1 W/m^2 °C.

Acknowledgement

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Development of Processed Cheese Spread Using Accelerated Ripened Cheddar Curd Slurries

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An acceptable processed cheese spread was developed using accelerated ripened cheddar curd slurries. Effect of pH control, agitation and addition of reduced glutathione, cobalt, manganese, riboflavin and diacetyl on flavour development in curd slurries as well as processed cheese spread were investigated. Alternate day agitation and addition of GSH had stimulatory effect. The flavour, body and texture improved by blending the slurries with 25% solids from fully ripened cheddar cheese. The slurries and processed cheese spread were analysed for proximate composition and sensory characteristics.

Cheese is an important dairy product. It may be ripened or unripened. Ripened varieties may be used as natural or processed cheese. It is estimated that about 45 per cent of natural cheeses are consumed as processed cheese. In India, ripened varieties are consumed as processed cheese and the most popular variety is cheddar. It requires a ripening period of 3-12 months at refrigeration temperature and controlled humidity. This adds considerably to the cost of production. Kristoffersen *et al*[1]. reported acceleration of cheese flavour development in cheddar curd slurries stored at 30°C. They claimed that quite a satisfactory level of cheese flavour developed in about a week which would ensure considerable amount of saving. The present study was undertaken to explore the feasibility of using such ripened cheddar curd slurries for preparation of process cheese spread. Some of the factors affecting flavour development in cheddar curd slurries were also investigated to ascertain their effect on the final cheese spread. These factors included agitation and pH control of curd slurries during ripening and incorporation of additives, like reduced glutathione (GSH), cobalt, riboflavin, manganese and diacetyl.

Materials and Methods

Preparation of cheddar curd slurries: Cow's milk obtained from the Experimental Dairy of the Institute was standardized (casein/fat ratio, 0.70) and pasteurized (63°C/30 min) and then used to prepare cheddar cheese. One hundred litres of milk was used for each trial. LF-40 culture consisting of *Str. lactis, Str. cremoris* and *Str. diacetilactis* was obtained from the Dairy Bacteriology Division of the Institute and used as starter culture. The cheddar curd was manufactured by the standard procedure[2]. After milling (0.5 per cent titratable acidity) and salting, the curd was left in the cheese vat overnight for the completion of lactic acid fermentation. From this, cheddar curd slurries were made as reported by Singh and Kristoffersen[3]. To accelerate flavour development, GSH (100 ppm.) and a mixture of cobalt (10 ppm.), manganese (5 ppm.), riboflavin (2 ppm.) and diacetyl (20 ppm.) were added to the curd slurries during grinding.

Preparation of processed cheese spread: After about 7 days of ripening, cheddar curd slurries were converted into processed cheese spread by the method of Kosikowski[4]. The processing was done with and without blending of slurry with regular cheddar cheese (25 per cent). The blend was heated at 88°C/5 min with 2.5 per cent trisodium citrate. The hot liquid cheese was homogenized at 1000 p.s.i. in a single stage homogenizer followed by packaging in 240 g lacquered tins and storing at 30°C for shelf-life evaluation. Sensory evaluation of slurries was done on an arbitrary scale of 0-8 points by an experienced panel of 5 members. The same members evaluated the product all the time in an air-conditioned sensory evaluation laboratory equipped with separate booths. At a time, not more than 5 samples were evaluated. The processed cheese spread was evaluated on a 9-point hedonic scale.

Analysis: The fat in milk was determined by the Gerber method[5] and casein by formal titration[6]. The cheddar curd slurries and processed cheese spread were analysed for moisture, fat, protein and salt. Agitation of some slurries was done daily whereas for others, on alternate days and samples were drawn for pH measurement and sensory evaluation.

Results and Discussion

Composition: The average composition of curd slurry and processed cheese spread is shown in Table 1. The curd slurries contained (in per cent) about 60 moisture, 20 fat, 13 protein and 3 salt. In the processed cheese spread without blending with natural cheddar cheese, the moisture content decreased whereas fat, protein and salt increased slightly. In cheese spread with blending, the moisture and fat increased almost to the level of curd slurries but protein and salt decreased.

Flavour characteristics: The effect of pH control and agitation on flavour characteristics of cheddar curd slurries and processed cheese spread is shown in Table 2. The initial pH of curd slurries was 5.2. The pH of one lot of slurry was adjusted to 5.2 with 50 percent NaOH solution whenever it dropped down during incubation.

There was no noticeable cheese flavour on 1st day of storage. The product showed acidic flavour. On 4th day, a distinct cheese flavour developed. The cheese flavour intensity increased further reaching an optimum level on 7th day. There was slight improvement in the intensity of flavour due to pH adjustment, but the effect was not marked. However, the character of flavour showed some variation. The samples without pH adjustment tended to be lipolytic whereas those with pH control showed a balanced flavour. The lipolytic character was an indication of Blue and Roquefort type cheeses. Alternate day agitation was found to be superior to daily agitation because of rancid flavour in the latter case.

It is quite apparent that the minor flavour differences of cheddar curd slurries disappeared on processing. All the samples were moderately liked (score 7). Similar scores were recorded for the body and texture and spreadability. The samples obtained from accelerated ripening were rated better than those made purely from the regularly ripened cheddar cheese (score 6.3).

The production of balanced flavour in pH adjusted slurries may be due to increased proteolysis. Baribo and Foster[7] observed that *lactobacilli* exhibit maximum proteolytic activity between pH 5.0 and 5.5 and above 30°C. The yeasts and molds may grow at lower pH and produce more lipolysis in control slurries. The lipolytic character of daily agitated slurries may be due to increased level of agitation and aeration which may encourage growth of yeasts and moulds. Singh[8] reported an increased concentration of C_4 and longer chain fatty acids in daily agitated slurries.

The effects of GSH and other additives on flavour development in cheddar curd slurries and cheese spread are shown in Table 3. Addition of GSH had a definite stimulatory effect on the flavour of slurries during ripening. The flavour score of slurries without GSH addition on 4th and 7th day were 2.5 and 5.0, respectively in contrast to the GSH added slurries (3.0 and 6.0). Other additives did not appear to have any significant effect on flavour development.

The intensity and type of cheese flavour in slurries

TABLE 1. PROXIMATE COMPOSITION OF CHEDDAR CURD SLURRIES AND PROCESSED CHEESE SPREAD

Constituents	Slurry	Processed cheese spread	
		Without blending	With blending
Moisture (%)	60.20	57.90	59.70
Fat (%)	20.10	21.00	20.33
FDM* (%)	50.30	49.90	50.50
Salt (%)	3.00	3.35	2.35
Total N (%)	2.05	2.06	1.75
Protein (%)	13.08	13.16	11.15

Each value is the average of five determinations
*Fat on dry matter basis

TABLE 2. EFFECT OF PH CONTROL ON THE FLAVOUR CHARACTERISTICS OF CHEDDAR CURD SLURRIES AND PROCESSED CHEESE SPREAD

Treatment	Days of storage						Cheese spread			
	1		4		7					
	CF* score	Perception	CF* score	Perception	CF* score	Perception	Flavour	Body text	Spread-ability	Perception
Control	0	Acidic	2.5	Slight lipolytic	5.0	Lipolytic	7.0	7.0	7.0	Lipolytic salty
pH 5.2	0	Acidic	2.5	Balanced	5.5	Balanced	7.0	7.0	7.0	Salty

*Cheese flavour score: 0-absent; 1-2 slight; 3-4 definite; 5-6 pronounced; 7-8 very pronounced.
Each value is the average of five determinations.

TABLE 3. EFFECT OF ADDITIVES ON THE FLAVOUR CHARACTERISTICS OF CHEDDAR CURD SLURRIES AND PROCESSED CHEESE SPREAD

Treatment	Days of storage						Cheese spread			
	1		4		7					
	CF* score	Perception	CF* score	Perception	CF* score	Perception	Flavour	Body text	Spread-ability	Perception
Control	0	Acidic	2.5	Slight lipolytic	5.0	Blue cheese like	6.75	7.0	7.1	Blue cheese like, salty
GSH**	0.5	Acidic	3.0	Bland	6.0	Pronounced	7.00	7.2	7.0	Balanced
Co**, Mn, Rib, Diacetyl	0	Acidic	2.0	Slight lipolytic	5.5	Balanced	7.00	7.2	7.0	—
GSH, Co, Mn, Rib, Diacetyl	0.5	Acidic	2.5	Bland	6.0	Pronounced	7.00	7.2	7.4	Balanced

*Cheese flavour score as in Table 1
**GSH-100 ppm: Cobalt-10 ppm; Manganese-5 ppm; Riboflavin-2 ppm; Diacetyl-20 ppm.
Each value is the average of five determinations.

increased with the addition of GSH. The GSH added slurries produced cheese spread with balanced cheese flavour in contrast to the lipolytic type in case of control slurries. However, the quality of processed cheese spread samples was not markedly influenced (score 7) by the additives.

The slight stimulatory effect of GSH in curd slurries may be due to the fact that lactic acid bacteria use GSH for their protein synthesis rather than cysteine, and glutathione is considered to be an ideal compound to supply sulfur compounds other than methionine for *L. casei*. Chandan and Shahani[9] reported stimulatory effect of GSH on lipase activity.

Effect of blending solids from fully ripened regular cheddar cheese is shown in Table 4. Blending was done in the proportion of 75 per cent total solids from curd slurries and 25 per cent from the one year old cheddar cheese. Cheese spread from 100 per cent slurry was observed to be slightly elastic and rubbery whereas that made from the blend showed quite balanced desirable characteristics. The control cheese spread prepared by replacing slurry with 4 months old cheddar cheese was rated to be inferior to that made from the blend of slurry and cheese solids.

TABLE 4. EFFECT OF BLENDING SOLIDS FROM REGULAR CHEDDAR CHEESE ON SENSORY CHARACTERISTICS OF PROCESSED CHEESE SPREAD

Curd slurry	Cheddar cheese	Flavour	Body & texture	Spread-ability
100	—	6.0	6.0	6.5
75	25	7.0	7.0	7.5
—	100	6.0	6.0	6.5

Each value is the average of five determinations.

Shelf-life of cheese spread: As per the Prevention of Food Adulteration Act (1954) of India as amended in 1976, process cheese tins stored at 30°C for 15 days should not bloat. The tins of process cheese spread kept in the present case at 30°C did not show any bloating upto 30 days. At refrigeration temperature (5-8°C) no deterioration in cheese flavour was observed upto 3 months.

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Characterisation of the Major Component in Thermostable Muscle Proteins

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The thermostable muscle protein (TMP) preparations obtained by ethanol (E) and 10% trichloroacetic acid (TCA) precipitation were compared with the ethanol precipitate of heated adrenal gland (TAP) by UV absorption studies and SDS-gel electro phoresis. The ethanol and trichloroacetic acid precipitates exhibited no UV absorption maxima at 280 and 260 nm, while the adrenal precipitates had a maximum at 260 nm. By ammonium sulphate fractionation, the bulk of the TMP was recovered in the 40-53% fraction. The SDS-gel electrophoresis patterns of TMP and TAP were similar but the former showed a greater intensity in the molecular weight range of 35,000-37,000 daltons corresponding to tropomyosin and troponin fractions. This major band was detected in both ethanol precipitate and trichloroacetic acid precipitated proteins. The antiserum to the whole TMP reacted only with pure troponin and not with tropomyosin in a precipitation test.

Thermostable protein preparations from adrenal glands are reported to be antigenic[1-3]. Shulman *et al.*[4] who worked on the characterisation of thermostable antigens of adrenal glands reported that these proteins were heterogeneous in nature and required further identification of one or more of the antigens of interest. In their attempt to characterise the bovine specific adrenal proteins, the same authors found evidence of these proteins being mucoproteins but Jones and Mortimer[5] found no such indications. Kang' ethe *et al.*[6] working with muscles of different kinds of animals reported that thermostable muscle preparations (TMP) were antigenic. In our investigations on TMP from sheep, goat, ox and buffalo, it was found that all the four preparations resolved into 4 distinct bands in SDS gel electrophoresis ranging in molecular weight from 16,000 – 37,000 daltons and that they were derived from the myofibrillar fraction of the muscle[7]. Since these TMP preparations were heterogeneous, attempts were made to fractionate them into components and identify the major fractions by means of UV absorption spectra, ammonium sulphate fractionation, SDS gel electrophoresis and by Ouchterlony's double diffusion method. For comparison, thermostable adrenal proteins (TAP) were used in some experiments. The results of these studies are reported here.

