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# ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS

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## Development and Evaluation of a Permanganate-based Ethylene Scrubber for Extending the Shelf Life of Fresh Fruits and Vegetables

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A shelf-stable and cost-effective granulated ethylene scrubber based on potassium permanganate impregnated in an inert matrix formulated using alumina and limestone was developed and evaluated against scrubber matrices like cement and silica gel *vis-a-vis* an imported trade scrubber (U.K.). The  $\text{Al}_2\text{O}_3$  – limestone and cement based formulations showed satisfactory overall ethylene absorption, granule firmness and shelf-stability comparable to the trade product. Among the two absorbents, the alumina based formulation performed better than the cement based. Laboratory and large scale (on board ships) storage trials with mango, tomato, brinjal and okra at ambient temperature and/or  $10^\circ\text{C}$  and banana under ambient temperature only using the scrubber sachets/blankets showed an overall extension in shelf life ranging from 3–8 days which is comparable to trade scrubber. The technique for production and packaging of the absorbent granules is simple, inexpensive and easy to adopt by small scale industry. The alumina and cement based scrubbers cost approximately Re. 0.18 and 0.06 respectively per kg fruit/vegetable to be stored as compared to Rs. 2.00 for the imported trade product.

Better horticultural practices and development of improved cultivars have resulted in increased production of fruits and vegetables in India in recent years. However, there is significant loss estimated at 20–40 per cent<sup>1</sup> attributable to improper handling, lack of low temperature storage and transportation facilities and high ambient temperatures. Especially in the case of Armed Forces, who consume large quantities of fresh fruits and vegetables, transportation under ambient temperatures and storage on board ships result in significant quality deterioration besides sizeable losses. Methods that have been developed to prolong shelf life include controlled and modified atmosphere storage, hypobaric storage, etc.<sup>2</sup>.

Ethylene ( $\text{C}_2\text{H}_4$ ), a natural plant hormone which plays a central role in the initiation of ripening, has been shown to damage several fruits and vegetables by accelerating senescence (ripening and ageing)<sup>2,3</sup>. Its removal from the storage atmosphere is known to extend shelf life, especially in mixed storage situations<sup>4</sup>. A method that has been in commercial practice is its oxidation by potassium permanganate impregnated in an inert matrix and a few proprietary formulations are in use in the West which have to be imported at high cost making their application commercially non-viable.

Identification and evaluation of a cost-effective and efficient matrix for permanganate impregnation is the foremost requirement in this regard. Earlier workers used vermiculite<sup>5</sup>, celite<sup>6</sup> and perlite<sup>7</sup> but have not emphasised on their absorption efficiency, storage stability and cost effectiveness.

Cement and expanded mica have also been used<sup>4</sup> besides silica gel and alumina pellets<sup>8</sup> but no extensive data are available on their application. The present study was, therefore, undertaken to develop a commercially viable  $\text{KMnO}_4$  based ethylene scrubber in granular form in suitable packaging and evaluate its efficacy under laboratory and field storage conditions.

### Materials and Methods

Three matrices impregnated with  $\text{KMnO}_4$  were formulated as follows:

a) *Matrix based on silica gel*: Coarse silica gel granules (BDH) were dipped in 3 per cent (w/v)  $\text{KMnO}_4$  (LR) solution and dried in ambient air to 7 per cent moisture. The process was repeated three times to achieve the optimum 2–3 per cent  $\text{KMnO}_4$  concentration.

b) *Matrix based on a mixture of alumina and limestone powder*: Roasted limestone procured from the local market was moistened by sprinkling water, cleaned free of stones, crushed, and sieved ( $< 18$  mesh,  $> 80$  mesh). The powder (50 per cent) was dry mixed with neutral active alumina ( $\text{Al}_2\text{O}_3$ ) (LR, 40 per cent), commercial grade plaster of Paris (7 per cent) and powdered  $\text{KMnO}_4$  (LR 3 per cent), as per formulation standardised to yield satisfactory granules. The mixture was moistened (30 per cent), mixed thoroughly and granulated.

c) *Matrix based on cement*: White portland cement (77 per cent, commercial grade) was dry mixed with clean sand ( $< 18$  mesh, 20 per cent) and powdered  $\text{KMnO}_4$

(3 per cent). The mix was moistened to 20 per cent, mixed thoroughly and granulated.

**Granulation and drying:** The  $Al_2O_3$ -limestone and cement based formulations at (b) and (c) above were granulated by extrusion using a mechanical mincer (Hobart, U.K.) fitted with a perforated disc having 4 mm diameter holes. The granules in the form of small beadlets were spread on metallic trays and allowed to dry in ambient air at room temperature (23-30°C) for 4 hr after which the trays were tightly covered with polythene sheet (300 gauge) and the granules allowed to set in the humid atmosphere for 16 hr for cement matrix and 32 hr for  $Al_2O_3$ -limestone matrix. Further moistening by spraying with water (100 ml/kg) was necessary for the optimum setting of  $Al_2O_3$ -limestone based granules after 20 hr of initial setting. Finally, the granules were dried in ambient air to a moisture content of 11 per cent in about 3 hr and packed in opaque pigmented polythene (LD, 300 gauge) pouches till used.

**Packaging as blankets and sachets:** The granules were packed in the form of blankets (120×70 cm with 12 horizontal tubular columns, net weight 7 kg) (Fig. 1) suitable for hanging inside store rooms/cold rooms of about 2 ton capacity and as sachets (7×7 cm, net weight 5 g) suitable for placing inside transportation containers, using a gas permeable polythene (HD) woven fabric. The blanket was lined with casing cloth along the margins with eyelets at the top to facilitate hanging. The granules were filled in the horizontal tubular columns formed between two layers of fabric by stitching and the mouths of columns finally sealed by stitching. In case of sachets, preformed pouches were filled and heat-sealed using an electrical heat sealer.

**Storage studies on fresh fruits and vegetables:** The efficacy of the developed formulations was tested by both laboratory storage studies and large scale storage trials on board naval ships. Laboratory studies were conducted using fresh mangoes, tomatoes and bananas at turning stage and brinjal and lady's finger (okra) of optimum eating quality. All fresh produce were procured from the local market. In

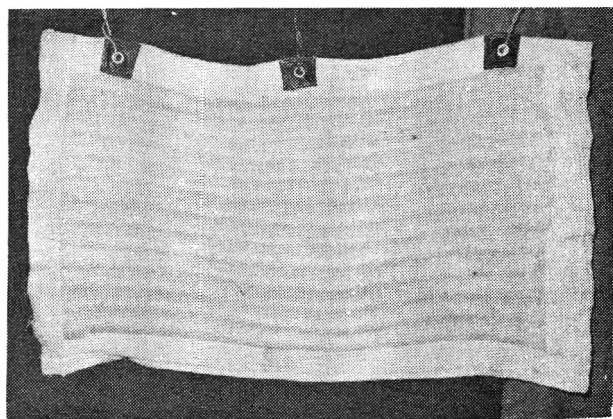


Fig. 1. Photograph of blanket containing  $Al_2O_3$ -limestone based ethylene scrubber granules.

large scale trials, fresh fruits and vegetables forming part of regular supplies to Navy, were used.

a) **Laboratory scale trials:** In the laboratory tests, both the  $Al_2O_3$ -limestone based and cement based formulations in sachets were evaluated during storage of fruits and vegetables in comparison with an imported proprietary trade formulation (U.K.). The selected commodities weighing 10 kg each were stored in a B.O.D. incubator maintained at  $10 \pm 1^\circ C$  and in plastic crates under ambient conditions along with the ethylene absorbent sachets. About 5 g scrubber was used for every 2 kg raw material as conventionally employed. A parallel control experiment without any ethylene scrubber was also conducted in each case under similar conditions. Data on brix/acid ratio, fruit/vegetable firmness, physiological loss in weight and per cent spoilage were collected periodically based on three experimental replicates on all commodities.

b) **Large scale trials:** Large scale storage trials were conducted using  $Al_2O_3$ -limestone based ethylene absorbent blankets in comparison with the imported trade product simultaneously in the cold rooms of two naval ships on routine sailing exercise. Approximately 200 kg each of mango, tomato, brinjal and lady's finger were stored in each ship's cold room maintained at  $10^\circ C \pm 1$  and RH of 85-90 per cent. The fresh produce were kept in wooden racks and the blanket was hung near the air exit of the cold room about 4 ft above floor level. Control without scrubber was simultaneously run inside a refrigerator maintained at  $10^\circ C$  with 10 kg of each commodity. Similar experiments on banana were conducted under ambient conditions.

**Analytical methods:**  $KMnO_4$  in the ethylene absorbent granules was estimated by titration with sodium oxalate<sup>9</sup>. pH was determined using a standard digital pH meter and the moisture content by drying in an air oven at  $100^\circ C$  to a constant weight.

$^\circ Brix$  in the fruit pulps/juices was recorded using a hand refractometer and their acidity estimated by titrating 5 g pulp with 0.1 N NaOH<sup>10</sup>.

The formulations were exposed to standard ethylene (99 per cent) and the amount of ethylene absorbed was determined using a gas chromatograph (Modular Gas Chromatograph Model ACC, CIC, Baroda) fitted with a porapak column (2 mm I.D and 200 cm length) and FID detector at a nitrogen gas flow rate of 40 ml/min and oven and injector temperatures of  $50^\circ$  and  $30^\circ C$  respectively. The reaction was conducted in a 250 ml stoppered bottle in which 1 g of absorbent was placed and 10 ml of ethylene gas was introduced through an injection septum. Gas samples (100  $\mu l$ ) were drawn from the bottle at regular intervals using a gas tight syringe and injected into the G.C. Ethylene level inside the bottle was measured till no more depletion occurred and the utilisation of  $KMnO_4$  from the granules was complete as judged by absence of colour in aqueous extract of sample drawn at specific time intervals. Ethylene

absorbance was expressed as ppm absorbed in 10 min as well as to complete utilisation of  $\text{KMnO}_4$ .

**Quality evaluation:** Firmness in fruits during storage was measured using a penetrometer (EFFIGI, Italy) and texture of vegetables using Warner Bratzler shear press and expressed as kg force.

Firmness of ethylene absorbent granules was measured by the force in kg required to crush a single granule using a Compression/Crush Tester (Karl Frank Model 936, W.Germany).

Sensory evaluation of fruits and vegetables was carried out during laboratory and large scale storage trials with a panel of 10 tasters selected from the laboratory staff/Service personnel (on ships) and expressed as scores on a 9-point Hedonic scale with 1 for extremely poor and 9 for excellent. The overall final rating was obtained by averaging the scores. A score of 5.5 and above was rated acceptable.

**Shelf stability studies on ethylene absorbent granules:** The storage stability of both  $\text{Al}_2\text{O}_3$ -limestone based and cement based formulations was studied by packing the sachets in an outer pack of opaque pigmented polythene (300 gauge) pouches and storing at room temperature and at  $37^\circ\text{C}$ . Samples were drawn at 4 month intervals and analysed for  $\text{KMnO}_4$  content and ethylene absorbance.

## Results and Discussion

**Comparative efficacy of scrubbers:** The  $\text{Al}_2\text{O}_3$ -limestone and cement based  $\text{C}_2\text{H}_4$  scrubbers showed 20 and 24 per cent lower ethylene absorption in 10 min respectively as compared to the imported trade product (Table 1). They were slower by 5 and 9 min respectively as compared to the latter in the time taken for complete reduction of  $\text{KMnO}_4$  in 1 g sample. This is possibly due to the lower surface area of the beadlets as compared to the trade sample which was in the form of spherical granules. However, in terms of

permanganate retention and overall ethylene scrubbing, both the formulations were at par with the trade product.

Between the two scrubbers, the alumina based absorbent was superior in rate of ethylene absorption p.p.m./g/min) calculated for 10 min as well as maximum absorption (Table 1) apparently due to higher  $\text{Al}_2\text{O}_3$  content (40 per cent compared to 10-12 per cent in white cement) which is known to impart micro-catalytic porosity to absorbents. The silica gel based formulation showed significantly lower absorption in 10 min as well as overall absorption besides being slower by 23 min in the latter as compared to the trade product and as such proved inferior.