Materials and Methods

Chemicals: Acetic acid, ammonium sulphate, bromophenol blue, ethanol, methanol, mercapto ethanol, hydrochloric acid (HCl), sodium chloride (NaCl), sodium dodecyl sulphate (SDS), sodium dihydrogen orthophosphate, disodium hydrogen phosphate, trichloroacetic acid, potassium chloride (KCl), potassium hydroxide (KOH) and urea were of the highest purity commercially available. Acrylamide, bisacrylamide, Tris, beef troponin and tropomyosin were products of Sigma Chemical Co., USA.

Adrenal glands from several sheep were obtained from the municipal slaughter house and frozen at –20°C until used. Fresh muscle from slaughtered animals was excised from the leg portion and used the same day.

Protein preparation: Proteins from 100 g each of both raw (uncooked) and autoclaved muscle of sheep were extracted in 0.15M NaCl and precipitated by ethanol as described previously[7]. The preparation from autoclaved extract was termed the thermostable muscle protein or TMP(E). Muscles of goat, ox and buffalo were similarly used for TMP(E) preparation. Ethanol precipitated preparations were obtained from raw as well as cooked adrenal glands (TAP). Heat stable muscle proteins were also precipitated by 10 per cent trichloroacetic acid [TMP (TCA)] and dissolved in 0.15M NaCl, the pH adjusted to 7.0 with 1N NaOH, and lyophilized.

Ammonium sulphate fractionation: TMP (E) was dissolved (1 mg/ml) in KCl-tris-HCl buffer (0.2M KCl, 10mM tris-HCl, pH 7.6) and subjected to ammonium sulphate fractionation[8]. The precipitates obtained from 0-40, 40-53, 53-60 and 60-80 per cent saturation levels were dissolved in the same tris-HCl buffer and lyophilized. The fractions obtained between 40 and 60 per cent saturation were further subjected to isoelectric precipitation at pH 4.6.

Preparation of tropomyosin and troponin from muscle: Crude tropomyosin and troponin were prepared from the autoclaved skeletal muscles of sheep following the procedure of Hartshorne and Mueller[8] but employing only one step isoelectric precipitation.

All pH measurements were made in a ELICO model L1-120 pH meter using a combined electrode. Protein was estimated by the biuret method[9].

Absorption spectra: The ultra violet absorption spectra of the different protein precipitates and fractions were obtained between 190 and 300 nm in a Shimadzu UV 240 recording spectrophotometer.

Electrophoresis: SDS-gel electrophoresis (10 per cent gel) of the different preparation was carried out as described previously[7] by the procedure of Weber and Osborn[10]. Gels of TMP (E) were stained with thymol-sulphuric acid by the method of Gander for glycoproteins[11].

Double immuno diffusion: Antiserum to beef whole TMP raised in sheep was used in Ouchterlony's[12] agar diffusion method against pure beef tropomyosin and troponin as test antigens.

Results and Discussion

UV absorption characteristics: The UV absorption characteristics of TMP(E), TMP(TCA) and TAP are shown in Fig.1. Neither TMP(E) nor TMP(TCA) exhibited absorption maxima at 280 nm and 260 nm.

TABLE 1. 260/280 RATIOS OF TMP(E) AND TAP(E) FROM DIFFERENT ANIMAL SPECIES

Animal	260/280 ratio of TAP (E)	260/280 ratio of TMP (E)
Sheep	1.70	1.29
Goat	1.60	1.20
Ox	1.64	1.40
Buffalo	1.66	1.32

TAP(E) exhibited an absorption maximum at 260 nm and in this respect differed from TMP. The TCA precipitated TMP, which is expected to give a nucleic acid free preparation also exhibited the same UV absorption spectrum as did the ethanol precipitated TMP. The 260/280 absorption ratio of the TMP(E) and TAP(E) obtained from sheep, goat, ox and buffalo muscles and adrenal glands are presented in Table 1. The TMP(E) from the four animal species had lower 260/280 ratios than that for TAP(E). The 260/280 ratio for bovine serum albumin is 0.66, but none of the test preparations showed such a low ratio.

The bulk (90 per cent) of the TMP was recovered in the 0-60 per cent ammonium sulphate fraction, the 40-53 per cent fraction alone accounting for 58 per cent of the total protein while 0-40, 53-60 and 60-80 per cent fractions yielded 26, 6 and 10 per cent protein respectively. The UV absorption spectra of the four fractions indicated (Fig. 2) that only the 40-53 fraction

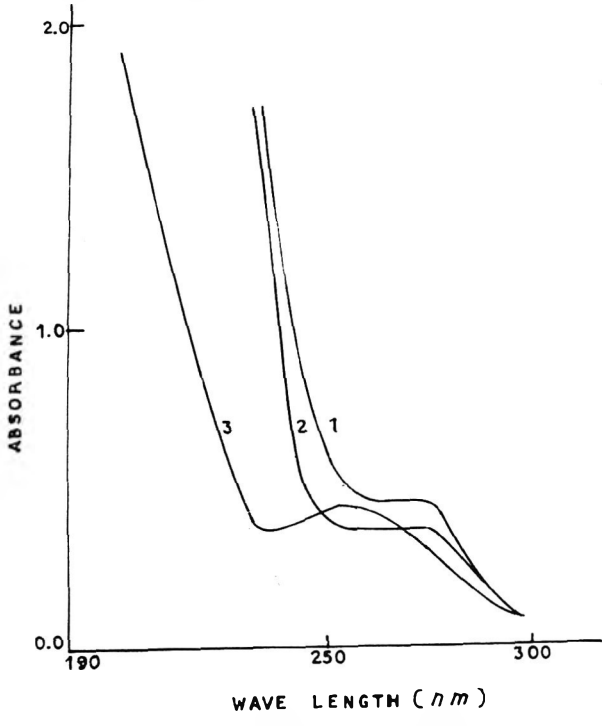

Fig. 1. Absorption spectra of 1. TMP (TCA), 2. TMP (E) and 3. TAP

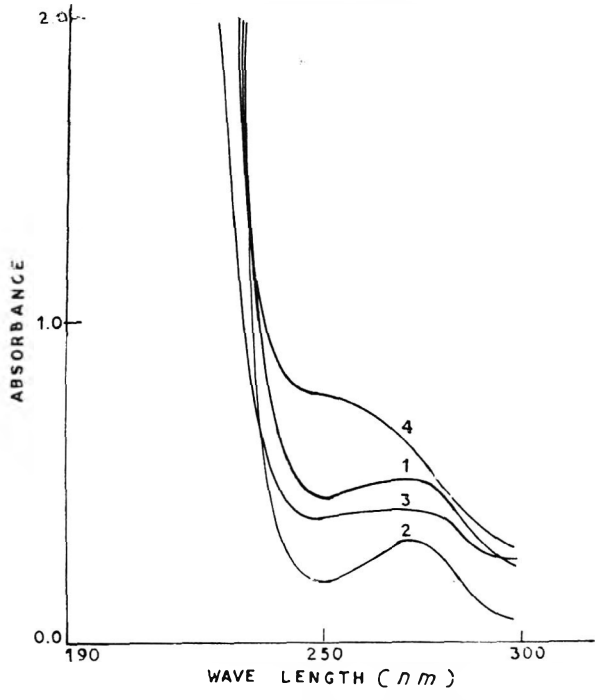

Fig. 2. Absorption spectra of TMP (E) fractions 1. 0-40%, 2. 40-53%, 3. 53-60% and 4. 60-80% ammonium sulphate.

showed an absorption maximum at 280 nm. The UV spectra for the 0-40 and 53-60 fractions showed similarities with that of whole TMP.

Electrophoretic patterns: The SDS gel electrophoretic patterns obtained for fresh raw (uncooked) muscle and adrenal gland extractives (FMP and FAP) were similar in the multiplicity of bands seen although they were not exactly comparable (Fig.3a and 3c). The relative intensities of the corresponding bands also varied. In the gel patterns obtained for TMP and TAP (Fig.3b and 3d) it was seen that the 35,000 – 37,000 dalton component exhibited greater intensity in the TMP than in TAP. The major band of 35,000 – 37,000 daltons was present in both TMP(E) and TMP(TCA). This band corresponded to the tropomyosin-troponin complex (Fig.4c and 4d). However, the region of pure troponin fraction in the gel at 37,000 daltons took up a faint stain and could not be photographed clearly (4d). The four ammonium sulphate fractions were similarly separated on the SDS gel (not shown). All the four ammonium sulphate fractions appeared to separate into similar bands in SDS-gel electrophoresis and had patterns comparable to that of whole TMP. Among the four fractions examined by SDS gel electrophoresis, in the 40-53 per cent fraction, the band corresponding to 35,000-37,000 daltons was the most intense and in the 60-80 per cent fraction this region appeared least intense.

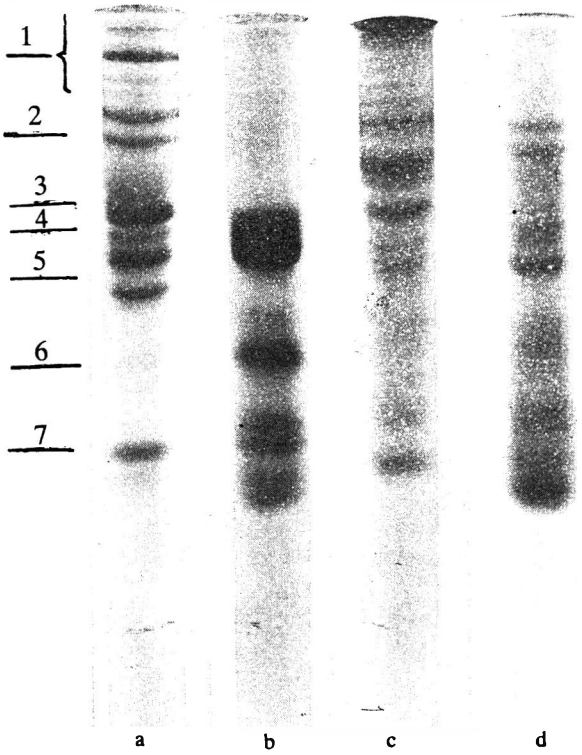

Fig. 3. Electrophoretic patterns of (a) FMP (b) TMP (c) FAP and (d) TAP
1. Myosin heavy chain; 2. α-actinin; 3. Actin 4. Troponin fraction; 5. Tropomyosin; 6. Troponin fraction; 7. Troponin fraction.

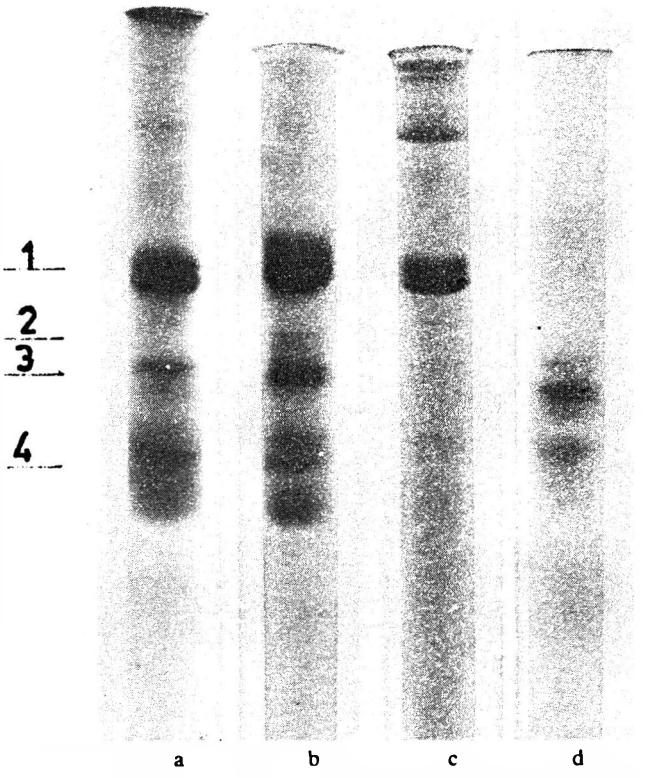

Fig. 4. Electrophoretic patterns of (a) TMP (TCA), (b) TMP (E), (c) Pure tropomyosin and (d) Pure troponin
Mol. wt. calculated (Daltons): 1. 35,000 – 37,000; 2. 28,000; 3. 26,000; 4. 16,000

Cheng and Parrish[13] reported that troponin and tropomyosin were more heat resistant than actin and myosin and could be extracted better upon mild heating (upto 80°C). Hofmann[14] reported that certain protein constituents of low molecular weight remained detectable in muscles heated to 120°C. Hartshorne *et al.*[15] in their studies on troponin reported that it showed an absorption maxima at 260 nm and was made up of two fractions – troponin A and B – with absorption maxima at 260 and 280 nm respectively. The variations among troponins were due essentially to differences in the proportion and properties of troponin A component. This may explain the differences seen in the absorption maxima in the TMP and TAP preparations.

The SDS gels were stained with thymol sulphuric acid by the procedure of Gander[11]. The gels stained negative in our experiment thereby indicating either the absence of carbohydrates or if present, that its concentration was below detection limits, even though Gander claimed the test to be sensitive to the presence of as low as 50 ng of carbohydrate. Our observations are in accordance with those of Jones and Mortimer[5] who reported that their TAP preparations from pork

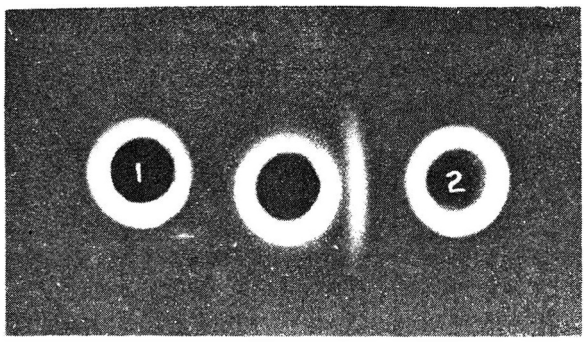

Fig. 5. Precipitation of beef troponin (well No.2) with antiserum to beef TMP (central well).
Tropomyosin (well No.1) did not show precipitation reaction.

stained negative as against observations of Shulman *et al.*[4] who found 8.4 per cent hexose in the TAP and classified the adrenal antigens to be mucoproteins.

Double immuno diffusion test: Since the major fraction of TMP appeared to consist of both tropomyosin and troponin (by the comparison of molecular weights), to find which one of them specifically would participate in a precipitation test against whole TMP antiserum, commercially available purified beef tropomyosin and troponin preparations were used in a double immuno diffusion test. Fig. 5 clearly indicates that troponin and not tropomyosin reacted with the antiserum against beef TMP. Therefore, the major antigenic portion of the TMP must reside in the troponin fraction.

While Sherikar *et al.*[16] were unable to obtain antisera in rabbits by injecting boiled (100°C) meat extracts, we could elicit antibody response in both rabbits and sheep by using ethanol precipitates of the autoclaved muscle extracts. These results are in conformity with the findings of Hayden[2] who used boiled ethanol precipitates of autoclaved adrenal gland extracts and of Kang' ethe *et al*[6] who prepared antisera successfully also by using ethanol precipitates of autoclaved skeletal muscle extracts. These studies indicate that irrespective of the alterations that may have occurred in the meat proteins due to severe heat treatment, their antigenic competence has not been totally lost.

The evidence obtained so far in our investigations indicates that the major band in SDS gel electrophoresis of TMP corresponded to a protein of molecular weight 35,000 – 37,000 daltons and that the major 40-53 per cent fraction obtained by ammonium sulphate saturation exhibited absorption maximum at 280 nm. Further, partially purified tropomyosin and troponin also exhibited gel patterns similar to that of TMP and the 40-53 per cent ammonium sulphate fraction. Similar characteristics have been observed in the TAP preparations. The major fraction obtained by both ammonium sulphate fractionation and SDS-gel electrophoresis appeared to be troponin and tropomyosin, but only purified troponin and not tropomyosin reacted with the whole TMP antiserum.

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Rheological Studies on a Protein-enriched Low-fat Spread

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Compositional characteristics such as fat-to-soy solids ratio and incorporation of glycerolmonostearate, trisodium citrate and guar gum in a soy-based oil-in-water spread were investigated with respect to spreadability and rheological properties. Increasing proportion of soy solids resulted in a less flowable spread when hot, but a more spreadable one when cold. Use of the monoglyceride and citrate retarded the viscosity at the processing temperature and oiling off of the finished product. While these two emulsifying agents had a softening effect, guar gum used as a stabilizer made the spread remarkably firm at the ambient temperature. Consistency of this visco-plastic product measured in terms of Cone Sress Index, yield value, viscosity and extruder thrust was more favourable than that of table butter both at ambient and refrigeration temperatures and this was confirmed by subjective data.

The most important rheological property of table spreads like butter and margarine is spreadability. Ideally, these products should be neither too difficult to spread nor too soft to be conveniently applied on a bread slice or handled after manufacture. Conventional butter, however, requires to be tempered appropriately (usually between 15° and 20°C) so that it is optimally spreadable, since it is too hard at its normal storage temperature (as in a domestic refrigerator) and too sloppy at ordinary temperatures. By virtue of their compositional characteristics, "soft" margarine and other spreads – dairy – and non-dairy type, have better spreadability than conventional butter. This is one of the main reasons for the growing popularity of such spreadable products in traditionally butter-consuming Western countries[1]. Despite several attempts to enhance the spreadability of butter by appropriately modifying the manufacturing conditions or using certain surfactants[2], the scope for a major breakthrough in this regard seems to be very limited. Prentice[3] concluded that there is little that can be done to modify spreadability of natural butter-fat substantially without altering its composition.

Non-dairy spreads of water-in-oil (W/O) type owe their spreadability largely to high moisture content (35-55 per cent) and composition of the fat phase which is usually tailor-made to impart the desired consistency to the product. Spreads representing oil-in-water (O/W) emulsions depend, for their improved spreadability, on the nature and concentration of the non-fat component as well. Little information is available in literature on rheological properties of such spreads, though several reports[2,4-6] relate to studies on rheology of butter and margarine. The present paper deals with the rheological aspects of a heat processed, soy-based, O/W type low-fat spread[7] as influenced by compositional parameters such as fat-to-soy solids (F/S) ratio, emulsifiers, stabilizers, etc.

Materials and Methods

Soy-based spread: Cleaned, dry soybeans (mixed varieties, 8 per cent moisture) were converted into a slurry using the Illinois process[8]. After homogenization in two stages (243 and 35 kg/cm^2) the slurry was acidified (pH 4.5) with 1:9 dilute hydrochloric acid and filtered through muslin to obtain a soy concentrate, which contained approximately 20 per cent solids, 10 per cent protein and 6 per cent fat. In order to prepare a spread, the concentrate was partially neutralized (pH 6.5) with 1N sodium hydroxide and blended with skim milk powder (SMP) or medium-calcium co-precipitate and processing adjuvants such as 0.25 or 0.50 per cent glycerol-monostearate (Kakker Scientific Store, Delhi) 1 per cent trisodium citrate (Sarabhai Chemicals) and guar gum (Pioneer Chemical Co., Delhi). Commercially available hydrogenated vegetable fat, (Vanaspati) (AOCS slip point, 36.3°C) was used as the principal fat source. The mixture, after processing at 85°C for 15 min, was ground in a hand-held homogenizer (C.W. Logeman, USA) or in a colloid mill (200 kg/hr; RAD Engineering, Calcutta). The hot (60-65°C) spread (45-47 per cent moisture, 39-40 per cent fat and 6-7 per cent protein) was poured into rectangular (13×8×4 cm) wooden trays with slide-opening smaller sides which, covered with glass plates, were then held overnight at refrigeration temperature (RT) of 5-10°C or ambient temperature (AT) of 25°-30°C before making rheological measurements or sensory evaluation.

Table butter: Sweet cream butter was manufactured in the Experimental Dairy (NDRI, Karnal) from pooled winter milk of the dairy herd maintained on the Institute's farm.

Rheological analysis: Firmness was determined using a cone penetrometer (Central Ignition Co., London) fitted with a cone of AOCS[9] specifications. Six readings (in tenths of a mm) were obtained for each sample and the mean penetrometer value (p) used to express firmness as Cone Stress Index[10] (Cv) as under:

$$Cv = \frac{C \times A^{-1.65}}{P^2} \times 10^6$$

where, C = Mass of the dropping assembly (92.5 g)
A = Cone angle (20°)
P = Corrected penetrometer reading

$$= p + \frac{\text{dia. of truncated tip of the cone}}{2 \tan \frac{A}{2}}$$

Viscosity of the spread during processing was determined by a constant-stress rotation disc viscosimeter (Associated Instruments Manufacturers, Bombay). Time in seconds for a single revolution using the large torque disc or 10 revolutions employing the small torque disc was multiplied by an appropriate constant viz., 50 or 100 when using 50 or 100 g weight with the large disc and 1 or 2 for the respective weights with the small disc. Plastic viscosity and yield stress determinations were made on the finished product as suggested by Chari and Avasthy[11] using the same viscometer.

Oiling off was determined at 25 ± 2°C as described by Dolby[12], employing a slice diameter of 5.5 cm (thickness, 1.6 mm) and Whatman No.1 filter-paper sheets (7 cm square). After a contact time of 1 hr, the filter-papers were dried at 100 ± 2°C for 2 hr, the absorbed fat extracted in a Soxhlet apparatus and its weight recorded for 4 slices of the spread. Extruder thrust and extruder friction were determined by using the FIRA-NIRD extruder (HA Gaydon & Co., England) fitted with cylinder No.2 and orifice 'C', and operated at speed '2'.

Spreadability evaluation: Subjective assessment of spreadability was carried out by a trained panel of seven judges selected from among the staff of the Division of Dairy Technology at the Institute. A specialized laboratory with necessary facilities such as individual booths, diffused white fluorescence lighting and air-conditioned environment[13] was used for the purpose. The panelists used a 5g sample to spread on slice of fresh sandwich bread using a kitchen knife. Samples were held in a cabinet at the required temperature before serving to the panel. A 9-point intensity rating scale ranging from 1 (extremely hard) to 9 (extremely sloppy) with the optimum at 5 was employed for spreadability scoring. Sensory data were subjected to statistical analysis using one-way layout with randomized blocks for variance analysis and t-test analysis for difference testing.[13,14]

Results and Discussion

An important aspect of the manufacture of processed O/W spreads is that the product should be flowable at the processing temperature. Fluidity of the product not only enables it to pass through a colloid mill or a homogenizer which imparts it the desired textural properties, but also facilitates pour-packaging of the hot product. At the same time, the composition of the spread should permit "setting" to a plastic body upon cooling. Thus, the temperature coefficient for product viscosity should be large enough to allow the desired change in consistency in the temperature range of 30 to 60°C or above, whereas it should be so small in the region of 5 – 30°C as to permit adequate pliability of the cold product.

Preliminary investigations on the effect of type of the not-fat component showed that with the normal moisture content of the spread (40-50 per cent), at similar protein concentrations, the co-precipitate failed to provide a satisfactory emulsion, while the fluidity of the co-precipitate-containing mixture was nearly the same as that of the SMP-added spread at the processing temperature.