Both scrubbers showed higher granule firmness (crush strength) compared to the imported trade product (Table 1). This showed that the granules would withstand the rigors of handling and transportation eliminating dust formation. The significantly higher firmness of cement based matrix, however, rendered it less porous and as such proved disadvantageous for absorption. Inclusion of 20 per cent sand was found to improve porosity.

The two formulations had a distinctly alkaline pH similar to the trade product. This may be considered beneficial for oxidation of  $\text{C}_2\text{H}_4$  by  $\text{KMnO}_4$  which requires alkaline conditions. The comparatively lower pH of 6.2 on the acidic side in silica gel based scrubber, being unfavourable for oxidation, is possibly one of the reasons, besides low  $\text{KMnO}_4$  retention, responsible for its low overall ethylene absorption.

**Storage stability of scrubber:** Data on the storage stability of  $\text{Al}_2\text{O}_3$ -limestone based scrubber at ambient temperature and  $37^\circ\text{C}$  are given in Table 2 which showed insignificant reduction in permanganate concentration and overall ethylene absorption at both temperatures. The cement based scrubber showed similar stability. In contrast, the silica gel scrubber had a shelf life of only 2 months (data not presented) which

TABLE 1. ETHYLENE ABSORBING EFFICIENCY AND PHYSICO-CHEMICAL CHARACTERISTICS OF THREE PERMANGANATE IMPREGNATED MATRICES COMPARED WITH AN IMPORTED TRADE SCRUBBER

Ethylene scrubbing formulation	Ethylene absorption in 10 min*		Maximum ethylene absorption**			$\text{KMnO}_4$ content (%)	Moisture (%)	pH	Crush strength (kg)
	Qty. (ppm/g)	Rate (ppm/g/min)	Qty. (ppm/g)	Time (min)	Rate (ppm/g/min)				
$\text{Al}_2\text{O}_3$ -lime stone matrix	1122 (54.8)	112.2	2048	19	107.8	2.48	11.2	12.1	17.6
White cement matrix	1069 (51.0)	106.9	2096	23	91.1	2.45	11.0	12.0	56.0
Silica gel matrix	496 (31.4)	49.6	1580	37	42.7	2.34	7.1	6.2	—
Trade product (imported)	1644 (75.3)	164.4	2184	14	156.0	2.62	9.3	9.1	9.5

\* Figures in parentheses represent per cent maximum absorption

\*\* Absorption by 1 g of granules till the  $\text{KMnO}_4$  is fully utilized.

TABLE 2. DATA ON STORAGE STABILITY OF  $Al_2O_3$ -LIMESTONE BASED ETHYLENE ABSORBENT PACKED IN OPAQUE LDPE (300 GAUGE) AT AMBIENT TEMPERATURE AND 37°C.

Storage period (months)	At room temp. (19-30°C)		At 37°C	
	KMnO <sub>4</sub> content (%)	Max C <sub>2</sub> H <sub>4</sub> absorption* (ppm/g)	KMnO <sub>4</sub> content (%)	Max C <sub>2</sub> H <sub>4</sub> absorption (ppm/g)
0	2.45	2096	2.45	2096
4	2.41	2077	2.36	2072
8	2.38	2057	2.31	2038
12	2.35	2048	2.29	2018

\*Absorption by 1 g of granules till the KMnO<sub>4</sub> is fully utilized.

may be attributed to lower initial KMnO<sub>4</sub> uptake due to lack of sufficient porosity and surface area.

*Storage studies on fruits and vegetables using the scrubbers:*

*Laboratory studies:* The  $Al_2O_3$ -limestone based formulation was found to increase the shelf life of mango, tomato brinjal and lady's finger by 6, 8, 5 and 7 days, respectively compared to 8, 6, 8 and 6 days by the imported commercial scrubber over the control at 10°C (Tables 3 and 4). Likewise, under ambient storage, shelf life of mango, tomato and banana were increased by 6, 4 and 3 days respectively compared to 7, 4 and 3 days by the imported scrubber over the control (Table 5). These results compare

with earlier reports on the accelerated senescence by accumulated ethylene in apples and pears by Gerhardt and Siegelman<sup>11</sup> and on the extension in shelf life by the action of ethylene scrubbers in apples by Knee<sup>12</sup> and Lin<sup>13</sup>. Ethylene scrubbing restricted the loss in firmness and physiological loss in weight and brought about slower changes in Brix/acid ratio in fruits and vegetables suggesting retarded senescence in the commodities (Tables 3, 4, 5 and 6). In case of lady's finger, toughening of material on moisture loss was minimised. Overall, the performance of  $Al_2O_3$ -limestone based scrubber was equal to that of the trade product and superior to the cement based formulation.

b) *Large scale storage trials:* The  $Al_2O_3$ -limestone based scrubber blanket extended the shelf life of mango, tomato, brinjal, lady's finger and banana by 5, 6, 5, 7 and 4 days respectively compared to 6, 5, 6, 6 and 3 days by the imported commercial scrubber blanket over the control (Table 6) as judged by rejections due to discolouration, microbial decay, softening, over-ripening etc. through visual and organoleptic evaluation. Ethylene scrubbing minimised the extent of microbial decay which is a major problem in the highly humid atmosphere prevailing on board ships. This was apparently due to delay in softening and senescence which is one of the main causes for fruit and vegetable infections<sup>6</sup>.

*Cost of manufacture of ethylene scrubber:* Manufacturing cost of the  $Al_2O_3$ -limestone based scrubber worked out to

TABLE 3. DATA ON THE LABSCALE STORAGE OF FRESH MANGO (Cv. BADAMI) AND TOMATO (Cv. PUSA RUBY) USING ETHYLENE SCRUBBER AT 10°C ± 1.

Scrubber type.	Physiol. loss in wt (%)	Firmness (kg)	°Brix	Acidity (% citric acid)	Brix/acid ratio	Spoilage rejection (%)	Shelf-life (days)	Overall acceptability*
<b>Mango</b>								
Initial	—	6.0	16.0	0.55	29.1	—	—	—
$Al_2O_3$ -lime stone based	4.2 (5.9)	3.6 (3.4)	18.0 (19.5)	0.34 (0.30)	53.3 (65.9)	11.2 (23.4)	21	7.4 (6.1)
Cement based	4.6 (6.8)	3.2 (2.6)	19.0 (19.5)	0.33 (0.28)	57.4 (69.6)	13.4 (25.6)	20	6.9 (5.8)
Trade Product (imported)	3.6 (6.3)	4.1 (3.2)	18.5 (19.5)	0.41 (0.30)	45.3 (64.8)	10.8 (22.6)	23	8.3 (7.2)
No scrubber (control)	6.1	2.4	20.0	0.29	69.0	28.8	15	3.7
<b>Tomato</b>								
Initial	—	3.8	3.0	0.37	8.1	—	—	—
$Al_2O_3$ -lime stone based	6.9 (9.2)	3.0 (2.5)	5.5 (6.5)	0.43 (0.32)	12.8 (20.4)	12.1 (23.1)	22	8.1 (7.2)
Cement based	7.8 (9.6)	2.8 (2.1)	5.5 (6.0)	0.40 (0.35)	13.6 (17.1)	15.2 (30.8)	20	7.8 (7.1)
Trade product (imported)	7.4 (10.3)	2.7 (2.4)	5.0 (6.0)	0.36 (0.34)	13.9 (17.7)	14.3 (28.2)	20	8.4 (7.5)
No scrubber (control)	9.8	1.9	6.0	0.35	17.2	34.4	14	4.3

Unbracketed and bracketed values represent observations on the day control was terminated and on the final day of storage respectively.

\*On 9-point Hedonic scale with 1 for extremely poor and 9 for excellent.



TABLE 4. DATA ON THE LABSCALE STORAGE OF FRESH BRINJAL (Cv. IRENGERE) AND LADY'S FINGER (Cv. PUSA SAWANI) USING ETHYLENE SCRUBBERS AT 10°C ± 1.

Scrubber type	PLW (%)	Firmness (kg)	Spoilage rejection (%)	Shelf life (days)	Overall acceptability score*
<b>Brinjal</b>					
Initial	—	1.5	—	—	—
Al <sub>2</sub> O <sub>3</sub> -lime stone based	7.7 (10.8)	1.3 (1.1)	14.1 (21.3)	14	7.4 (5.5)
Cement based	7.9 (11.4)	1.0 (0.95)	15.3 (23.5)	14	7.1 (5.9)
Trade product (imported)	6.0 (8.5)	1.4 (1.2)	11.2 (19.6)	17	8.2 (6.9)
No scrubber (control)	8.4	0.9	34.2	9	3.8
<b>Lady's finger</b>					
Initial	—	2.6	—	—	—
Al <sub>2</sub> O <sub>3</sub> -lime stone based	6.2 (11.8)	3.3 (3.8)	11.3 (30.6)	19	8.4 (7.1)
Cement based	7.9 (12.8)	4.1 (4.4)	17.2 (31.4)	16	7.9 (6.8)
Trade product (imported)	7.6 (12.4)	3.6 (4.1)	14.1 (28.3)	18	8.2 (6.4)
No scrubber (control)	12.2	4.5	30.1	12	4.2

Unbracketed and bracketed values represent observations on the day control was terminated and on the final day of storage respectively.

\*On 9 point Hedonic scale.

TABLE 5. DATA ON LABSCALE STORAGE OF FRESH FRUITS USING ETHYLENE SCRUBBER AT AMBIENT TEMPERATURE (19-30°C)

Scrubber type	PLW (%)	Firmness (kg)	°Brix	Acidity (% citric acid)	Brix/acid ratio	Spoilage rejection	Shelf life (days)
<b>Mango</b>							
Initial	—	6.4	15.5	0.51	30.4	—	—
Al <sub>2</sub> O <sub>3</sub> -lime stone based	7.5 (10.4)	4.3 (3.2)	17.6 (19.2)	0.39 (0.32)	45.1 (60.0)	19.7 (36.4)	16
Cement based	8.5 (10.9)	3.8 (3.1)	19.4 (19.8)	0.32 (0.31)	60.6 (63.9)	29.4 (41.8)	14
Trade product (imported)	6.9	4.7 (3.4)	17.1 (19.5)	0.40 (0.31)	42.8 (62.9)	17.2 (32.3)	17
No scrubber (control)	11.7	2.9	19.6	0.31	63.2	37.2	10
<b>Tomato</b>							
Initial	—	4.1	3.1	0.37	8.4	—	—
Al <sub>2</sub> O <sub>3</sub> -lime stone based	7.2 (9.3)	3.0 (2.1)	5.4 (5.9)	0.38 (0.35)	14.2 (16.9)	20.4 (37.2)	12
Cement based	8.7 (11.1)	2.9 (2.0)	5.4 (5.8)	0.37 (0.34)	14.6 (17.1)	25.7 (37.2)	11
Trade product (imported)	6.4 (9.8)	3.2 (2.3)	5.1 (5.9)	0.38 (0.36)	13.4 (16.4)	19.2 (35.8)	12
No scrubber (control)	12.8	2.2	5.8	0.36	16.1	39.8	8
<b>Banana</b>							
Initial	—	5.2	13.6	—	—	—	—
Al <sub>2</sub> O <sub>3</sub> -lime stone based	11.9 (15.8)	4.2 (2.6)	15.2 (18.1)	—	—	19.8 (34.3)	7
Cement based	13.4 (17.1)	3.8 (2.2)	17.6 (18.7)	—	—	24.8 (37.6)	6 7
Trade product (imported)	11.4 (16.2)	4.3 (2.0)	16.3 (18.1)	—	—	21.2 (36.5)	7
No scrubber (control)	17.9	2.2	18.4	—	—	39.3	4

Unbracketed and bracketed values represent observations on the day control was terminated and on the final day of storage respectively.