Fat-to-soy solids ratio: Soy solids from the soy concentrate contributed protein and insoluble carbohydrates which by virtue of their water-binding ability[15], might influence the spread consistency. Table 1 shows that as the F/S ratio is decreased, the viscosity of the spread during processing increased i.e. the flowability decreased. This indicates that the vegetable fat tended to impart greater flowability to the product when hot, whereas soy solids had the opposite effect. Further, with the decreasing F/S ratio,

TABLE 1. CONSISTENCY CHARACTERISTICS AND SPREADABILITY SCORE OF SOY SPREAD AS A FUNCTION OF FAT-TO-SOY SOLIDS (F/S) RATIO

F/S ratio	Viscosity* ($\times 10^4$ cP)	Cone stress index		Spread. score	
		Refrig. temp.	Ambient temp.	Refrig. temp.	Ambient temp.
5	3.0	25.20	11.16	3.5[a]	5.4[a]
4	4.1	22.09	11.82	4.0[b]	5.6[a]
3	4.8	17.53	12.46	4.2[b]	5.6[a]

Average values of duplicate determinations
Means with different superscripts in the same column differ significantly ($P \leq 0.05$)
* Viscosity during processing

the spread became appreciably softer (decreased Cv) at the refrigeration temperature but remained almost unaffected at the ambient temperature. A similar effect was also noticed on subjective spreadability; it increased with decreasing F/S ratio, the increase being significant ($P \leq 0.05$; critical difference, 0.44) at RT but not at AT. The greater influence of the F/S ratio on spread consistency at RT reflected enhanced softening effect of soy solids in the product containing extensively solidified fat at this temperature. This may be taken to account for the observed higher temperature dependence of firmness of the system with an F/S ratio of 5 than that of the system with a ratio of 3. The smaller firmness difference between RT and AT for the lower F/S ratio indicated that soy solids could desirably narrow down the consistency gap in this temperature range although it had an adverse effect on fluidity of the hot product.

A significant ($P \leq 0.01$) correlation ($r = -0.96$) between Cv and sensory spreadability score was evident. This corroborated the finding of Dixon and Parekh[10] who reported that Cv accounted for 91 per cent of the variation in the subjectively determined spreadability of butter.

The spread with an F/S ratio of 4 appeared to have an optimum consistency; a higher ratio would tend to make the product too firm at lower temperatures while a lower one would restrict its flowability during heat processing.

Effect of emulsifier: Highly lipohilic emulsifiers such as glycerolmonostearate (GMS) are essential in the preparation of W/O spreads[16], but they are seldom used in O/W spreads, in which hydrophilic emulsifiers or emulsifying agents such as protein are relied upon. However, as Table 2 indicates, surfactant-emulsifiers like GMS could considerably influence the spread rheology. GMS substantially enhanced the flowability of the spread during processing as is evident from decreased viscosity, the viscosity decrease being nearly proportionate to the level of the emulsifier. Reduced oiling off is indicative of increased emulsification owing to the addition of GMS. This enhanced emulsification, presumably causing greater dispersion of fat droplets which, in turn, would result in a weaker network of solidified fat droplets, may be considered responsible for the decreased firmness of the spread containing GMS (Table 2). It should, however, be pointed out that use of higher levels (e.g. 1.0 per cent) of the emulsifier had a destabilizing effect on the emulsion to the extent that no homogeneous product could be obtained.

Role of sodium citrate and guar gum: Emulsifying or melting salts, such as trisodium citrate and disodium phosphate are well known auxiliary emulsifiers for processed cheese and cheese spreads. These salts are believed to effect emulsification in an indirect manner[17]. It is evident from Table 3 that sodium citrate markedly decreased (38.5 per cent) the viscosity of hot spread, while guar gum, a non-ionic hydrocolloid, greatly increased the viscosity (317.9 per cent). The citrated spread exhibited satisfactory flowability during processing, while the gum-added product had a considerably reduced flowability. Viscosity increase effected by the gum was somewhat retarded by the citrate, but the resulting product was still much too viscous (nearly three times the control).

Added individually, sodium citrate and guar gum diminished oiling off of the spread but when used together, these additives had no perceivable effect. Interestingly, the zone of water absorption on the filter-papers (marked in pencil after fat extraction, Fig.1) used for determination of oiling off suggested that, while both the citrate and the gum appreciably retarded the extent of water absorption (reflecting increased water binding in the spread), their combined effect being much more pronounced. Thus, the water holding characteristic of the product was favourably modified by the peptizing salt and the sabilizer.

It can be noticed from Table 3 that firmness of the spread was perceptibly decreased by sodium citrate, the decrease being greater when no stabilizer was

TABLE 2. EFFECT OF GLYCEROLMONOSTEARATE (GMS) ON THE EMULSIFICATION AND CONSISTENCY PROPERTIES OF SOY SPREAD

GMS added (%)	Viscosity* (x 10^4 cP)	Oiling off (g)	CSI at RT (Cv)
0.00	3.6	0.41	20.38
0.25	1.5	0.37	14.14
0.50	0.7	0.15	10.97

Average values of duplicate determinations
F/S ratio, 4; CSI = Cone stress index
*Viscosity during processing

TABLE 3. EMULSIFICATION AND CONSISTENCY CHARACTERISTICS OF SOY SPREAD AS INFLUENCED BY SODIUM CITRATE AND GUAR GUM

Guar gum (%)	Sodium citrate (%)	Viscosity* (x 10^4 cP)	Oiling off (g)	Cone stress index	
				Refrig temp.	Ambient temp.
0.00	0.00	3.9	0.52	26.50	13.03
0.00	1.00	2.4	0.33	19.53	9.67
0.25	0.00	16.3	0.22	53.88	22.60
0.25	1.00	10.8	0.52	46.85	20.38

Average values of duplicate determinations
* Viscosity during processing

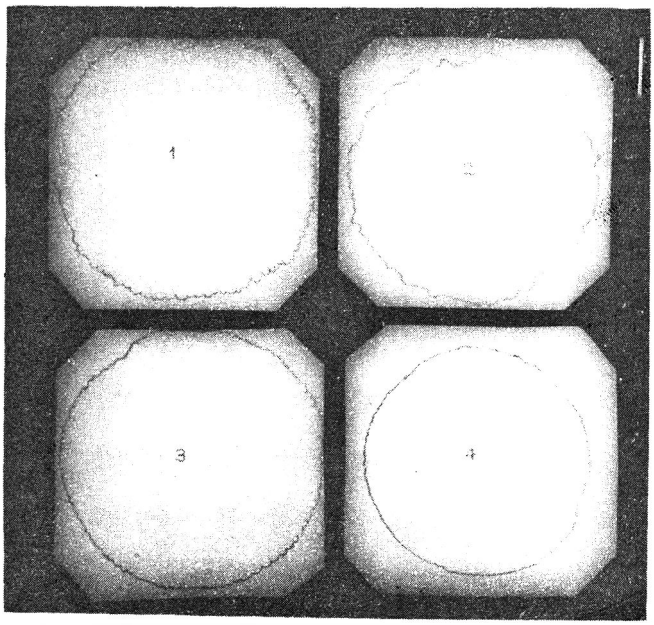

Fig. 1. Pattern of moisture absorption on filter-papers as an indication of the water binding characteristic of soy spread containing (1) no emulsifier or stabilizer, (2) 1% sodium citrate, (3) 0.25% guar gum and (4) 1% sodium citrate + 0.25% guar gum

added (26.3 per cent at RT and 25.8 per cent at AT) than when 0.25 per cent guar gum was used (13.0 and 9.8 per cent). Moreover, sodium citrate reduced the temperature-dependent difference in firmness from 13.5 to 9.9 Cv units in absence of guar gum and from 31.3 to 26.5 Cv units when the gum was present.

Rheology of the soy spread versus table butter: The finished soy spread with its high moisture and non-fat solid contents was envisaged to have the desired spreadablity without losing its physical stability over a wide temperature range. Rheological data presented in Table 4 show that as compared to table butter, the soy spread had considerably improved consistency characteristics viz., lower values for Cv, viscosity, yield stress and extruder thrust at RT, and appreciably greater values for all these parameters at AT. Sensory data (Table 4) revealed that the spread was nearly optimally spreadable both at RT and AT, whereas butter was too hard at RT and had too little firmness at AT, the difference between the two products being statistically significant ($P \leq 0.001$ at RT and $P \leq 0.01$ at AT).

TABLE 4. RHEOLOGY OF SOY SPREAD AS COMPARED TO TABLE BUTTER

Characteristic	Soy spread*		Table butter	
	Refrig temp.	Ambient temp.	Refrig temp.	Ambient temp.
Cone stress index	36.74	13.34	188.02	2.35
Plastic viscosity (x 10^6 cP)	5.00	4.10	**	1.00
Yield stress (x 10^3 dynes/cm^2)	1.10	0.60	**	0.30
Extruder thrust (kg)	1.17	0.56	3.97	0.13
Extruder friction (g/cm)	35.00	20.60	288.40	4.00
Spreadability score	5.70	5.90	1.60	7.70

Average values of triplicate determinations
*F/S ratio, 4; sodium citrate, 1 per cent; guar gum, 0.1 per cent
**Too high to be conveniently measured by the instrument

The observed physical stability together with spreadability of the spread over the desired range of temperature can obviously be ascribed to the non-fat solid components capable of holding a substantial quantity of water in the continuous aqueous phase and thus retarding the hardening effect at RT and softening effect at ordinary temperatures due to a relatively high temperature coefficient of fat for consistency. Kulkarni and Rama Murthy[18] have demonstrated the role of increased moisture in enhancing the pliability of conventional butter which is a W/O emulsion. Nevertheless, an increase in the solids-not-fat content (from 1 to 3 per cent) hardened the butter. The type of emulsion and the great difference in non-fat solids as well as moisture contents of the two systems might be responsible for this observed rheological difference between them.

Extruder friction, an index of adhesiveness or stickiness of the product, was much lower (approximately one-eighth) for the spread than for butter at RT (Table 4). However, the reverse was true at AT presumably because of the lubrication effect of the oiled-off surface fat in butter at this temperature. Though of minor consequence in comparison with firmness, adhesiveness is one of the several physical properties which determines the actual spreadability of a visco-plastic product[5]. Thus, the differential stickiness of the soy spread and butter appeared to make the former more favourable from the spreadability point of view than latter at the lower temperature.

It could thus be concluded that the compositional variables studied in relation to consistency characteristics of the soy-based spread had a significant bearing on the temperature-dependent changes in its consistency. While soy solids imparted spreadability to the product at RT, vegetable fat rendered it flowable at the processing temperature, both of them contributing stability at AT. Additives such as emulsifying and stabilizing agents could also play a decisive role in spread rheology.

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Consumer Response to a Low-cost Butter-like Spread

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A butter-flavoured low-fat soy spread developed based on soy concentrate and vegetable fat was subjected to consumer acceptance studies. A consumer survey involving 191 individual respondents indicated that the product was highly acceptable as a bread spread. The average hedonic response of consumers representing different economic groups of the society was between "Like very much" and "Like moderately". A limited number of families regularly consuming table butter confirmed the potential of the spread as a low-cost substitute for butter. They rated the product only "Slightly less suitable" than conventional butter and most of them showed willingness to buy it at half the cost of butter.

Stagnated or declining consumption of traditional table butter in many of the milk affluent countries has given rise to a variety of new spreadable or plastic products[1]. These products, having appreciably different compositional characteristics, are supposed to be similar to butter in their sensory properties or "eating" quality. A distinct feature of spreads, besides being low in their calorie content, is spreadability at the refrigeration temperature. In other words, a spread can be taken out from the domestic refrigerator and straight applied on a bread slice, unlike conventional butter which requires to be tempered before being able to spread. Another major advantage associated with such spreads is their low cost primarily due to reduced fat and high moisture contents. Hence, low-fat spreads have a great potential even in developing countries where consumption of butter is limited on account of its high cost.

Most low-fat spreads claim to resemble table butter in their eating quality, and often such claims are substantiated by laboratory sensory tests. However, consumer opinion is vital in determining actual acceptability of a product. The significance of reliable consumer opinion in new product development cannot be over-emphasized[2]. Nevertheless, not much published data are available in respect of butter spreads. One of the rare reports is of Seas *et al*[3]. who studied consumer preferences for colour, salt, flavour and texture in a low-fat dairy spread. No such information has been documented in relation to non-dairy or soy-based low-fat spreads. The present paper discusses consumers' response to such a low-fat, low-cost product based on soy concentrate, skim milk powder and vegetable fat, which has been developed at this Institute[4].

Materials and Methods

Manufacture of spread: The soy-based spread was manufactured as described earlier[5]. It was coloured by using 15 mg/kg/ β-carotene (Sigma, USA) and 2 ml/kg butter annatto (Sonarome Chemicals, Bangalore), and flavoured with 0.3 per cent (v/w) Butardol, a liquid flavour concentrate (Naarden India, Bombay). Added with preservatives (sorbic acid and potassium sorbate each at 0.05 per cent level) the processed spread was packaged hot in 200 ml screw-capped wide-mouth glass jars ('Yera', Alembic Glass Works, Baroda), cooled at room temperature for 6-8 h and finally transferred to a refrigerator.

Consumer survey: The soy spread was served with fresh sandwich bread (approximately 13 g of the product spread between two 8 cm square slices) to each of 191 consumers included in the study. These individuals, randomly selected from among the staff of the Institute, represented nearly a cross-section of the society. Following the single-sample presentation approach[6], servings were made at tea time i.e. around 11.00 a.m. or 4.00 p.m. at the place of work. The questionnaire used in this survey briefly indicated the purpose of this excercise. It also sought information as to whether the respondent was familiar with traditional butter. The 9-point Hedonic scale included in it ranged from "Dislike extremely" (score, 1) to "Like extremely" (score, 9). Those individuals who disliked butter were not included in the survey.

Household survey: Eleven randomly selected butter-consuming families (sampled from the consumer survey) were provided with the soy spread (200 g) along with instructions on how to use it. The ballot employed to know the respondent's opinion carried a 5-point rating scale ("Much less suitable than

TABLE 1. CONSUMER ACCEPTANCE OF THE SOY SPREAD

Score	No. of respondents familiar with butter			No. of respondents willing to buy			
	Yes	No	Total	Yes	No	Indifferent	Total
5	3(2)	2(4)	5(3)	1(20)	2(40)	2(40)	5(100)
6	6(4)	4(8)	10(5)	5(50)	3(30)	2(20)	10(100)
7	47(33)	14(29)	61(32)	53(87)	3(5)	5(8)	61(100)
8	72(51)	20(41)	92(48)	85(92)	1(1)	6(7)	92(100)
9	14(10)	9(18)	23(12)	18(78)	0(0)	5(22)	23(100)
Total	142(100)	49(100)	191(100)	162(85)	9(5)	20(10)	191(100)

*Figures in parentheses indicate per cent of respondents

butter", 1 to "Better than butter", 5) and aimed at finding out if the consumer would actually buy the spread instead of butter and if she/he had preference for a specific type of packaging – glass bottles *versus* cheaper plastics tubs.

Results and Discussion

Consumer response: A considerable number (26 per cent) of the respondents were not used to consuming butter. These "unfamiliar"-with-butter respondents belonged to the low-income group of the populace. As regular consumers, the "familiar"-with-butter group (74 per cent) had a fairly good idea of what it tasted like.

Table 1 shows that a majority of respondents in both familiar and unfamiliar groups liked the spread "very much". The high acceptability rating for the product was further reflected by the "Like extremely" response of an appreciable number of the sampled consumers. Only a very small percentage of the respondents indicated an acceptance level of "Like slightly' or lower. The mean Hedonic score of 7.6 from all the respondents is indicative of a high acceptability of the product. The nearly equal acceptance of the spread by both the familiar and unfamiliar respondents (average rating, 7.6 for each) thus suggested the possibility of substituting the spread in place of butter even for those who, because of the cost factor, cannot afford to buy the latter.

The willingness of consumers to buy the soy spread, indicates the potential of the product to be a low-cost substitute for butter (Table 1). Obviously, respondents exhibiting a greater liking for the product, as indicated by the Hedonic score, tended to show more willingness to buy it. Since it was offered at its estimated cost, which was about half the cost of butter, the acceptance of the spread could be interpreted essentially as a low-cost alternative to butter. It is, however, interesting to note that even in the group showing "Like extremely" response on appreciable number of respondents were 'reluctant' to buy the spread, presumably, more due to prejudice against a 'synthetic' or soybean product rather than its cost, as could be seen from the comments made by such consumers.

Acceptance by families: A new product visualized as a substitute for a conventional one can be assessed for its real potential only by actually replacing the latter with the former as the housewife uses it, so that she will be in a position to judge for herself how good the substitute is. As shown in Table 2, nearly 64 per cent of eleven families found the soy spread "Slightly less suitable than butter", while 27 per cent of them found it either "As good as butter" or "Better than butter", the average rating being 3.3.

It was observed that the spreadability rating for the spread was distinctly higher than that for butter. Nevertheless, some respondents commented that its appearance was somewhat less attractive and flavour slightly less desirable. Four out of eleven families indicated their willingness to buy the spread at Rs.4.50 per 200 g in glass bottles i.e. at 70 per cent of the cost of butter. If the cost was reduced to Rs.3.50 using cheaper packaging such as plastic tubs, most

TABLE 2. FAMILIES' RESPONSE TO THE SOY SPREAD AS A BUTTER REPLACER

Overall response	Score	No. of families*
Better than butter	5	1(9)
As good as butter	4	2(18)
Sightly less suitable than butter	3	7(64)
Moderately less suitable than butter	2	1(9)
Much less suitable than butter	1	0(0)
Total		11

*Figures in parentheses indicate per cent of families

respondents would prefer the spread to butter. The market potential of the product was thus amply evidenced by this consumer reaction.

It is thus concluded that according to consumers' Hedonic rating for the soy spread, it was highly acceptable. Very favourable response from respondents not familiar with butter was indicative of the potential of the product in institutional feeding programmes too. Willingness shown by most respondents to purchase the spread when made available at about half the cost of butter was a clear indication of the acceptability of the product as a low-cost substitute for butter, which is so expensive that very few people can afford to buy it.

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COMPOSITION OF UNCOMMON FOODS

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Estimation of proximate principles and some of the essential nutrients in fifteen uncommon foods of Uttar Pradesh (India) revealed that several of these foods contain high amounts of important dietary factors. These include fat in Hemp seeds, *(Cannabis saliva)* Musk melon *(Cucumis melo) and in Jakhya (Polanisia viscosa)* protein and energy in Hemp seeds, Musk melon seeds, Nasturtium *(Thopaeolum maius)* seeds and in Jakhya; fibre in Hemp seeds; β-carotene in Amlora *(Rumex hestatus)* leaves, Indian sorrel *(Oxalis corniculate)* leaves, Hisalu *(Rubus ellipticus)* fruit and in Kilmora *(Berberis asiatica)* flower; ascorbic acid in Kaphal *(Myrica esculenta)* fruit, Kilmora flower and Indian sorrel leaves; and certain minerals in fruits of Kilmora, figs *(Ficus roxburglii)* and Black nightshade *(Salanum nigrum)* in leaves of India sorrel, in Hemp seeds and in Jakhya.

There are several foods which are consumed by people in different regions on a very limited scale. Composition of many of these foods is not known. This study was conducted to estimate the composition of fifteen such foods of the Hill Campus, Ranichauri and of Pantnagar, Uttar Pradesh. The foods were classified as (1) leaves (2) fruits, (3) flowers (4) seeds and (5) spices. The leaves studied were (1) Garlic (*Allium sativum*), (2) Yellow oxalis or Indian sorrel (*Oxalis corniculate*), (3) Grape (*Vitis vinifera*) and (4) amlora (*Rumex hastatus*). The fruits included were (1) Black nightshade (*Solanum nigrum*) (2) Kilmora (*Berberis asiatica*) (3) Hisalu (*Rubus ellipticus* (4) Kaphal (*Myrica esculenta*) and (5) Fig. (*Ficus roxburghii*). The flower studied was Kilmora (*Berberis asiatica*). The seeds included were (1) Nasturtium, dry (*Thopaeolum maius*), (2) Musk melon (*Cucumis melo*) and (3) Hemp (*Cannabis sativa*) Spices studied are (1) Bay leaves (*Laurus nobilis*) and (2) Jakhya seeds (*Polanisia viscosa*).

Fresh samples taken from Ranichauri were preserved in the solution containing 5 ml acetic acid, 30 ml formaldehyde, 30 ml ethyl alcohol and 50 ml distilled water.

Hemp seeds, musk melon seeds, bay leaves and Jakhya were studied for proximate composition, iron, calcium and phosphorus only, whereas the others were studied for these components as well as for ascorbic acid and β-carotene. Samples were analysed for proximate principles by standard AACC procedures[1]. Iron content was estimated by the method of Elvehjan[2], calcium and ascorbic acid were estimated by standard AOAC procedures[3] and phosphorus and β-carotene were estimated by the method of Ranganna[4].

TABLE 1. PROXIMATE COMPOSITION OF UNCOMMON FOODS

Foods										
Name	Part	Moisture (%)	Total ash (%)	Acid insolube ash (%)	Crude fat (%)	Crude fibre (%)	Crude protein (%)	±SD	Carbohydrates (by diff.) (%)	Energy (kcal/100 g)
Garlic	leaves	84.1	1.12	0.19	0.79	0.94	3.17	0.10	9.8	59
Indian Sorrel	leaves	84.1	1.52	0.29	1.05	1.09	1.67	0.12	10.5	58
Amlora	leaves	81.9	1.11	0.14	0.31	0.98	3.39	0.05	12.3	68
Grape	leaves	79.4	1.99	0.44	1.34	1.21	2.97	0.05	13.0	76
Black nightshade	fruits	74.4	1.43	0.04	2.19	1.18	2.99	0.06	17.8	103
Kilmora	fruits	88.1	0.25	0.07	0.52	0.67	1.22	0.03	9.2	46
Hisalu	fruits	94.2	0.19	0.07	0.69	0.16	0.52	0.01	4.2	25
Kaphal	fruits	71.2	0.67	0.17	1.46	1.11	4.58	0.08	21.0	115
Figs	fruits	79.4	1.15	0.23	0.77	1.26	1.70	0.05	15.8	17
Kilmora	flower	78.2	0.49	0.03	0.59	1.39	2.38	0.10	17.0	83
Nasturtium (dry)	Seeds	8.4	5.79	0.31	9.97	3.02	24.15	-	48.7	389
Musk melon	Seeds	9.1	4.39	0.71	41.91	1.65	30.92	0.50	12.0	549
Hemp	Seeds	7.7	6.47	1.53	30.63	18.12	24.65	0.25	12.4	424
Bay	Leaves	6.0	3.96	0.13	2.13	2.47	10.45	0	73.0	362
Jakhya	Seeds	6.0	2.43	0.53	14.82	1.46	16.19	0.44	58.0	430

*As is basis

TABLE 2. MINERAL AND VITAMIN CONTENTS OF UNCOMMON FOODS*

Foods		Iron		Calcium		Phosphorus		Ascorbic acid		Carotene	
Name	Part	(mg/100g)	±SD	(mg/100g)	±SD	(mg/100g)	±SD	(mg/100g)	±SD	(mg/100g)	±SD
Garlic	leaves	7.43	0.05	100.5	1.83	64.3	1.37	18.39	1.99	747	11.55
Indian Sorrel	leaves	12.81	0.00	132.3	1.83	142.9	0.00	114.93	3.98	3,947	23.09
Amlora	leaves	8.33	0.10	101.8	0.00	77.9	0.52	85.05	1.99	5,740	575.85
Grape	leaves	7.41	0.16	253.1	0.00	103.4	0.59	62.66	2.31	1,707	23.09
Black nightshade	fruits	3.85	0.13	68.4	2.96	99.2	0.74	50.57	1.99	533	46.19
Kilmora	fruits	3.98	0.11	37.3	1.37	28.4	0.34	46.24	1.86	453	23.09
Hisalu	fruits	1.41	0.02	27.4	0.67	25.9	0.17	53.77	1.86	1,867	23.09
Kaphal	fruits	3.33	0.16	19.0	3.30	91.4	0.00	70.11	1.99	307	23.09
Figs	fruits	2.99	0.06	88.4	2.36	35.8	0.60	3.45	0.00	147	23.09
Kilmora	flowers	4.89	0.19	39.1	0.00	37.0	0.00	35.50	3.23	1,293	23.09
Nasturtium (dry)	seeds	49.50	0.00	200	0.00	838.3	2.89	–	–	–	–
Nasturtium (fresh)	seeds	–	–	–	–	–	–	13.33	2.31	213	23.09
Musk melon	seeds	19.5	0.50	113.3	11.55	563.3	2.89	–	–	–	–
Hemp	seeds	62.0	0.00	213.3	11.55	900.0	–	–	–	–	–
Bay	leaves	74.0	–	340.0	–	290.0	–	–	–	–	–
Jakhya	seeds	44.66	0.29	772.3	11.55	750.0	–	–	–	–	

*As is basis

Three samples of each food were analysed for each component.