TABLE 6. DATA ON LARGE SCALE STORAGE OF FRESH FRUITS AND VEGETABLES USING ETHYLENE SCRUBBER BLANKETS AT  $10 \pm 1^\circ\text{C}$  ON BOARD SHIPS

Scrubber type	PLW (%)	Firmness (kg)	$^\circ\text{Brix}$	Spoilage rejection (%)	Shelf life (days)	Overall acceptability score +
<b>Mango</b>						
Initial	—	5.8	15.1	—	—	9.0
Al <sub>2</sub> O <sub>3</sub> -lime stone based	5.5 (7.1)	3.6 (2.9)	17.1 (19.2)	14.2 (28.7)	18	8.4 (7.6)
Trade product	4.2 (6.9)	3.9 (3.1)	16.4 (19.7)	12.4 (25.2)	19	8.3 (7.1)
Control	7.8	2.2	20.1	33.2	13	4.1
<b>Tomato</b>						
Initial	—	3.2	3.0	—	—	9.0
Al <sub>2</sub> O <sub>3</sub> -lime stone based	7.3 (10.8)	2.5 (2.2)	4.5 (4.0)	13.1 (24.2)	18	8.7 (7.4)
Trade product	8.4 (11.2)	2.5 (2.3)	4.0 (4.5)	16.3 (28.4)	17	7.3 (6.2)
Control	13.2	1.3	4.0	29.5	12	3.3
<b>Brinjal</b>						
Initial	—	—	—	—	—	9.0
Al <sub>2</sub> O <sub>3</sub> -lime stone based	11.4 (14.6)	—	—	24.6 (34.1)	12	7.8 (6.1)
Trade product	9.1 (11.2)	—	—	18.9 (33.8)	13	8.2 (6.4)
Control	12.8	—	—	36.5	7	4.2
<b>Lady's finger</b>						
Initial	—	—	—	—	—	9.0
Al <sub>2</sub> O <sub>3</sub> -lime stone based	7.1 (11.6)	—	—	10.4 (31.7)	18	8.1 (6.7)
Trade product	8.2 (12.8)	—	—	13.2 (30.4)	17	7.2 (6.1)
Control	11.4	—	—	27.7	11	3.4
<b>Banana*</b>						
Initial	—	4.7	14.0	—	—	9.0
Al <sub>2</sub> O <sub>3</sub> -lime stone based	11.3 (15.3)	3.2 (2.3)	16.5 (18.7)	14.8 (26.1)	7	7.2 (5.4)
Trade product	12.4 (15.9)	2.8 (2.5)	17.1 (17.5)	21.2 (31.4)	6	7.4 (6.3)
Control	15.2	1.9	18.3	40.3	3	2.8

Unbracketed and bracketed values represent observations on the day control was terminated and on the final day of storage respectively.

\* Stored at ambient temp (25–30°C).

+On 9-point Hedonic scale.

Rs. 70.00 per kg and that of the cement based scrubber Rs. 22.00 per kg. A blanket weighing 7 kg meant for hanging inside a conventional store/cold room with an estimated life of approximately 12 weeks is estimated to cost Rs. 500.00 with Al<sub>2</sub>O<sub>3</sub>-limestone based scrubber and Rs. 150.00 with cement based scrubber while a sachet of 5 g meant for about

2 kg fresh produce will cost Re. 0.35 and 0.11 respectively. The cost of Al<sub>2</sub>O<sub>3</sub> based scrubber can be reduced further if commercial grade active alumina is used instead of L.R. grade employed in the present studies.

In contrast, the imported commercial scrubber costs inclusive of air freight, insurance and customs duty Rs.11,000

for a 7 kg blanket and Rs. 4.00 for a 5 g sachet calculated based on a price of £ 50.00 per blanket and £ 33.00 per 1000 sachets in the year 1987.

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# Influence of Different Treatments, Storage Temperature and Period on Some Physico-chemical Characteristics and Sensory Qualities of Indian Honey

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Colour darkening of honey was significantly affected by the storage temperature and period. Storage of honey at 40°C resulted in deterioration of colour, increase in colloidal contents and complete inhibition of granulation. Addition of KMS reduced the darkening effect of honey at room temperature, but did not affect the samples at 5°C. Heat treatment also reduced the darkening of honey and prevented granulation both at room temperature and 5°C for 60 and 90 days, respectively. The various treatments and storage period employed affected the sensory qualities of honey to a variable extent. Honey stored at 40°C was not liked due to perceivable after taste. In sensory qualities, unheated honey stored at 5°C was found to be the best.

Honey contains about 80 different components and 95 to 99 per cent of the total solids are sugars. From the consumers' point of view, colour, flavour and aroma are important quality attributes. Darkening in colour of honey is one of the major changes that occurs during storage, especially at elevated temperatures<sup>1</sup>. It is also influenced by processing temperature, moisture content, pH, free amino acids and reaction between amino acids and reducing sugars<sup>2,4</sup>. Although raw honey is the best honey, its processing is needed to meet the market requirements like retention of its liquid nature and an attractive appearance.

Studies on the changes which occur in honey during storage and processing, have been reported<sup>1,2,5-7</sup>, but each type of honey is affected differently by the length of storage and storage temperature. Such studies are limited for Indian honeys<sup>8</sup>. Moreover, most of the workers have not studied the effect of storage temperature as well as storage period and processing, on the granulating tendency and sensory qualities of honey. The present studies were, therefore, undertaken to know the changes occurring in colour, colloidal contents, granulating tendency as well as sensory qualities of multifloral honey of exotic bee *Apis mellifera* after processing and storage at different temperatures.

## Materials and Methods

The present investigations were carried out in the Department of Entomology and Apiculture, UHF, Solan, Himachal Pradesh, India, during 1987-88. The honey used

was extracted during October-November, 1987 from *Apis mellifera* colonies. It was strained through a cheese cloth and packed in clean 500 g glass jars. The different treatments given to honey were: addition of potassium metabisulphite (KMS, 80 p.p.m.), heating at 60°C for half an hour in a water bath while honey without any treatment served as a control.

Samples of the treated honey, were stored at three storage temperatures viz. room temperature (7 to 30°C), 5°C and 40°C. Three replicates were maintained in all the treatments. The honey was analysed at 0,2,4 and 6 months of storage. Colour was determined as its optical density without dilution of honey<sup>9</sup>. Colloidal contents were determined as per method described by Deans<sup>10</sup> and the results are expressed in per cent of colloidal contents.

Granulation of honey is expressed in terms of per cent body of honey granulated (visual observations). Honey was also evaluated for its sensory qualities by a panel of ten trained judges. The method followed was the scoring of samples for various attributes as described by Ranganna<sup>11</sup>. Each attribute was given a maximum score of 20. The coded samples were presented in random to the judges. Statistical analysis of the data for physico-chemical characteristics and sensory evaluation were carried out by completely randomized block design and randomized block design, respectively.

## Results and Discussion

The colour of honey was significantly affected by the storage temperature and period. Maximum deterioration in

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TABLE 1. EFFECT OF DIFFERENT TREATMENTS AND STORAGE PERIODS ON COLOUR OF HONEY

Treatment	Optical density at 560 nm, at indicated storage periods (months)				Mean	Mean for temp.
	0	2	4	6		
<b>Room temp. (7-30°C)</b>						
Unheated	0.35	0.43	0.43	0.42	0.41	0.38
Unheated (KMS)	0.35	0.42	0.36	0.39	0.38	
Heated	0.35	0.35	0.37	0.37	0.36	
<b>5°C</b>						
Unheated	0.35	0.39	0.41	0.42	0.39	0.38
Unheated (KMS)	0.35	0.36	0.41	0.41	0.38	
Heated	0.35	0.36	0.36	0.35	0.36	
<b>40°C</b>						
Unheated	0.35	0.57	0.78	1.27	0.74	0.75
Unheated (KMS)	0.35	0.58	0.77	1.28	0.75	
Heated	0.35	0.63	0.80	1.27	0.76	
Mean	0.35	0.45	0.52	0.69		
CD (P = 0.05)	Treatments at different temp.		= 0.01			
	Storage temp.		= 0.01			
	Storage period		= 0.01			
	Interaction (Treat/Storage)		= Significant			
	Any pair of combination		= 0.03			

TABLE 2. THE EFFECT OF DIFFERENT TREATMENTS AND STORAGE PERIODS ON COLLOIDAL CONTENTS (PER CENT) OF HONEY

Treatment	Storage period (months)				Mean	Mean for temp.
	0	2	4	6		
<b>Room temp. (7-30°C)</b>						
Unheated	0.26	0.25	0.27	0.30	0.27	0.27
Unheated (KMS)	0.26	0.25	0.27	0.31	0.27	
Heated	0.26	0.26	0.27	0.31	0.26	
<b>5°C</b>						
Unheated	0.26	0.27	0.27	0.31	0.28	0.27
Unheated (KMS)	0.26	0.26	0.27	0.30	0.27	
Heated	0.26	0.25	0.27	0.30	0.27	
<b>40°C</b>						
Unheated	0.26	0.36	0.44	0.53	0.40	0.41
Unheated (KMS)	0.26	0.35	0.47	0.56	0.41	
Heated	0.26	0.35	0.43	0.58	0.41	
Mean	0.26	0.29	0.33	0.39		
CD (P = 0.05)	Treatments at different temp.		= N.S.			
	Storage temp.		= 0.01			
	Storage period		= 0.01			
	Interaction (Treat./Storage)		= Significant			
	Any pair of combination		= 0.03			

colour was observed in honey stored at 40°C (Table 1), while there was no difference in the colour of honey stored either at room temperature or at 5°C. Period of storage, irrespective of the treatments and storage temperature, significantly increased the colour intensity of honey which could be attributed to the Maillard reaction resulting in the formation of coloured pigments and higher colloidal contents.<sup>1,12,13,6,7</sup>

Among the samples stored at room temperature, maximum darkening was observed in unheated samples without KMS and least in the samples given heat treatment, indicating that heat treatment and/or addition of KMS retarded the darkening effect. But such effect was not visible in honey stored at low temperature, possibly low temperature itself could have retarded the Maillard reaction. The samples which were

heated initially, showed comparatively less darkening than those with or without KMS and whether stored at room temperature or 5°C. Wootton *et al.*<sup>1</sup> have reported retardation of the Maillard reaction by use of sulphur dioxide in the form of sodium metabisulphite at 5000 and 10000 p.p.m. which lightened the honey colour by bleaching pigments. Although 80 p.p.m. of KMS used in our study did not lighten the original colour of honey, it retarded the rate of darkening in storage. It can be concluded that storage is definitely increasing the darkening of honey, while heat treatment and KMS can reduce it.

The colloidal contents of honey increased both due to temperature of storage and period. The honey samples stored at 40°C had more colloidal contents than those at room temperature or 5°C (Table 2). Addition of KMS or heat treatment did not change the colloidal contents of honey. Breakdown of macromolecules and resulting products might have increased the colloidal contents of honey due to storage temperature and period.

There was no granulation at all in honey stored at 40°C in contrast to those stored at room temperature at 5°C. The onset of granulation was instant in honey containing KMS and stored at room temperature (Fig. 1). The granulation was complete after 30 days. Honey without KMS started granulating only after 30 days which was complete in 90 days of storage. The honey stored at 5°C whether containing KMS or not, granulated completely in 60 days.

The results on granulation further indicate that the honey, which otherwise started granulating after 30 days at room temperature, could further be prevented from granulation for two more months by giving the heat treatment and then storing at 5°C. The heat treatment is known to destroy the crystal nuclei consisting of fine crystals of dextrose, dust particles or pollen grains<sup>5</sup>. Thus, if the winter period of three months, which is most favourable for granulation, could be skipped over by giving the heat treatment and storing at 5°C,

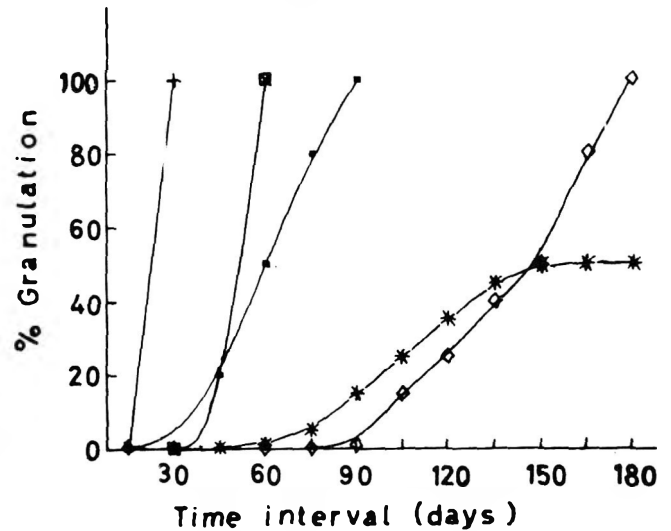


Fig.1. Effect of different treatments and storage periods on the extent of granulation of honey.

- x- Unheated honey with KMS and stored at 5°C
- Unheated honey without KMS and stored at 5°C
- \*- Honey given heat treatment and stored at room temperature
- +- Unheated honey with KMS and stored at room temperature
- Unheated honey without KMS and stored at room temperature

the honey may remain in liquid form in the extended storage, because the most favourable temperature reported for honey granulation lies between 10°C and 16°C and both lower and higher temperatures are less effective<sup>5</sup>.

**Sensory evaluation:** The results of sensory evaluation of honey, determined after 180 days of storage (Table 3) revealed significant differences in various quality parameters studied. Unheated honey with or without KMS, stored at 5°C and unheated honey stored at room temperature, had the best colour and appearance whereas that stored at 40°C was the least acceptable though it had the highest score for consistency. The honey stored at 5°C got low score due to granulation.

Unheated honey and honey with KMS stored at 5°C had the best taste while that stored at 40°C was unacceptable

TABLE 3. THE EFFECT OF DIFFERENT TREATMENTS ON THE SENSORY CHARACTERISTICS OF HONEY AFTER SIX MONTHS OF STORAGE

Treatment	Colour	Consistency	Taste	Aroma	No defect	Overall qualities
<b>Room temp. (7-30°C)</b>						
Unheated	18.8	16.7	17.3	17.8	18.0	17.8
Unheated (KMS)	17.3	16.5	17.6	17.1	18.3	18.2
Heated	15.8	17.2	16.1	16.7	15.7	15.6
<b>5°C</b>						
Unheated	19.3	15.4	19.2	18.9	19.4	19.6
Unheated (KMS)	18.6	15.0	18.6	17.5	18.3	18.4
Heated	16.0	16.2	16.5	17.6	15.4	15.5
<b>40°C</b>						
Unheated	14.4	19.1	13.8	14.0	14.1	13.7
Unheated (KMS)	14.0	18.7	14.2	13.5	14.1	13.9
Heated	14.2	19.0	13.9	14.2	14.1	13.8

CD (P = 0.05) = 1.0

because of after-taste. Best aroma was perceived in unheated honey stored at 5°C. In contrast, honey stored at 40°C lacked the desirable honey aroma.

In overall quality evaluation, unheated honey stored at 5°C was adjudged the best followed by honey with KMS stored at 5°C and room temperature. Honey stored at 40°C was unacceptable.

It is thus revealed from the present studies that though in sensory qualities, unheated honey stored at 5°C was adjudged to be the best, in countries where liquid honey is preferred to the granulated one, the honey could be given the heat treatment and then stored at 5°C, at least for the period when temperature conditions favourable to granulation exist.

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## Hydrolysis of Fish Protein by *Bacillus megaterium* Cells Immobilized in Radiation Induced Polymerized Wood

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The immobilization of *Bacillus megaterium* cells in radiation-induced polymerized wood was studied for hydrolysis of trash fish protein. The optimum conditions and reaction kinetics for hydrolysis of protein by free and immobilized cells were found to be similar. Maximum hydrolysis occurred at 50°C and at pH 7.5 with 15-20% (w/v) of immobilized matrix. The soluble content of the resultant hydrolysate was about 2.4% (w/v).

Low cost fish is considered as one of the major sources for the production of easily digestible nutritionally adequate protein<sup>1,2</sup>. Several investigations have been carried out to solubilize trash fish protein by the action of proteases<sup>3,4</sup>. However, for economic reasons, it is necessary to immobilize either enzyme or cells producing proteases for repeated use. Venugopal *et al.*<sup>5</sup> have shown that cells of *B. megaterium* immobilized in alginate beads could serve as a source of proteases for the solubilization of fish protein. However, alginate beads disintegrate in fish protein slurry after only two cycles of its use<sup>5</sup>. Hence, there exists a need to develop a suitable matrix for protein solubilization. The present paper deals with the use of polymerized wood matrix for immobilization of *B. megaterium* cells for protein hydrolysis.

### Materials and Methods

**Immobilization of *B. megaterium* cells with wood:** Teak wood chips (size 6.3×6.3×6.3 mm) were refluxed at 80°C for 4 hr in a mixture of benzene and toluene (1:1); dried overnight at 105°C to a constant weight and then exposed to a radiation dose of 5 kGy in the presence of air in a package irradiator at a dose rate of 0.05 kGy/min. Irradiated wood chips (60g) were then mixed with *B. megaterium* cells (20g of wet cells) and 20 ml of 2EHA (2 ethylhexyl acrylate) followed by irradiation at a dose of 35 kGy. *B. megaterium* cells were prepared as described earlier<sup>5</sup>. The cells bound to polymerized wood chips were then washed repeatedly with 10 per cent ethanol solution in water to remove unadhered cells and unreacted monomer. The matrix was then dried at room temperature for 18 hr to remove the adhered ethanol from the matrix. The final moisture content of the matrix was about 6-8 per cent (w/v).

**Preparation of fish protein:** Fish protein was prepared according to the method of Venugopal and Lewis<sup>3</sup>. Croaker

(*Johnius dissumeri*), a trash fish, obtained from local market was dehoned and boiled with 1 per cent acetic acid for 15 min and pressed in a screw press to remove water. The press cake was dried in a tunnel drier at 55°C for 18 hr. It was then pulverized and passed through a cheese cloth. The moisture content of the powder was about 8-10 per cent (w/v).

**Solubilization of fish protein:** The fish protein powder (5g) was suspended in 50 ml of water at pH 7.5, transferred to 100 ml conical flask containing different concentrations of

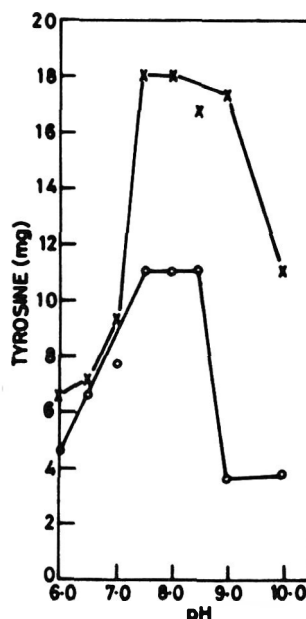


Fig. 1. Effect of pH on solubilization of fish powder by free and immobilized cells. Reaction condition for free cells (O), 24 mg of cells in 50 ml of 10% (w/v) fish powder suspension for 4 hr at 55°C. For immobilized cells (X), 2 g of immobilized matrix containing 20% cells in 50 ml of 10% (w/v) fish powder suspension for 4 hr at 55°C.



immobilized cells in wood matrix (0 to 30 per cent) and kept at 50°C in a Gallenkamp shaker water bath. During the process of hydrolysis, pH was maintained by adding 1N NaOH. Samples were withdrawn at different time intervals and the extent of solubilization of protein was determined by the tyrosine value<sup>6</sup> and expressed as mg of tyrosine formed per ml of solution.

### Results and Discussion

The optimum conditions for the solubilization of fish protein by free and immobilized cells in polymerized wood have been standardized. It is evident from Fig.1 that solubilization in terms of tyrosine content was maximum between pH 7.5 and 8.5 in both the cases.

The effect of reaction temperature on solubilization of protein by free and immobilized cells is shown in Fig.2. The optimum temperature observed in both the cases was at 60°C.

The extent of solubilization by free and immobilized cells at a reaction temperature of 50°C and at pH 7.5 is shown in Fig.3. It was observed that hydrolysis attained its maximum peak after 5 hr of reaction in both the cases. Thereafter, there was no further increase in the rate of hydrolysis.

Repeated cycle studies showed that there was no change in the activity of the immobilized cells matrix after 10 cycles.

Biocatalyst concentration plays an important role in any enzymatic reaction. To optimise the ideal concentration of the matrix with respect to fish slurry, reactions were carried out using different concentrations of matrix. It was observed in the wood matrix. The resultant matrix formed by irradiation

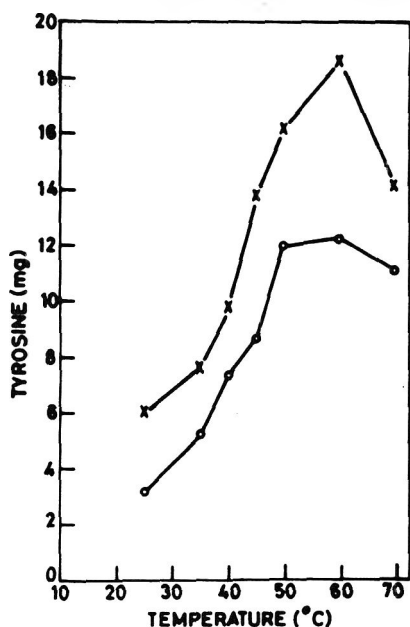


Fig. 2. Effect of temperature on solubilization (fish powder) by free and immobilized cells. Reaction condition : For free cells (O) 24 mg of cells in 30 ml of 10% (w/v) fish powder suspension for 4 hr at pH = 7.5. For immobilized cells (X), 2 g immobilized matrix containing 20% cells in 30 ml of 10% (w/v) fish powder suspension for 4 hr at pH = 7.5.

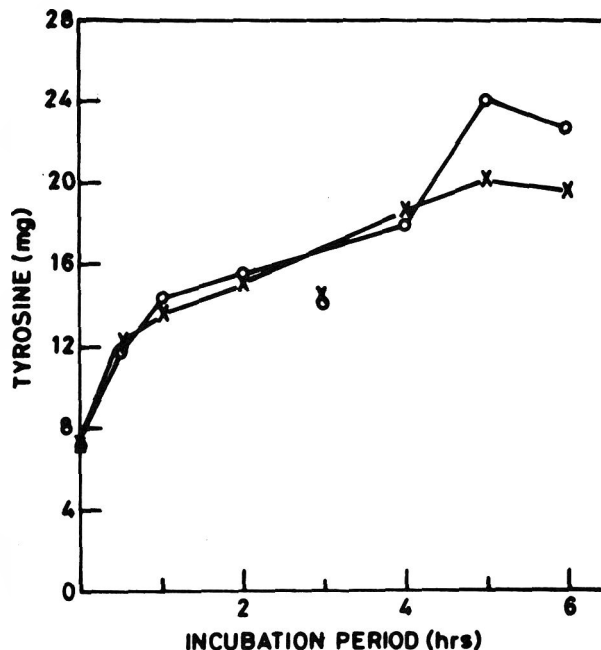


Fig. 3. Effect of incubation period on solubilization (fish powder) by free and immobilized cells. Reaction condition : For free cells (O), 100 ml of 10% (w/v) fish powder suspension and 2 gm free cells (wet basis) at a temp. of 50°C and pH = 7.5. For immobilized cells (X), 100 ml of 10% (w/v) fish powder suspension and 2 g immobilized cells containing 20% cells at a temp. of 50°C and pH = 7.5.

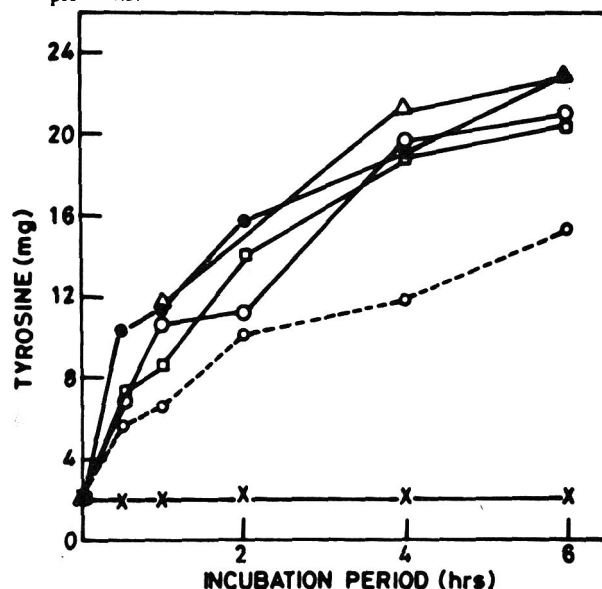


Fig. 4. The rate of hydrolysis of fish protein by different concentrations of biocatalyst matrix with respect to time. Reaction condition : 100 ml of 10% (w/v) fish powder suspension in water and different concentrations of immobilized cells (beads) at a temperature of 50°C and pH = 7.5. (—X—) no beads, (—O—) 10% beads, (—□—) 15% beads; (—●—) 20% beads, (—●—) 25% beads; (—Δ—) 30% beads. that for a 10 per cent (w/v) fish protein slurry, matrix concentration of 15-20 per cent (w/v) was ideal (Fig.4).