Proximate composition: The proximate composition and the calculated energy values of the fifteen uncommon foods are presented in Table 1. All leaves, fruits and flower showed low contents of proximate principles. Seeds, in general, were rich in proteins, fat and energy.

Mineral content: Iron, calcium and phosphorus values of the uncommon foods are presented in Table 2. Leaves had fair to good values of the minerals. Indian sorrel leaves were particularly rich in iron and grape leaves in phosphorus. The seeds, in general, had high mineral content. The values for iron and phosphorus in Hemp, Nasturtiun and Jakhya can be considered very high. Jakhya also has a very high calcium content. Mineral value for fruits and the Kilmora flower were comparatively low; however considering that the quantity of fruits consumed is large, their contribution of minerals may not be insignificant.

Vitamin content: The ascorbic acid and β-carotene values of eleven uncommon foods are also presented in Table 2. Both the values are very low for figs. All the other fruits, leaves and flower have good ascorbic acid values. β-carotene content in the leaves of Amlora and Indian sorrel are very high like some of the commonly eaten dark green leafy vegetables.

Results of this investigation show that several of the uncommonly eaten leaves and fruits may be recommended as rich dietary sources of minerals and vitamins and the seeds can be recommended for edible purposes. However, before such a recommendation is made it may be necessary to study the biovailability of nutrients from these foods.

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CLOSTRIDIA IN SWEETENED CONDENSED MILK AND THEIR ASSOCIATED DETERIORATIVE CHANGES

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Clostridial counts ranging between 0 and 5.9×10^2/100 g were recorded among twenty-eight commercial samples of sweetened condensed milk (SCM). Among the 20 isolates of clostridia, the species distribution included *C. perfringens* (20%), *C. butyricum* (75%) and unidentified (5%). Introduction of *C. perfringens* at a level of 1×10^3/g into SCM, resulted in both saccharolytic and proteolytic changes, while with that of *C. butyricum* only saccharolytic changes were observed during the storage of samples at 37°C upto a period of 30 days. The extent of degradation of lactose by *C. butyricum* was as high as 65% at the end of the incubation period. There was an increase in titratable acidity from 0.15% at 0 day to 0.21% at the end of 30 days. Total volatile acids production was higher in case of *C. perfringens* as compared to *C. butyricum*.

Presence of clostridia in canned milk products is a matter of concern to the food processors and public health authorities[1-3]. Once the anaerobic spore formers enter the milk, it is difficult to destroy them due to formation of heat resistant spores.[4] Besides, milk is perhaps the most protective medium.[5] Hence these clostridial spores continue to be present even after pasteurization of milk and are carried over finally to the manufactured milk products.[6] Although the incidence of clostridia in milk and milk products has been reported by several workers[2,3,7-13], information on saccharolytic and proteolytic changes[14] by clostridia in sweetened condensed milk is scanty. Therefore, the present investigation has been undertaken to study the occurrence of clostridia in sweetened condensed milk (SCM) and the deteriorative changes they may bring about in this product.

Samples: Samples comprised a total of twenty-eight cans of a commercial brand of SCM of different batches.

Enumeration of clostridia: MPN method[15] using differential reinforced clostridial medium (DRCM) was followed for the estimation of total clostridial counts in SCM. The inoculated DRCM tubes were incubated at 37°C up to 7 days. Confirmation of the presumptive tubes was done by heating at 80°C for 10 min and then inoculating into fresh DRCM broth tubes which after culturing indicated positive results.

Isolation of clostridia: The positive MPN tubes of DRCM cultures were subsequently used for isolation of clostridial colonies on DRCM agar by roll tube technique[16]. The isolated colonies of clostridia were subcultured, purified and were examined for various characteristics. Maintenance of clostridial isolates was done by weekly transfer in DRCM containing neomycin sulphate (100μg/ml).

Characterisation of clostridial isolates: Clostridial isolates obtained from samples of SCM were characterized on the basis of microscopic examination, fermentation of carbohydrates, end-products of fermentation by gas-liquid chromatography[17] and other characteristics such as gelatin liquefaction[18], indole production[19] and nitrate reduction[20]. The microscopic examination especially the motility and spore examination was by conventional laboratory techniques. Location of spores was checked microscopically after staining. The motility of the culture was examined by capillary method[21]. In case of fermentation of carbohydrates, namely glucose, lactose, maltose, sucrose, cellobiose, fructose, galactose, mannose, raffinose and xylose and a sugar alcohol mannitol, the individual carbohydrate/sugar alcohol was added to give a final concentration of 1 per cent to the basal medium containing tryptone, 10.0g; bromothymol blue (2 per cent) 15 ml; sodium chloride, 5.0 g on 1000 ml of distilled water.

Estimation of deteriorative changes by clostridia in SCM: Cells of *Clostridium perfringens* and *C. butyricum* were inoculated into sterile SCM cans of 240 g each to give a final concentration of 1×10^3/g and after seaming, the cans stored at 37°C and analysed for lactose[22], and deteriorative changes such as titratable acidity[23], proteolytic activity[24] and total volatile fatty acids[25] on 0, 15 and 30 days of storage.

Out of a total of 28 samples of SCM, the range of clostridial counts was 0 to 5.9×10^2/100 g. However, for convenience, the clostridial counts of the samples have been arbitrarily categorised into four ranges viz. <1, 1-10, 11-100 and > 100/100 g (Table 1). In SCM samples, the corresponding per cent distribution was 7.1, 0.0, 53.6 and 39.3. Although this level of clostridia may not be sufficient for spoilage of SCM directly due to high sugar content (41 per cent), its further use for conversion into other dairy products or confectioneries may pose spoilage problems.

The results of clostridial counts revealed that there was no uniform pattern regarding their occurrence in

TABLE 1. MOST PROBABLE NUMBER (MPN) OF CLOSTRIDIA SWEETENED CONDENSED MILK AND THE PER CENT DISTRIBUTION OF SPECIES OF CLOSTRIDIUM

Range of clostidia/100g	Samples +ve (No.)	Species of *Clostridium*
< 1	2(7.1)	*C. butyricum* (75)
1-10	0(0.0)	*C. perfringens* (20)
11-100	15(53.6)	Unidentified (5)
>100	11(39.3)	

Figures in parenthesis indicate per cent distribution.

SCM but based on their characteristics, the distribution of the type of clostridial species was significant (Table 1). Of the various species of clostridia reported earlier in milk and milk products[26-28], *C. perfringens* and *C. butyricum* were the two species which were predominant in this study with percentages of 20 and 75, respectively. In an earlier study[29], mainly *C. butyricum* was encountered in SCM.

The fermentation pattern and other characteristics of clostridia checked in the present study were very much similar to those reported by Holdeman *et al.*[30].

Since there were variations in the number of clostridia in SCM, a higher number (1×10^3/g) as used in the present study may cause spoilage problems. The deteriorative changes brought about by clostridial species in SCM are presented in Table 2. It may be seen that there was a progressive increase in lactose degradation, starting from 100 per cent at the inoculum stage to as low as 85 and 35 per cent in the case of *C. perfringens* and *C. butyricum* at the end of 30 days of storage, respectively. The present study has shown higher saccharolytic activity in the case of *C. butyricum* compared to *C. perfringens*. The degradation of lactose was accompanied with increase in titratable acidity. According to the ISI recommendation[23] increase in acidity is permitted to 0.03 per cent and hence with this standard the product may be considered unsatisfactory after 15-30 days of storage at 37°C if the initial level of clostridial contamination was 10^3/g. Apart from the saccharolytic changes by both the organisms *C. perfringens* and *C. butyricum*, proteolytic changes were observed by *C. perfringens* only. Such results are not unexpected in view of the fact that *C. butyricum* is a strong saccharolytic organism and does not have any proteolytic activity[14]. However, increase in proteolytic activity of *C. perfringens* was progressive only upto 15 days and thereafter no proteolytic activity was observed.

TABLE 2. DETERIORATIVE CHANGES BROUGHT ABOUT BY CLOSTRIDIAL SPECIES IN SWEETENED CONDENSED MILK DURING STORAGE STORAGE

Type of change	*Cl. perfringens* during indicated storage days			*Cl. butyricum* during indicated storage days		
	0	15	30	0	15	30
Lactose degraded (%)	100	92	85	100	59	35
Titratable acidity (%)	0.20	0.22	0.23	0.15	0.18	0.21
Proteolytic activity (mg of tyrosine)	22.50	25.50	16.00	14.20	13.50	13.10
Total volatile acids[4] (ml of 0.1 N NaOH)	10.00	44.00	62.00	10.00	37.50	48.00

The present data have revealed that metabolic activity of clostridia in a dairy product like SCM may take place even with high sugar content. This has been well reflected by increase in total volatile fatty acids production (Table 2) leading to sensory changes in the product. It appears that the saccharolytic and proteolytic activities of *C. perfringens* were accompanied by higher total volatile fatty acids production compared to *C. butyricum* which exhibited only saccharolytic activity.

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PREPARATION OF CRYSTALLIZED AND GLAZED CITRUS PEELS

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Crystallized glazed citrus peels were prepared from five citrus varieties. Among the crystallized and glazed products those from seville orange were found to be the best.

Candied fruits and peels are products which are impregnated with enough sugar to preserve them. In crystallized and glazed fruits and peels the process is carried out a little further and the fruits or peels are either given a coating of sugar crystals or glazed with sugar. These products require pretreatments such as brining and cooking in boiling water before syrup treatment[1].

Preparation of candied crystallized and glazed peels from citrus peels would serve as an important outlet for utilization of by-products in a citrus processing plant[2]. Additional benefit of making these products from citrus peels is that the essential oil present in them would impart a natural pleasing flavour which is not apparent in such products from other fruits[3].

Materials: Mature and well developed citrus fruits with orange or yellow colour were collected from the local market. Green fruits or fruits having patches were discarded. The following citrus fruits were used: (1) Sour orange (Seville orange, *Citrus aurantium* L) (2) Citron (*Citrus onedica*), (3) Pummelo (*Citrus grandis*) (4) Coorg orange (*Citrus reticulata* Blanco), (5) Rough Lemon (*Citrus jambhiri* Lush). Cane sugar from local market Glucose syrup (82° Brix and 42 DE) (from M/s Anil Starch Ahmedabad) and commercial table salt were used. Tartrazine and sunset yellow F.C.F. (ABRAC) were used to impart yellow or orange colour to the citrus peels as desired.

The preparation of candied peels from citrus fruits is shown in the flow-sheet given.

Preparation of crystallized and glazed citrus peels: The candied peels were immersed in the super saturated sugar syrup of 75° brix and left in it for 16 hr. By this time, crystals of desired size were obtained. In another batch, the candied peels were immersed in 80° brix for 2 min for glazing and the glazed citrus peels were drained, dried and packed in LDPE bags[4].

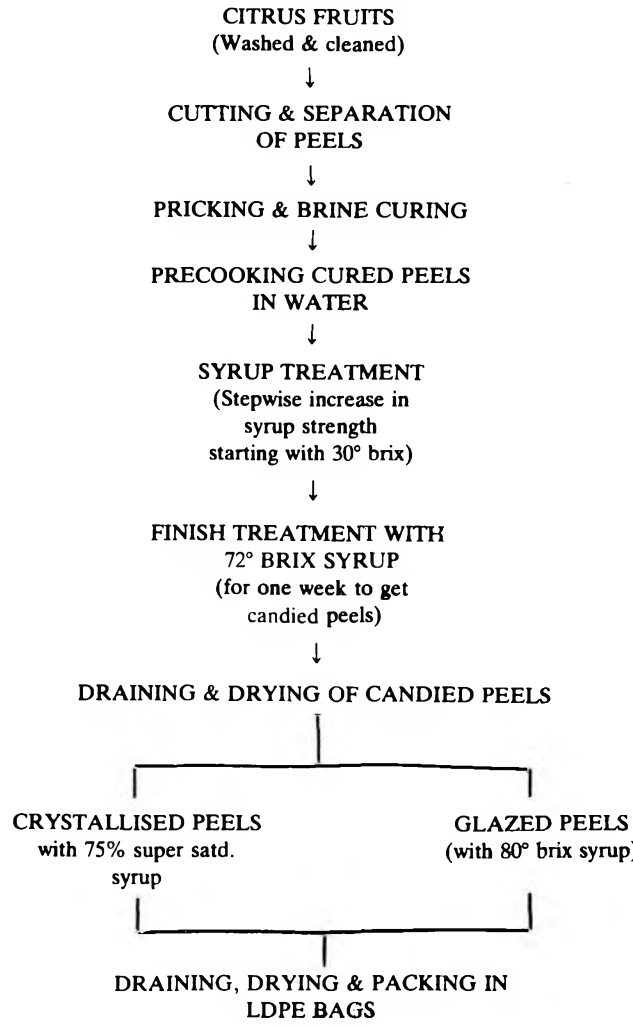

Fig. 1. Flowsheet showing various steps involved in the preparation of candied peels from citrus fruits.

Effect of various treatments: A flow sheet showing various treatments is shown in Fig. 1. The effect of brine curing was that the cured peels were slightly more translucent and fairly tender and pliable. One hour cooking of the brine cured citrus peel was sufficient to tenderize the peel and leach out the salt. Glucose syrup was used beyond 60° brix because this overcame the undesirable crystallization of sucrose and also imparted translucency/transparency to the syruped peel. When the drained crystallized peel was spread on trays and dried at room temperature for 24-30 hr, beautiful sugar crystals were observed on the peel surface.

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TABLE 1. YIELD OF GLACED AND CRYSTALLIZED PEELS FROM CANDIED PEEL

	wt of peels used (g)	Glaced peel obtained (g)	Per kg of candied citrus peel* (kg)	Candied peel used (g)	Crystallized peel obtained (g)	Per kg of candied peel* (kg)
Coorg orange	300	300	1.00	315	320	1.02
Pummelo	440	495	1.13	500	590	1.18
Rough lemon	330	335	1.01	330	340	1.03
Citron	475	4485	1.02	500	515	1.03
Seville orange (with albedo)	1000	1085	1.09	1000	1165	1.17
Seville orange (no albedo)	380	390	1.03	650	720	1.10

*Calculated values are means of three replicates.

Crystallized peel: The yield of crystallized peel varied from 1.02 to 1.13 kg of candied peel (Table 1). The yield of crystallized peel from candied pummelo peel and seville orange peel (with albedo) were higher (1.18 and 1.17 kg) respectively per kg of candied peel as compared to others.

Glazed peels: Proper glaze was obtained in to 2 min of immersion time of the candied peel in the 80° brix syrup, and longer duration of immersion imparted a thick white crust of sugar on the surface of the peel which was not attractive. The yield of glazed peel varied from 1.00 to 1.13 kg from 1 kg of candied peel. It was the highest in the case of glazed pummelo peel (1.13 kg) and lowest in glazed Coorg orange peel (1.00 kg) Seville orange peels gave the best products in both crystallized and glazed citrus peels.

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CHANGES IN QUALITY OF KEW PINEAPPLE FRUIT AT DIFFERENT TIMES

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Pineapple fruits harvested during different months of the year showed variation in the time taken to attain maturity, per cent juice content, total soluble solids and acidity of the juice. Fruits harvested during July to November matured early and were very juicy and sweet, while those harvested during December to February were sour and those harvested during January to May were late maturing and less juicy.

Pineapple fruits harvested in different months of the year are not of uniform quality. Some of the important characters are dependent on climate[1,2]. Though fruits of winter months had very appealing colour, they had higher acid content. Fruits in the drier months were less juicy. Fruits of March to June took very long time to mature. The present investigation was undertaken to examine changes in quality of the kew pineapple fruits harvested at different times of the year.
year.

Half ripe fruits of Kew variety of pineapple were harvested in different months from the general plantation of the pineapple research project of Assam Agricultural University, Jorhat, during 1982-83 and 1983-84. The local climate is characterised by a marked dry season from November to March and wet season from April to August. The average annual rainfall during the experimental period was 2100 mm. The minimum and maximum temperatures were 11° and 22°C during December-January and 24° and 32°C from May to September, respectively.

Flowering plants were labelled at different times. From January to June, in fact, harvesting due to natural flowering was nil. So the plants which attained about 40 to 45 leaf number with D leaf (Young, Physiologically mature) size of about 75 × 5.5 cm were induced to flower during August to January with ethrel applied at 25 ppm mixed with 0.04 per cent calcium carbonate and 2 per cent urea (50 ml solution was sprayed on the heart of each plant). Days taken from emergence of inflorescence to harvest were taken as the time for fruit maturity. Fruiting is the period from blossoming of first flower to last flower in the inflorescence.

Ten fruits in three lots (total 30) in each month were tested for juice content and acidity according to methods given in Manual of Analysis of Fruits and Vegetable products[6] and total soluble solids (TSS) were read in a hand refractometer.

Days required for fruit maturity were significantly influenced by different time of the year. Fruits harvested during August, September and October required about 150 days for maturity, whereas those harvested during July to November required a few days more (Table 1). From December onwards to June the trend was for more days to maturity and it took 197 days in May with a downward trend from June onwards. In most of the countries, pineapple

TABLE 1. CHANGES IN QUALITY OF PINEAPPLE FRUIT HARVESTED AT DIFFERENT MONTHS

Month of harvest	Maturity in days		Juice (%)		TSS(%)		Acidity (%)	
	I yr	II yr	I yr	II yr	I yr	II yr	I yr	II yr
January	165	168	67	67	15.7	15.6	1.01	1.00
February	166	168	68	69	16.2	15.5	0.90	0.89
March	172	177	68	68	15.1	15.6	0.66	0.68
April	180	188	66	66	15.4	16.0	0.67	0.69
May	196	197	68	67	15.4	14.8	0.57	0.59
June	178	177	70	69	15.7	15.6	0.50	0.55
July	156	159	73	72	15.2	14.9	0.51	0.51
August	150	152	73	74	14.8	15.5	0.53	0.49
September	148	151	73	74	15.2	15.0	0.51	0.52
October	148	151	74	74	15.3	15.5	0.57	0.60
November	156	157	73	73	15.6	14.5	0.64	0.67
December	163	164	74	74	14.6	14.7	0.88	0.88
S.E. diff.	3.8	4.07	2.44	2.00	N.S.	N.S.	0.052	0.042
CD at 0.05%	7.87	8.43	5.05	4.14			0.108	0.088

N.S.: Not significant;

fruits are generally harvested in summer months and these take around 150 days for maturity[3]. The longer duration required for maturity from December to June was due to the effect of cold weather with shorter winter days during fruiting and developing period of the fruit. This was also observed by Hope[4]. The juice content of the fruit harvested during January to May was significantly less than those harvested in most of the other months of the year (Table 1). Cold weather with dry spell is not favourable for pineapple fruit growth and it affected juice accumulation. Some fruits harvested in February – March were found to have cracks.

Fruits harvested in summer months were having normal titrable acid range from 0.5 to 0.6 per cent, but when matured in December, January and February, the acid contents in juices were significantly higher than those matured at other times. The cooler temperature and low solar radiation are reported to produce fruits with higher acid content[5].

There were no significant differences in TSS contents between juices of the fruits harvested in different months.

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BOOK REVIEWS

Environmental Health Criteria, 63; Organophosphorus Insecticides: A general Introduction, WHO, Geneva, 1986; pp 181.

This publication is the outcome of the meeting of the WHO task group on Environmental Health criteria for organophosphorus insecticides held at Geneva during 1985 followed by the draft prepared by Dr. M.K. Johnson of the United Kingdom, Medical Research Council with the efforts of many others. WHO/FAO have reviewed at least 100 organophosphorus insecticides while preparing this document. The book under review prepared at the instance of IPCS (International Programme on Chemical Safety) and published under the joint sponsorship of the United Nations Environmental Programme, the International Labour Organization, and the World Health Organization gives an authoritative, consistent and up-to-date account of organophosphorus insecticides (OPI) and serves the basic purpose of giving valuable information.

The necessary prerequisites of OPI in general are covered in 7 sections: Summary and recommendations; Properties and Analytical methods; sources of Human and Environmental Exposure; Environmental Transport and Distribution; Exposure levels, Metabolism and mode of Action; Effects on Animals and Effects on man. It is made comprehensive by supplementing with 14 tables, 8 figures and 297 references. Each section is self-contained. Notable among the sections are:-.

a) Sources of human and environmental exposure reviewing the mode of transport and distribution in the environment, bioaccumulation and degradation and occupational exposures;
b) Metabolism and mode of action dealing with dermal uptake of OPI, absorption from gastrointestinal tract and through inhalation, tissue binding and elimination and mode of action;
c) Effects on animals covering the acute and chronic toxicity and determination of neurotoxicity, structure and activity relationship, mutagenic, carcinogenic and teratogenic mechanisms and effects on immune system;
d) The last section deals with the effects on humans, the clinical picture of OPI intoxication, diagnosis, methods for assessing absorption and effects, biochemical methods for measuring the effects, electrophysiological procedures adopted, monitoring whole blood acetylcholinesterase effects on the nervous and neuromuscular systems due to acute or long-term exposures of OPIs.

While annex I includes 77 OPI compounds arranged alphabetically detailing the name, chemical structure, molecular formula and molecular weight, the involvement of task force in preparing the document with a wide range of agencies and sources like JMPR reviews, IARC, FAO/WHO data sheets, IRPTC profile and legal files covering 57 compounds included in annex II is commendable. Data on LD_{50} and no-observed-adverse-effect levels in animals for 32 compounds with references indicated in annex III are useful inclusions. The biotransformations of OPI in general, through oxidative desulfuration, N-dealkylation, O-dealkylation, thioether oxidation, triester hydrolysis, hydrolysis of functional groups are well depicted in seven figures besides the inhibition of esterases by OPI presented in Fig. 8. Details of the chemical structure with common names, consumption patterns in Africa, North, Central & South America, Asia and Europe, toxicity of 45 OPIs to aquatic organisms, OPIs causing delayed neuropathy in hens and in humans depicted in fourteen tables are valuable additions.

This book covers all major aspects and is well presented. Valuable insights have been provided relating to the aspects of measuring the delayed neuropathic effects and measurement of neuropathic target esterases (NTE) in human lymphocytes as a predictive monitor.

It is a valuable addition to any library catering to the information needs of research workers and also to the personal collection of individuals for reference. This document is an illustration of the earnest effort made to update the facts and issues involved in OPI at International level by the task force group.

M. K. KRISHNAKUMARI
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Response Surfaces: Design and Analyses: by Andre I Khuri and John A. Cornell, Marcel Dekker Inc, 270, Madison Avenue, New York, NY 10016, Basel, Statistics Text-books and Monographs Series Vol.81; 1987; pp:427, Prices: $45 (US & Canada; $54 (All other countries).

A well produced book, with current references, which can serve as a text-book as well as a reference book for professional users as well as researchers in various fields presents, in total, up-to-date techniques in Response Surface Methods.

The first chapter introduces the Response Surface Method lucidly, and effectively covers the various terminologies, their definitions and the need for systematic designing of experiments so that the optimum can be reached fast. It is a well illustrated chapter which should be convincing to any research worker, regarding the need to adopt the Response Surface Methods.

The second chapter provides the basic requirements in matrix algebra and statistical methods without which one can not proceed further. The useful designs and the appropriate analysis of variance and testing of hypothesis are covered with several examples.