Radiation-induced polymerization of monomers have been employed for immobilization of cells, enzymes and other bioactive substances<sup>7,9</sup>. However, matrices formed by such

polymerization have the inherent drawbacks of low mechanical strength, low density and possibility of cell leakage during operations.

Wood impregnated with monomer results in graft polymerization when exposed to gamma radiation<sup>10</sup>. This technique of graft polymerization was exploited to bind cells of cells, monomer and wood has high mechanical strength, low water absorption capacity and resulted in the improvement in dimensional stability.

Basically, wood is a complex natural substance which in addition to cellulose and lignin contains many other substances. Among the constituents of wood, cellulose is most sensitive to radiation and yields free radicals. These free radicals and the polyfunctional compounds of cellulose react with the acetyl group of the monomer and the outer surface of cells results in cross linking. A radiation dose of 35 kGy was employed to achieve complete polymerization of the monomer added to wood and cells mixture.

There was no change in the temperature and pH optima of *B. megaterium* cells after immobilization, thus suggesting that active sites of the biocatalyst were not disturbed due to the polymerization reaction.

The immobilized preparation lost its activity after 24 hour of operation at 60°C. Therefore, subsequent experiments were carried out at 50°C. It was observed that the enzymes were stable at 50°C and even after 10 cycles of operation, there was no change in the reaction kinetics.

The free and immobilized cells showed (Fig.3) almost same amount of tyrosine formation. In both the cases, same amount of cells (2g) was used, indicating that the activity of cells did not alter due to immobilization. A proteolytic activity of 16 mg tyrosine was observed in both the cases.

Thus, the radiation-induced polymerized wood containing *B. megaterium* cells can serve as a biocatalyst for hydrolysing fish protein. This hydrolysed protein may find wide application as health foods for special diet formulations, therapeutic use and in personal care products. The resultant hydrolysate was clear and transparent with a solid content of about 2.4 per cent (w/v).

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## Solubility and Electrophoretic Characteristics of Proteins in Different Regions of Coconut Endosperm

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A marked concentration gradient in the increasing order was observed for proteins from the inner to the outer regions of coconut endosperm. The testa and coconut water showed lower levels of protein concentration. However, non-protein nitrogen did not show any appreciable variation. Fractionation of proteins showed that more than 80% of the proteins from the different regions were soluble in aqueous and salt media. Solubility of the proteins in water and 5% NaCl (0.86 M) was determined in the pH range 1-10. The outer and testa regions showed different solubility characteristics both in aqueous and in salt media. Electrophoresis of the major albuminous and globular proteins showed the presence of 6 and 4 protein bands of molecular weights ranging from 14,000 to 52,000 and 17,500 to 45,000 respectively. The various regions from the inner to the outer had the same electrophoretic profile, but showed significant differences in their quantities with respect to the major and minor protein bands. However, the testa region showed maximum variations for all the parameters studied.

Fresh coconut kernel contains 44 per cent oil by weight and 5.5 per cent protein. The abundance of its oil composition outweighs its protein in the commercial consideration of the fruit. Whereas coconut oil has been well characterized<sup>1</sup>, not enough is known about the nature of coconut protein apart from the studies on its amino acid composition and nutritional value<sup>2,3</sup>. Earlier workers<sup>4,7</sup> studied the extractability and nitrogen solubility profile of coconut meal proteins and also the effect of heat treatment on protein characteristics of coconut meats and coconut meal. Attempts made<sup>8-14</sup> on the characterization of coconut proteins of coconut skim milk, copra meal, fresh coconut kernel and coconut protein isolate obtained by ultrafiltration revealed the occurrence of protein subunits of mol. wt. ranging from 13,000 to 57,000 on electrophoresis<sup>15,16</sup>. Apart from these studies, no detailed investigation was carried out on the differential distribution of protein fractions, nitrogen solubility index and electrophoretic patterns of major protein fractions of coconut endosperm. Results from our laboratory<sup>17,18</sup> on the distribution of major chemical constituents and fatty acids in different regions of coconut endosperm showed a marked gradient in the concentration across the endosperm, from the inner region enclosing the water cavity through middle and outer regions and testa. This paper describes the results of the study on the various protein fractions, nitrogen solubility index and electrophoretic pattern of major protein fractions from different regions of coconut endosperm.

### Materials and Methods

Fully matured coconuts (12 months) were harvested from 10 healthy coconut trees ('West Coast Tall' variety), one nut

from each tree. The nuts were split immediately and the endosperm was separated from the shell. First, the brown testa (region 4) was scraped off from the endosperm with care to avoid contamination of the white endosperm. The remaining endosperm was divided into three equal portions based on thickness of the endosperm as indicated in Fig.1. These formed the four regions, namely, inner (region 1), middle (region 2), and outer (region 3) of the white endosperm, and testa (region 4). The respective regions separated from the 10 nuts were pooled together. A second set of 10 nuts was collected from the same trees as before and used as reference for whole endosperm. The pooled

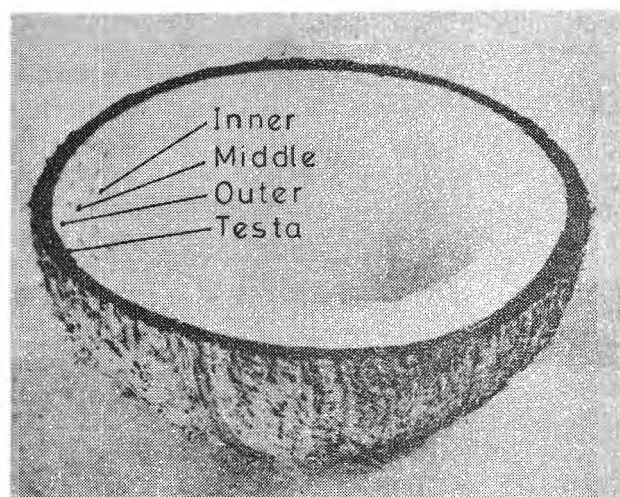


Fig.1. Regions of coconut endosperm: Inner (region 1); middle (region 2); outer (region 3), testa (region 4).

samples of the respective regions and the whole endosperm were blended in a waring blender and dried under vacuum at 40°C to a moisture content of 3 per cent. The dried samples were defatted with hexane using a Soxhlet apparatus. The ground dried defatted samples were passed through a sieve of mesh size 52. The coconut water (liquid endosperm) collected from these nuts were pooled and preserved.

**Protein classification:** Depending upon the solubility characteristics, the different protein fractions were separated by the method of Sauvare *et al*<sup>19</sup>. Four different solvents [distilled water, 5 per cent NaCl (0.86 M), 70 per cent ethanol and 0.05N (NaOH)] were used successively with 20 ml solvent per gram sample.

The samples were stirred in the respective solvent for 30 min at room temperature with a magnetic stirrer. The extracts were centrifuged at 10,000×g for 20 min and the supernatants filtered through Whatman No.1 filter paper. For each solvent, three extractions were carried out and the supernatants were pooled. Nitrogen content of the supernatants was determined by micro Kjeldahl method<sup>20</sup>.

For non-protein nitrogen (NPN), the fat free sample was extracted with 10 per cent TCA. The supernatant containing NPN was filtered out. The filtrate was analysed for nitrogen content following the micro-Kjeldahl method.

**Electrophoresis:** Polyacrylamide disc gel electrophoresis (PAGE) was performed as described by Weber and Osborn<sup>15</sup>. Lysozyme (MW 14,300 daltons),  $\beta$ -lactoglobulin (MW 18,400 subunit), trypsinogen (MW 24,000 daltons), pepsin (MW 34,700 daltons), ovalbumin (MW 45,000 daltons) and bovine plasma albumin (MW 66,000 daltons) were employed as molecular weight markers. Samples and standards were electrophoresed on disc gels using phosphate buffer. Resolving gels of 10 per cent SDS-polyacrylamide were used. The gels were run at 8 mA/tube until marker dye (bromophenol blue R) was 1 cm from anodic end of gel. The gels were stained overnight with 0.25 per cent coomassie brilliant blue R after immersing the gels in a fixative solution of methanol:acetic acid:water (40:7:53) for 10 hr. Destaining was done in methanol:acetic acid:water (5:7.5:87.5). The relative mobility ( $R_f$ ) of protein was determined for standards and experimental samples. Molecular weights of the proteins were determined from a standard curve of relative mobility *versus* the molecular weights of protein standards.

The protein concentrations were measured by eluting the dye with dimethyl sulphoxide and reading in a colorimeter at 595 nm by the method of Makino *et al*<sup>16</sup>.

**Determination of nitrogen solubility index (NSI):** The nitrogen solubility or extractability was determined using aqueous and 0.86M (5 per cent) NaCl solutions whose pH had been adjusted from 1 to 10 with 0.5N HCl or 0.5N NaOH. After 30 min of vigorous stirring (solvent to sample ratio of 20:1) followed by centrifugation at 10,000 r.p.m. for 20 min, the supernatants were analysed for their nitrogen contents by the micro-Kjeldahl method. The protein solubility expressed as nitrogen solubility index (NSI) was calculated as the percentage of protein extracted, at the given pH.

## Results and Discussion

**Distribution of protein and NPN in the coconut endosperm:** The percentage distribution of protein and non-protein in the different regions are presented in Table 1. The results indicated a marked concentration gradient for the constituents across the endosperm from the region surrounding the water cavity to the testa (regions 1 to 4). Coconut water (liquid endosperm) contained the lowest amount of protein and the outer region had the highest. The testa (region 4) also had a very low level of protein. Non-protein nitrogen, apparently did not show appreciable variation.

**Fractionation of protein:** Results on the protein fractions from the different regions after sequential extractions with the four different solvents following this classification are presented in Table 2. Most of the proteins in the different regions of coconut endosperm were soluble in aqueous and salt media and it can be seen that over 80 per cent of the proteins would be classified as albumins and globulins. The composition of region 4 was altogether different from the other regions in that it contained the highest amount of glutelin and residual proteins.

**Nitrogen solubility index:** Studies were made on nitrogen solubility or extractability of samples from different regions of coconut endosperm in aqueous and salt media over a range of pH and results are shown in Fig. 2 and 3. In the aqueous media, nitrogen solubility increased with increasing acidity and alkalinity. Under the conditions of extraction, maximum solubility was observed at pH 1 for all the different regions and minimum solubility at pH 3. The solubility profile of the

TABLE 1. DISTRIBUTION OF PROTEIN AND NON-PROTEIN NITROGEN (WT PER CENT) IN DIFFERENT REGIONS OF COCONUT ENDOSPERM\*

	Whole endosperm	Coconut water	Regions of endosperm (fractional wt %)			
			Inner	Middle	Outer	Testa
Protein**	26.66	0.24	3.58	8.10	13.77	0.71
NPN	0.39	0.16	0.10	0.11	0.13	0.31

\* Values are average of three determinations expressed on moisture fat-free basis.

\*\*N × 6.25

TABLE 2. FRACTIONAL PERCENTAGE OF PROTEIN FRACTIONS OF VARIOUS REGIONS OF COCONUT ENDOSPERM\*

Extraction solvent	Protein type	Whole endosperm	Regions of coconut endosperm			
			Inner	Middle	Outer	Testa
H <sub>2</sub> O	Albumin	7.850	1.110	2.290	4.390	0.080
5% NaCl	Globulin	14.090	1.810	4.500	6.980	0.050
70% EtOH	Prolamine	0.720	0.090	0.080	0.120	0.050
0.05N NaOH	Glutelin	2.040	0.250	0.590	0.950	0.190
10% TCA	NPN	0.006	0.003	0.001	0.001	0.002
Residue		1.580	0.230	0.540	1.200	0.330

\* Values are mean of three determinations of dry defatted material.