The third chapter deals with first order models and designs. Several designs viz., the 2^k factorial, fractional factorial, simplex and Plackett-Burman designs have been dealt with. The Plackett-Burman designs should have been given in more detail as these are very useful in screening experiments with large number of factors. Also, the analysis part of the Plackett-Burman designs with one replicate per combination and more than one replicate would have been more useful in industry and research.

The fourth chapter deals with second order designs, orthogonal and rotatable. The factorial, Box-Behnken, Central composite, equiradial, cylindrically rotatable, asymmetric rotatable and other second order designs have been dealt with. The more widely used central composite designs with the constants for fitting orthogonal polynomials and standard errors would have provided the user complete information. Box-Behnkan designs are more popular in several fields. As such, the inclusion of an example would have been very useful.

The next chapter presents the methods of determination of optimum conditions:- An excellent treatment with good figures of contours of various types, canonical equations, their interpretation and ridge analysis which are of immense value to the user. Also, a short account of Nelder-Mead simplex method which is a derivative free optimisation method has been dealt with.

The methods of estimating response surfaces that rival least squares based on integrated mean squared error criterion has been adequately dealt with in chapter 6, which does not usually find a place in books of this type.

Analysis of Multiresponse experiments is a very useful chapter which helps the user to identify the minimax response region with respect to several responses jointly. This is a very valuable addition for practical use. In chapter 8, non-linear and partially linear response surface models are considered which are very necessary for growth, biological potency of drugs/enzymes, rate of reaction and concentration, econometric models of supply and demand and so on.

Mixture designs find a place in chapter 9 which are very interesting and useful with adequate examples. Many materials that one comes across in every day life are mixtures of several ingredients and hence the methods of handling and optimisation of these is a worthwhile inclusion.

Last but not least, an interesting discussion on directions for further research in response surface methods has been included which is food for thought for every experimenter in the field. Overall, the get up of the book is very good with well reproduced figures. The references and bibliography provide a lot of material for identifying the right type of techniques for specific uses.

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Accelerated Processing of Meat: Ed. by A. Romita, C. Valin and A.A. Taylor, Elsevier Applied Science Publishers Ltd., Crown House, Linton Road, Barking, Essex, England; 1987; pp:291; Price: not mentioned.

Meat is an expensive item of food. It is also unique in that although it is edible immediately after slaughter of the animal, yet on practical considerations it is aged in order to impart desirable sensory characteristics to it. Since meat is also susceptible to deteriorative changes during storage, it is one of the food items which continues to be investigated intensely by scientists and technologists. One of the areas of study is the accelerated processing of meat. The latter term includes the entire gamut of operations from slaughter of animals right through to the final product as it reaches the consumer.

The book is a compilation of the various papers presented at the first Workshop of the new Standing Committee for Agricultural Research, Agro Food Programme for European Meat Scientists, held in Rome on 29-31 October 1985. Twenty one specialists drawn from 8 European countries and the USA participated in the Workshop. There were five sessions including the concluding session.

Seven papers were presented during Session I dealing with the Basis of Accelerated Processing. They were: Optimal chilling and ES parameters for hot boning; Factors influencing protease activity; Chilling of hot bones muscles; Rapid cooling of hot boned meat; In line chilling of beef carcasses; Influence of accelerated processing on production of clean beef carcasses and Microbiological aspects of accelerated processing.

In Session II, dealing with Accelerated Processing of Pork, four papers were presented. They were: Hot boning of pig carcasses – influence of chilling on quality; Skimming *vs* scalding; 'Warm' boning of pigs and Developments in the USA.

Session III was on Accelerated Processing of Beef in which five papers were presented viz. Effect of advanced boning on beef tenderness; Hot boning and beef quality; Accelerated Processing and meat quality aspects; Meat characteristics of low voltage ES cows and Meat characteristics of cows related to ES.

Session IV was on Assessment and/or Prediction of meat quality characteristics. The five papers in this session dealt with evaluation of some quality characteristics, rapid counting methods and microbiological implications of accelerated processing.

The concluding Session summarised the papers and recommended proposals for coordinated activities in the field.

Although the emphasis is on beef and pork, the compilation should serve as a useful guide reflecting the recent trends in accelerated processing of meat.

Some grammatical and typographical errors have crept in, presumably originating from the Authors' manuscripts.

L. A. RAMANATHAN
D.F.R.L. MYSORE

Modern Carbohydrate Chemistry: by Roger W. Binkley; Mercel Dekker Inc., New York 10016; 1988; pp: 343; Price: not mentioned.

This book is an attempt to summarize comprehensively the current understanding of carbohydrate chemistry, both from synthetic and mechanistic points of view. However, the inclusion of 'Modern' in the title is a bit paradoxical as none of the modern methodologies – techniques – developments in carbohydrate research are dealt with in the present book. Also, the author assumes the readers to have some familiarity with classical organic chemistry, although the initial five chapters are meant to bridge partly this vital gap.

Carbohydrates, in general, contain three types of hydroxyl groups, viz., anomeric (C1), primary (C2/C3/C4) and secondary (C6); each of them possessing specific reactivities. Accordingly, a very broad spectrum of reactions can be performed on them and a host of new compounds/derivatives be prepared. The book under review contains wealth of information on specific reaction types, comprehensive discussion of reaction mechanisms, description of reagents and approaches used to solve synthetic problems and the *pros* and *cons* of new and established synthetic reactions.

Of the 14 chapters in this book, the first six have been devoted to a brief discription of the basic terminology and fundamental concepts of carbohydrates. Narration of conformation analysis, in chapter 6, particularly describing the 'anomeric effect' in terms of energy differences, dipole-dipole interactions, molecular geometry properties, hydrogen bonding and solvent effects is easily understandable, simple and effective too.

Chapters 7 and 8 discuss the modern and classical concepts relating to properties of protected and unprotected sugars. Sugars being polyfunctional molecules, need to be selectively substituted/protected before envisaging a suitable strategy for a particular synthesis. The fate of reducing sugars in solution, the various reactions involving anomeric and non-anomeric carbon atoms, the formose reaction and protection of sugars by acetal, ketal, ester, ether and other linkages are all described with specific examples. Reaction mechanisms are given for each of these reactions.

Chapters 9 through 13 orient respectively on nucleophilic substitution, red-ox reactions, addition and elimination reactions. In multistep synthetic procedures, all these reactions are commonly encountered. Numerous examples are given under each section. Oxidation-reduction reactions are the most important and extensively studied, both in synthetic and naturally occurring carbohydrates. In the latter, they perform a key structure role. For this reason alone the periodate oxidation details could have been further amplified for the benefit of readers.

The final chapter covers the state-of-the-art in oligosaccharide synthesis. A host of reactions and reagents have been used and high stereoselectivity has been achieved in many cases. Nevertheless, it should be emphasized that each oligosaccharide synthesis remains an independent problem, and that there are no defined set of conditions for oligosaccharide synthesis.

Overall, the aims of the book have been reasonbly well met. The book is of interest to both the student and the working scientist, and should be appealing to any one interested in the chemistry of carbohydrates. The general appearance, layout, and design of the book are good. Inclusion of pertinent references at the end of the chapters adds additional value to the book, although some of the references cited are not easily available. The book is also provided with an excellent index and content section (for each of the chapters).

Of course, the book is not without any typographical errors. For example, on page 10, line 9 ketoses is

written as ketones; page 37 Table I, line 13, methylsulfonyl group is wrongly named as trifluoromethyl; page 63, Fig 16, α-anomer is mentioned as β-anomer, etc.

Not withstanding these, the book will be a useful reference text for all those engaged in carbohydrate research.

R. N. THARANATHAN
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ADDITIONAL INSTRUCTIONS FOR REPORTING RESULTS OF SENSORY ANALYSIS

1. *Objectives:* The objective of the study should be stated clearly.

2. *Sensory test methods:* The methods are classified under two major categories; Analytical and Affective. Laboratory analysis with trained or semi-trained panels must use analytical methods. Affective methods can be used in consumer studies. Adequate details and references should be provided regarding methods used for pattern of collection, analyses and interpretation of data.
Analytical (Trained Panel): The Major types of tests which can be made use of are:
Discriminative. Difference or similarity testing and sensitivity assessments using differences tests, ranking, thresholds, dilutions etc.,
Descriptive/Quantitative: Flavour profiles, Texture profiles, Interval Scaling, Ratio Scaling, Descriptive Quality Scoring etc.,
Affective (Untrained/consumer Panels): Difference/Preference, Hedonic rating, FACT ratings-Preference rankings etc.,

3. *Experimental designs:* The designs used are to be clearly stated. e.g. Randomized block, Latin squares, Factorials, Fractional factorials, incomplete blocks and so on.

4. *Panel:* For analytical tests, the source of panel, whether inhouse or outside organisation to be indicated. The number of panelists should be stated, which should be normally not less than 15. Also whether the same panelists or different panelists have participated in testing the samples has to be indicated. Information on the composition (age, sex, etc) of the panel to be provided. The panel should be trained to function as a human analytical instrument, with periodic re-orientation and at required sensitivity.

For affective test, the panel (sample of population) should be representative of target population selected on the basis of defined sampling procedures. The number should not be less than 200. The composition (age, sex, income group, etc) of the panel should be indicated.

5. *Physical requirements:* For the analytical tests, the laboratory set up should be reported e.g. conducted in a booth with soft neutral shade walls or separators, without distraction from external sound or odour, with comfortable room temperature (22° – 25°C) and relative humidity conditions (35-40%) and suitably illuminated.

The equipment and methods of sample preparation, testing temperature conditions, sample size and number of samples evaluated per panelist and per session should be reported.

The time of evaluating and sequence of testing and data entry carriers, if any, and nature of palate-clearing agents used should be indicated.

6. *Statistical analysis:* The data handling procedure should be appropriate to the design, and should be clearly indicated including any transformations or derivations that are carried out, e.g. assignment of numbers to intervals, categories and the like. The type of analysis carried out, categories, the level of significance and the decisions made are to be provided with appropriate tables and graphs. Appropriate and adequate data should be provided to justify conclusions and enable repeatability. For e.g. while reporting results of tests of significance, the relevant tests like F, x^2, t, r, Rank sum, Mann-whitney, Rank correlations and so on. The probability levels, degrees of freedom, the observed value of the test criterion, the direction of the effect and the decision based on these are to be indicated.

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INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (in triplicate) should be typewritten in double space on one side of bond paper. They should be complete and in final form. The paper should not have been published or communicated for publication anywhere else. Research Notes should clearly indicate the scope of the investigation and the salient features of the results. Only *invited* review papers will be published.

2. The typescript should be arranged in the following order: Title (to be typed in capital and small letters for Research Papers and all capitals for Research Notes), Authors' names (all capitals) and Affiliation (capitals and small letters). Also give a short running title not exceeding 10 words as a footnote.

3. **Abstract:** The abstract should indicate the principal findings of the paper and typed in single space. It should not be more than 200 words and in such a form that abstracting periodicals can readily use it.

4. Use names of chemical compounds and not their formulae in the text. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Footnotes especially for text should be avoided as far as possible.

5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables should be typed on *separate* sheets. Nil results should be indicated and distinguished clearly from absence of data, which is indicated by '——' sign. Tables should not have more than *nine* columns.

6. **Illustrations:** Graphs and other line drawings should be drawn in Indian ink on tracing paper or white drawing paper preferably art paper not bigger than 20 cm (OY axis) × 16 cm (OX axis). The lettering should be twice the size of the printed letter. Photographs must be on glossy paper and must have good contrast; **three copies** should be sent.

7. **References:** Names of all the authors along with title of the paper should be cited. Abbreviations such as *et al.*, *ibid*, *idem* should be avoided. References should be serially numbered as superscripts in the order they are cited in the text and the same order should be maintained in the reference list. The titles of all scientific periodicals should be abbreviated in conformity with the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.

   Citation should be as follows (note the underlines also):

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

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

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

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

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

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

8. Consult the latest issue of the *Journal* for guidance. For "Additional Instructions for Reporting Results of Sensory Analysis" see **issue No. 1** of the Journal.

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Research Notes

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