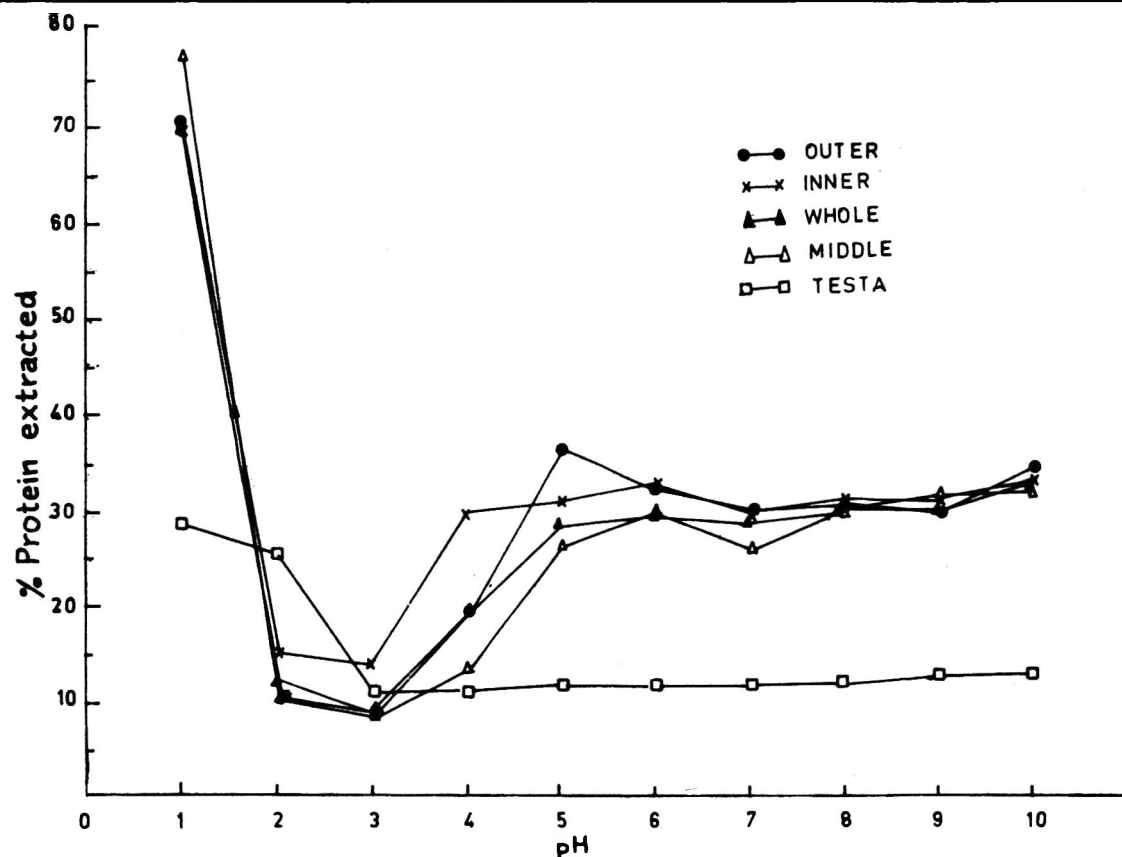


Fig. 2. Nitrogen solubility profiles of different regions of coconut endosperm in aqueous media at different pH.

testa region was different from all other regions in that it did not show any change after pH 3.

The solubility profile of different regions of coconut endosperm in the NaCl solution exhibited an interesting pattern. As against the aqueous medium, the proteins were least soluble at pH 1 in the salt solution. A steady increase in the solubility was observed for all from pH 1 to 3. This was followed by a plateau (pH 3-6) and a subsequent peak at pH 7. The peak solubility for inner and middle was more than 80 per cent whereas for the outer, it was only 55 per cent at pH 7. Testa had the least solubility in the entire pH range resembling aqueous media. The pH solubility values reported here for various regions of coconut endosperm were

in close agreement with the solubility profile reported by Samson *et al.*<sup>5</sup> for whole coconut endosperm.

**Electrophoresis of water-soluble and salt-soluble proteins:** The results obtained with albuminous (water soluble) and globular (salt soluble) proteins from different regions of coconut endosperm are shown in Tables 3 and 4. As could be seen from the electrophoretic pattern (Fig. 4 and 5) the albumins resolved into 6 bands with mol. wt. ranging from 14,000 to 52,000 (Table 3). Globulin had three major bands corresponding to molecular weights 45,000, 23,000 and 17,5000. While the major protein bands (water soluble) showed an increase in their concentrations (relative percentages) from region 2 to 3, it was in the decreasing order

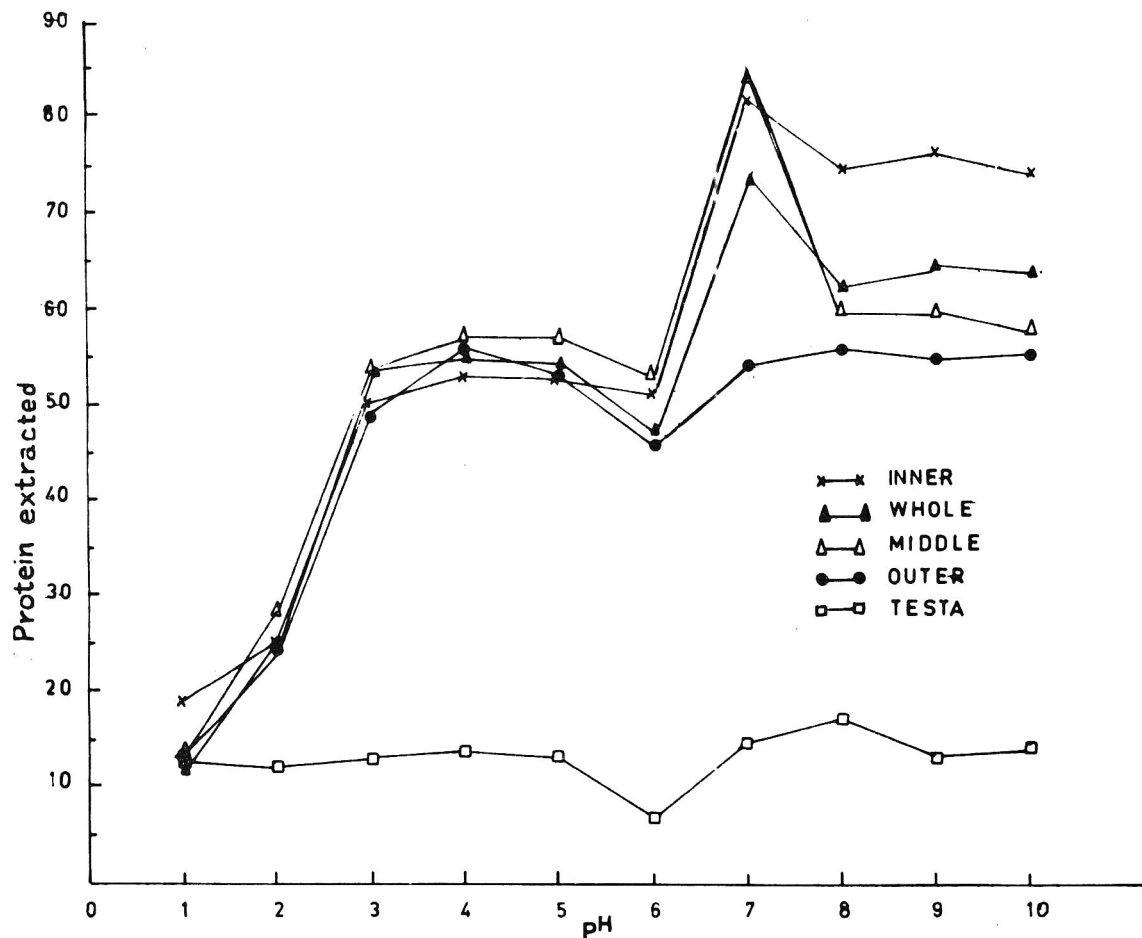


Fig.3. Nitrogen solubility profiles of different regions of coconut endosperm in salt media at different pH.

TABLE 3. ELECTROPHORETIC PATTERN OF ALBUMIN FRACTION BY PAGE-SDS<sup>a</sup>

Band No.	$R_f$	Molecular wt	Whole endosperm	Regions of coconut endosperm (relative %)				Coconut water
				Inner	Middle	Outer	Testa	
1	0.31	52,000	23.46	25.15	25.85	20.25	17.01	36.62
2	0.35	46,000	—	—	—	—	9.31	—
3	0.40	39,000	7.76	9.43	8.71	5.13	7.55	—
4	0.45	35,000	7.76	9.43	8.71	5.13	26.74	—
5	0.52	29,000	13.09	15.72	9.14	10.26	20.51	26.51
6	0.63	22,000	24.35	21.38	25.90	26.66	—	—
7	0.69	18,500	—	—	—	—	18.87	—
8	0.81	14,000	23.51	18.86	21.68	32.56	—	36.86

a Values are average of three determinations

TABLE 4. ELECTROPHORETIC PATTERN OF GLOBULIN FRACTION BY PAGE-SDS\*

Band No.	$R_f$	Molecular wt	Whole endosperm	Regions of coconut endosperm (relative %)			Testa
				Inner	Middle	Outer	
1	0.36	45,000	15.56	7.60	18.18	21.81	—
2	0.55	27,000	10.32	—	10.33	12.27	—
3	0.61	23,000	41.10	50.14	41.21	36.36	—
4	0.71	17,500	33.02	42.25	30.28	29.54	—

\* Values are mean of three determinations

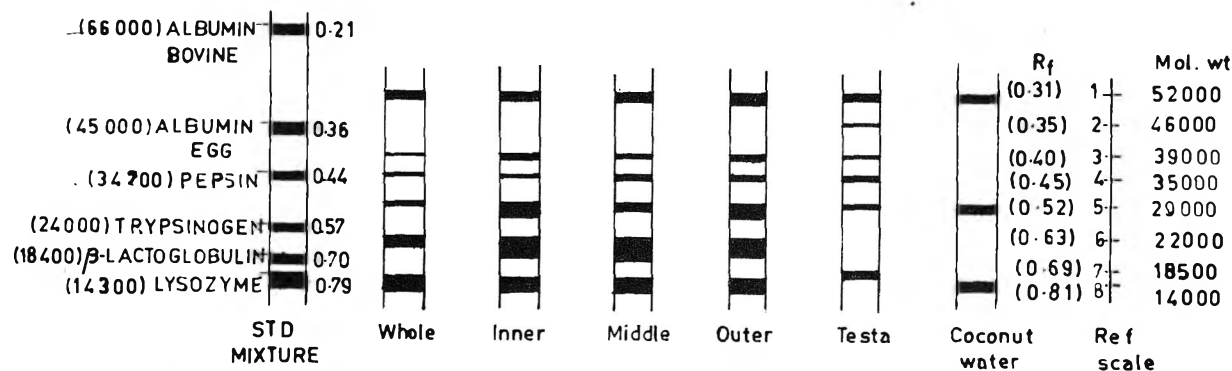


Fig.4. Electrophoretic patterns of water soluble proteins in different regions of coconut endosperm.

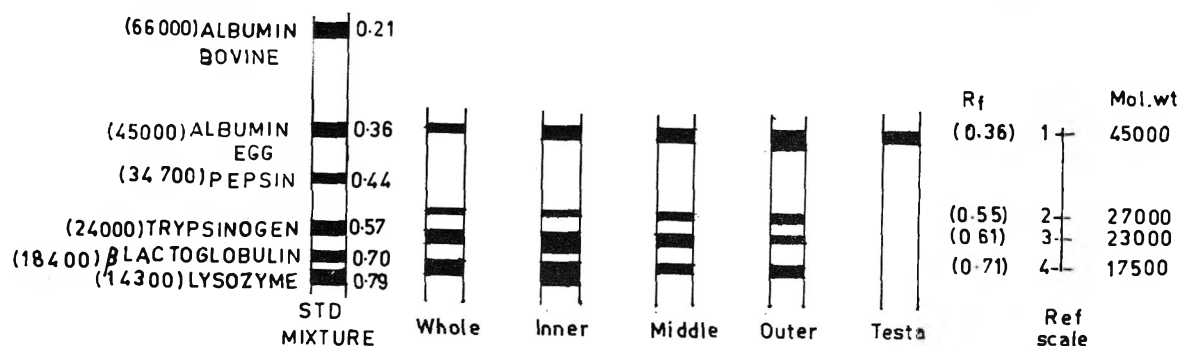


Fig.5. Electrophoretic patterns of salt soluble proteins in different regions of coconut endosperm.

for the minor protein fractions. But it may be noted that only the outer region showed a lesser concentration for the major protein band with molecular weight 52,000. In the case of testa, two major protein bands corresponding to mol. wts. 22,000 and 14,000 were absent and protein bands corresponding to molecular weights 46,000 and 18,5000 were obtained. Coconut water proteins (water soluble) showed the presence of only 3 bands with molecular weights 52,000, 29,000 and 14,000. In general, for the albuminous proteins, the various regions from 1 to 3 had the same electrophoretic profile, but showed significant differences in their quantities with respect to the major and minor protein fractions. The testa region showed a different electrophoretic pattern. The major protein bands (salt soluble) with high molecular weights also showed an increasing concentration gradient from regions 1 to 3 whereas for the low mol. wt. bands, this was in the decreasing order.

The present investigation did not reveal any major differences in the general electrophoretic pattern amongst the different regions except testa. The variations in number, type and concentration of protein bands probably contribute towards the importance of the particular stage of development of the fruit. Further studies may have to be carried out to elucidate changes in protein patterns during various stages of development of the coconut endosperm.

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## Nutrient Composition and Relationship Between Physico-chemical and Sensory Qualities of Sorghum Genotypes\*

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The content of amino acids, tannins and physico-chemical characteristics were estimated in eight sorghum genotypes viz. 'CSV-10', 'CSV-11', 'SPV-386', 'SPV-736', 'UPFS-3', 'UPFS-11', 'P-37' and 'P-151'. The chapatis made from the flours of all the genotypes were evaluated for sensory properties. The genotypes 'UPFS-11' had the lowest leucine (0.63 g per 100g) whereas 'P-151' had the highest. The maximum lysine was observed in 'UPFS-3'. Tannins ranged from 48 ('SPV-736') to 336 mg per 100 g ('P-37'). Correlation studies revealed significant positive correlation between grain hardness and texture of chapati. Amylose had significant positive correlation with overall acceptability of chapati.

The crop sorghum (*Sorghum bicolor* (L.) Moench) has many merits and demerits when compared with other cereal crops. Not only the sorghum grain but also the plant is useful to farmers. Crop is hardy, can withstand weather vagaries. Quantitatively its nutrient composition is *at par* with wheat and rice but quality of protein is poor mainly due to its high leucine and tannin contents<sup>1</sup>. Eating quality is comparatively poor and this relates with physico-chemical characteristics<sup>2</sup>.

Hence in the light of these facts, it is necessary to explore genetically diversified material to evolve high yielding nutritionally sound sorghum varieties with good eating quality. In the present study, eight sorghum genotypes were evaluated for amino acid profile, tannins, physico-chemical properties and chapati making quality.

### Materials and Methods

Sorghum genotypes viz. 'CSV-10', 'CSV-11', 'SPV-386', 'SPV-736', 'UPFS-3', 'UPFS-11' were obtained from the Department of Plant Breeding of this University and 'P-37' and 'P-151' from the Department of Entomology, Indian Agricultural Research Institute, New Delhi.

**Physico-chemical characteristics:** The sorghum grains of various genotypes were compared for their physical characteristics including grain colour<sup>2</sup>, grain volume, thousand kernel weight, grain hardness<sup>2</sup> and hydration capacity. The hydration capacity was calculated as the difference in weight of 100 grains after soaking for 24 hr. It was expressed as weight per grain. Moisture and protein were determined using standard procedure of AACC<sup>3</sup>.

Amylose content was estimated according to the procedure of Juliano<sup>4</sup>. The modified vanillin-HCl method was used to estimate tannin content<sup>5</sup>.

**Amino acid analysis:** Samples containing 3.25 mg nitrogen were hydrolysed for 24 hr at 110°C in 13 ml of 6 N HCl in sealed and evacuated tubes. The resultant hydrolysate was lyophilized to dryness and dissolved in 0.2 M citrate buffer, pH 2.0. The analyses were done by ion exchange chromatography on a TSM amino acid analyser. Tryptophan was not estimated.

**Dough hardness and chapati making quality:** Dough hardness was measured by Instron Testing machine (Model 6021). Dough piece of 1 cm<sup>2</sup> was deformed to 3/4th of its height using cross head speed of 50 mm/min and full scale load of 20 kg. The deformation curve was obtained and the peak force was recorded as dough hardness. Chapaties were prepared from the dough using standard laboratory procedure of Murty and Subramanian<sup>6</sup>. The sensory properties of chapaties were evaluated using a trained lab panel consisting of 7 members using the following score card: Taste: 5-good; 4-fair; 3-average; 2-bad; 1-very bad; Texture; 5-very soft; 4-soft; 3-average; 2-hard; 1-very hard; Aroma; 3-pleasant; 2-moderate; 1-unpleasant; Overall acceptability; 5-good; 4-fair, 3-average; 2-bad; 1-very bad.

For statistical analysis of the data on nutritional quality and physico-chemical properties, CRD was employed whereas for sensory evaluation of chapaties, randomised block design was used.

\*Part of M.Sc. thesis of the first author.

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TABLE 1. PHYSICAL CHARACTERISTICS OF EIGHT SORGHUM GENOTYPES

Genotype	Grain colour	1000 kernel wt* (g)	Hydration capacity* (ml/seed)	Grain hardness** (kg)
CSV-10	2.5 Y 8/2 white	25.35	0.011	8.26
CSV-11	2.5 Y 8/4 pale yellow	22.34	0.007	7.19
SPV-386	2.5 Y 8/4 pale yellow	22.87	0.007	7.86
SPV-736	2.5 Y 8/4 pale yellow	22.98	0.011	7.91
UPFS-3	2.5 Y 8/6 yellow	22.33	0.006	7.50
UPFS-11	2.5 Y 8/4 pale yellow	22.45	0.006	6.26
P-37	2.5 Y 6/4 light yellowish brown	20.61	0.010	3.36
P-151	2.5 Y 6/6 olive yellow	21.90	0.009	5.10
Mean		22.72	0.008	6.68
C.D. (P = 0.05)		0.37	0.001	1.17

\*Average of triplicate values reported

\*\*Average of ten replicates reported

## Results and Discussion

From the results of Table 1, it can be observed that the genotype 'CSV-10' showed whitish colour and genotype 'P-151' the olive yellowish. The colour of various genotypes fell in the range reported by Subramanian *et al.*<sup>7</sup> Genotype 'CSV-10' was rated superior to other genotypes on the basis of colour. The thousand kernel weight of various sorghum genotypes ranged from 20.61 to 25.35 g, maximum being for 'CSV-10' and minimum for 'P-37'. These values are in the range of 19.6 to 32.9 and 20.0 to 26.0 g reported by Maxon *et al.*<sup>8</sup> and Sullins and Rooney<sup>9</sup>. The genotypes 'CSV-11', 'UPFS-3' and 'UPFS-11' did not differ significantly from each other.

The hydration capacity (HC) of the grain is an important attribute which affects the cooking quality and in turn sensory characteristics of the product<sup>10</sup>. The genotypes 'UPFS-3' and 'UPFS-11' showed minimum HC whereas 'CSV-10' and 'SPV-736' the maximum. The differences may be due to the genetic variations in bran layer<sup>11</sup>. Genotypes 'P-151' and 'P-37' differed significantly ( $P < 0.05$ ) from all other genotypes with respect to HC.

Genotype 'CSV-10' showed maximum grain hardness and 'P-37' the minimum. The grain hardness of former differed significantly ( $P < 0.05$ ) from that of 'UPFS-11', 'P-37' and 'P-151'. These values are in the range reported by Subramanian and Jambunathan<sup>7</sup>.

TABLE 2. CHEMICAL CHARACTERISTICS AND DOUGH HARDNESS OF EIGHT SORGHUM GENOTYPES

Genotype	Moisture (%)	Crude protein* (%)	Amylose (%)	Tannins* (mg/100g)	Dough hardness (kg)
CSV-10	9.09	12.02	14.17	144	3.67
CSV-11	12.00	10.60	11.55	108	4.30
SPV-386	11.75	8.77	13.93	96	2.60
SPV-736	11.21	7.21	17.92	48	3.12
UPFS-3	11.92	8.97	14.70	144	3.79
UPFS-11	11.62	7.58	10.53	276	3.21
P-37	9.27	9.74	14.40	336	4.21
P-151	10.30	9.19	11.55	324	5.14
Mean	10.89	9.26	13.59	184.5	3.76
C.D. (P=0.05)	0.30	0.30	0.51	87.64	0.64

\*Average of triplicate values reported.

The contents of moisture and protein of various sorghum genotypes are within the range reported earlier<sup>12-14</sup> (Table 2). With regard to moisture content, the differences were significant ( $P < 0.05$ ) in all the genotypes except between 'CSV-10' and 'P-37' and among 'SPV-386', 'UPFS-3' and 'UPFS-11' whereas with regard to protein content, all genotypes differed significantly except 'SPV-386' and 'UPFS-3'.

The values for amylose content obtained in the present study are in accordance with those reported by Ring *et al.*<sup>15</sup>.

The tannin content of the sorghum genotypes varied from 48 to 336 mg per 100 g sample being minimum for 'SPV-736' and maximum for 'P-37'. These values are covered in the range of 10 to 2050 mg and 10 to 2056 mg per 100 g reported by Nair and Radhakrishnan<sup>1</sup> and Sivaprasad<sup>16</sup>, respectively. Dough hardness ranged from 2.6 kg ('SPV-386') to 5.14 kg ('P-151'). A value of 1.88 kg for wheat flour dough hardness was recorded. A good quality dough is sticky and easily rollable into chapati without any breakage. The cause of variation in dough hardness may be attributed to water soluble components<sup>2</sup>.

The values obtained for all amino acids in the eight genotypes evaluated fell within the range reported by earlier workers<sup>17-19</sup> (Tables 3 a and 3 b). The lowest leucine content of 0.63 g per 100 g sample was observed in 'UPFS-11' and the highest value of 1.06 g per 100 g sample in genotype 'P-151'. Isoleucine ranged from a minimum of 0.21 ('UPFS-11') to a maximum of 0.41 g per 100 g sample, while range for lysine was observed to vary from 0.22 ('SPV-736') to 0.37 g per 100 g sample ('UPFS-3').

Sensory evaluation of the chapatis showed that on the basis of scores obtained for taste, texture, aroma and overall acceptability of chapati, the genotypes 'SPV-736', 'CSV-10' and 'SPV-386' were more acceptable than others (Table 4). The dark coloured genotypes 'P-37' and 'P-151' were not acceptable due to their bitter taste.

Correlations between the physico-chemical and sensory characteristics of different sorghum genotypes showed

TABLE 3a. ESSENTIAL AMINO ACIDS (G PER 100 G SAMPLE) OF EIGHT SORGHUM GENOTYPES

Genotype	Lys	Leu	Iso	His	Phe	Met	Thr	Val
CSV-10	0.32	0.88	0.33	0.32	0.15	0.16	0.39	0.33
CSV-11	0.30	0.81	0.32	0.30	0.44	0.18	0.37	0.36
SPV-386	0.33	0.94	0.37	0.26	0.44	0.18	0.41	0.41
SPV-736	0.22	0.74	0.33	0.26	0.34	0.14	0.30	0.33
UPFS-3	0.37	0.91	0.35	0.45	0.45	0.19	0.42	0.41
UPFS-11	0.26	0.63	0.21	0.21	0.31	0.10	0.28	0.27
P-37	0.36	1.01	0.41	0.47	0.54	0.19	0.41	0.46
P-151	0.32	1.06	0.32	0.40	0.49	0.19	0.46	0.48
Mean	0.31	0.87	0.33	0.28	0.39	0.17	0.38	0.38

TABLE 3b. SEMI-ESSENTIAL AND NON-ESSENTIAL AMINO ACIDS OF EIGHT SORGHUM GENOTYPES

Genotype	Semi-essential amino acids* (g/100 g sample)					Non-essential amino acids* (g/100 g sample)			
	Arg	Tyr	Cys	Gly	Ser	Asp	Ala	Pro	Glu
CSV-10	0.47	0.30	0.20	0.32	0.35	0.77	0.74	0.74	2.08
CSV-11	0.39	0.35	0.49	0.36	0.29	0.65	0.69	0.95	1.57
SPV-386	0.48	0.29	0.49	0.38	0.45	0.61	0.80	0.65	2.08
SPV-736	0.39	0.41	0.29	0.30	0.26	0.63	0.65	0.71	1.50
UPFS-3	0.40	0.37	0.28	0.36	0.38	0.74	0.90	0.79	1.94
UPFS-11	0.43	0.23	0.14	0.25	0.26	0.49	0.55	0.59	1.46
P-37	0.57	0.37	0.23	0.38	0.42	0.70	0.84	0.98	2.14
P-151	0.56	0.37	0.21	0.35	0.43	0.83	0.89	0.87	2.19
Mean	0.46	0.34	0.29	0.34	0.35	0.67	0.75	0.78	1.87

significant positive correlation between grain hardness and texture of chapati (Table 5). Grain hardness relates to endosperm texture. Corneous endosperm is harder compared to flourey endosperm. Hydration capacity, dough hardness, moisture and protein contents had significant positive

TABLE 4. MEAN SCORES FOR SENSORY PARAMETERS OF CHAPATIES PREPARED FROM EIGHT SORGHUM GENOTYPES

Genotype	Taste*	Texture*	Aroma*	Overall acceptability*
CSV-10	3.71 <sup>ab</sup>	3.29 <sup>abc</sup>	2.29 <sup>abc</sup>	3.57 <sup>a</sup>
CSV-11	3.57 <sup>ab</sup>	3.57 <sup>a</sup>	2.14 <sup>bc</sup>	3.29 <sup>ab</sup>
SPV-386	3.14 <sup>bc</sup>	3.29 <sup>ab</sup>	2.43 <sup>ab</sup>	3.57 <sup>a</sup>
SPV-736	4.00 <sup>d</sup>	3.71 <sup>a</sup>	2.57 <sup>a</sup>	3.57 <sup>a</sup>
UPFS-3	2.86 <sup>c</sup>	2.71 <sup>bc</sup>	2.00 <sup>c</sup>	3.00 <sup>b</sup>
UPFS-11	3.14 <sup>bc</sup>	2.57 <sup>a</sup>	2.00 <sup>c</sup>	3.14 <sup>b</sup>
P-37	1.86 <sup>d</sup>	2.57 <sup>c</sup>	1.71 <sup>cd</sup>	2.00 <sup>c</sup>
P-151	2.00 <sup>d</sup>	2.43 <sup>c</sup>	1.43 <sup>d</sup>	2.29 <sup>c</sup>
Mean	3.03	3.01	2.07	3.05
C. D. (P=0.05)	0.58	0.63	0.39	0.48

\*Any two figures in the same column with similar superscripts do not differ significantly at P=0.05.

TABLE 5. CORRELATIONS BETWEEN PHYSICO-CHEMICAL AND SENSORY QUALITIES OF EIGHT SORGHUM GENOTYPES

	Taste	Texture	Aroma	Overall acceptability
1000 kernel wt	-0.306	-0.418	-0.986*	0.907*
Hydration capacity	-0.200	0.505	0.998*	-0.858*
Grain hardness	0.603	0.882*	0.583	-0.062
Dough hardness	0.437	0.286	0.934*	-0.954*
Moisture	-0.186	0.512	0.999*	-0.848*
Protein	-0.533	0.248	0.898*	-0.944*
Tannins	-0.596	-0.933*	-0.608	0.104
Amylose	0.459	-0.065	-0.830*	0.847*

\*Significant at 5% level of significance.

correlation with aroma of chapati whereas significant negative correlation with overall acceptability of chapati was observed. Amylose had significant positive correlation with overall acceptability of chapati as reported earlier<sup>2</sup>.

The genotypes 'SPV-386' and 'UPFS-3' seem to be promising as they have high lysine contents and produced chapaties of acceptable quality. However, no particular genotype showed positive results for all the above parameters.

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## SPECTROPHOTOMETRIC DETERMINATION OF BUTYLATED HYDROXY ANISOLE (BHA) IN OILS

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Two simple spectrophotometric methods were developed for the determination of butylated hydroxy anisole in commercial samples and oils. The methods are based on the reaction of BHA to form coloured species with either of two reagents, Fe (III) – 2,4,6-tripyridyl-S-triazine (TPTZ) or triphenyl tetrazolium chloride (TTC).

Butylated hydroxy anisole (BHA) is an antioxidant permitted to be added to edible oils in a concentration not exceeding 0.02 per cent either individually or in combination with gallic acid derivatives<sup>1</sup>. The chromogenic agents reported to be useful for the determination of BHA include Fe (III) – dipyridyl<sup>2,3</sup>, diazo sulphanilic acid<sup>4</sup>, Gibbs reagent<sup>5</sup>, sodium nitrite<sup>6</sup>, Metol in conjunction with various oxidants<sup>7,8</sup>, F-C reagent<sup>9</sup> and MBTH<sup>10</sup>. Most of the reported methods suffer from one disadvantage or the other such as low sensitivity, low absorption maximum and lack of specificity. We have now for the first time developed two simple, rapid and accurate methods for the determination of BHA at the microgram level utilising the reagents, Fe (III) – 2,4,6-tripyridyl-S-triazine (TPTZ) or triphenyl tetrazolium chloride (TTC). Both these methods are based on the reduction of the oxidant by BHA. In method A, BHA reduces the oxidant Fe (III) to the Fe (II) state. TPTZ then forms a violet coloured complex<sup>11</sup> ( $\lambda_{\max}$  590 nm) with the Fe (II) after masking the excess of Fe (III) with phosphoric acid. In method B, BHA reduces the oxidant (TTC) to yield formazaan<sup>12</sup> which is self coloured ( $\lambda_{\max}$  480 nm). These methods are sensitive and useful in the purity assays of BHA and its estimation in oil samples.

*Preparation of solutions and reagents:* All the chemicals and reagents used were of G.R. grade. TPTZ solution (Loba, 0.312 per cent) and FeCl<sub>3</sub> solution (0.27 per cent) were prepared in GR methanol. Orthophosphoric acid solution was prepared by diluting 1.27 ml of the reagent to 1000 ml with methanol. TTC solution (E. Merck, 0.3 per cent) and sodium hydroxide solution (I N) were prepared with double distilled

water. Standard solutions of BHA 500  $\mu$ g/ml (for method B) and 50  $\mu$ g/ml (for method A) were prepared in methanol.

Spectral absorbance measurements were made with a Systronics model 105 (MK1) spectrophotometer.

*Method A (Fe (III) – TPTZ):* To each of a series of 25 ml graduated tubes containing 20–100  $\mu$ g of BHA, 0.5 ml of ferric chloride and 1 ml of TPTZ solutions were added successively. The volume was brought to 8 ml with distilled water and the tubes were heated for 15 min in a boiling water bath. The tubes were removed from the water bath, cooled and 1 ml of phosphoric acid solution was added to each tube. The volume was made upto the mark with distilled water after mixing well. The absorbance of the violet coloured complex was measured at 590 nm against a reagent blank after 2 min and before 60 min.

*Method B (TTC-NaOH):* Aliquots of BHA solution (ranging from 100–1000  $\mu$ g) were taken in a series of 60 ml Pyrex separating funnels. Two ml of TTC solution was added to each funnel. After 5 min, 1 ml of NaOH solution and 10 ml of n-butyl alcohol were added to each funnel and kept aside for 10 min with occasional shaking. The absorbance of n-butanol layer was measured at 480 nm against a reagent blank after 2 min and before 60 min.

*Determination of BHA in oils:* Ten g of oil containing BHA was dissolved in 100 ml of petroleum ether and extracted with four 20 ml portions of acetonitrile. The combined acetonitrile extract was evaporated to dryness by heating in a boiling water bath and the residue was then dissolved in an appropriate volume of aqueous methanol (1:1). The proposed procedure was then followed for estimation of BHA.

The optimum parameters found for each method were incorporated in the recommended procedures. The Beer's law limits ( $\mu$ g/ml) and molar absorptivity ( $1 \text{ mole}^{-1} \text{ cm}^{-1}$ ) were found to be 0.5–4.0 and  $3.78 \times 10^4$  for method A and 10.0–100.0 and  $1.18 \times 10^3$  for method B. The slope, intercept and correlation coefficient found by linear least squares analysis of the results were found to be 0.2124, 0.0027 and 0.9999 respectively for method A and 0.0066, 0.0002 and 0.9998 respectively for method B. The per cent relative standard deviation for methods A and B was found to be 1.17 and 1.58 respectively. Comparison of the values of recovery

TABLE 1. RECOVERY (PER CENT) OF BHA FROM OILS

Sample (mg)	BHA added	Method A	Method B method <sup>10</sup>	Reported
Sunflower oil	5	98.1	96.9	96.56
Coconut oil	5	96.1	96.2	95.80
Cotton seed oil	5	97.4	97.1	97.60
Groundnut oil	5	96.8	97.3	97.00

experiments of BHA in various oils obtained by the proposed and reported methods<sup>10</sup> reveal good recovery (Table 1). Among the proposed methods, method A was found to be the most sensitive reported so far for determination of BHA. Both the proposed methods are simple, rapid and accurate and can be used for routine determination of BHA in commercial samples and oils. Since the two methods are based on the reducing properties of BHA, other compounds such as propyl gallate (PG) and di-tertiary butyl hydroquinone (TBHQ) with similar reducing properties when present in the oil sample under analysis, interfere with the proposed procedure. Preliminary separation by TLC (Stationary phase : Silica gel G-Kieselguhr (2:1) impregnated with EDTA is necessary for the determination of BHA in such samples. Benzene – n-butanol (12:1) and toluene – dioxan (6:1) solvent systems are found to effect good separation of PG and TBHQ from BHA. The  $R_f$  values in the former system are found to be 0.35, 0.96 and 0.72 and in the latter system 0.38, 0.92 and 0.53 for PG, BHA and TBHQ respectively.

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**MICROBIOLOGICAL STATUS OF INFANT FOODS**

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**Microbiological status of infant foods available in Gujarat market was studied with special reference to the presence of fungi. Most of the infant foods were found to contain fungi. The main contaminating fungus was found to be *Aspergillus*.**

Mother's milk has been accepted as an ideal food for infants. However, certain situations require the use of prepared (readymade) infant foods. In this country, mainly infant milk food, infant formulae and milk-cereal based weaning foods are available. However, specific standards for infant milk foods only appear in the Prevention of Food Adulteration Rules<sup>1</sup>. Rules lay down for microbiological standards as, "Infant milk food shall not show Standard Plate Count of more than 50,000 per g. and coliform shall be absent in 0.1g of the powder". As this laboratory is involved in the routine market surveillance of food samples under the P.F.A. Act and Rules, the routine analysis of the samples of infant foods is also carried out. During this routine analysis, it was observed that besides bacteria, some samples also showed the presence of various types of fungi. Like some bacteria, certain fungi also have capacity to produce pathogenic reaction in humans when taken orally. *Aspergillus* causes *Aspergillus*-infiltrated ulcer in ileum and colon<sup>2</sup>. Furthermore, fungi like *Aspergillus*, *Fusarium* and *Penicillium* produce mycotoxins which are considered to be potential hazard to health. It is also known that *Mucor* can cause intestinal mucormycosis, which is a rapidly progressing fatal disease<sup>2</sup>. Infants and children, in particular, appear to be highly susceptible to this disease<sup>3</sup>.

Thirty samples of 3 categories of infant foods manufactured by 7 manufacturers were studied. The standard plate count and coliform count were determined by the usual microbiological procedure<sup>4</sup>. For the isolation of fungi, the samples were diluted tenfold with water<sup>5</sup> and 0.1 ml of the diluted sample was streaked on malt agar medium (with antibiotic)<sup>6</sup> and DYPBS medium<sup>7</sup>. The plates were incubated at 22° C for 48 to 72 hr. Individual colonies were further purified by transfer to appropriate media. The fungi were identified by studying the colony characteristics and by microscopical examination on slide cultures<sup>8,9</sup>.

Table 1 gives microbiological status of 18 samples of 5 manufacturers of infant milk foods. Only one sample showed more than 50,000 standard plate count per g as prescribed by the P.F.A. Rules. However, there were 8 samples which showed standard plate count as high as 2.4 × 10<sup>3</sup> to 1.04 × 10<sup>4</sup> per g. Out of the samples tested, 2 samples showed the

TABLE 1. MICROBIOLOGICAL STATUS OF INTANT MILK FOODS

Manufacturer code	SPC/g	Coliform count/0.1g	Fungi isolated
A	3.07 × 10 <sup>2</sup>	Nil	<i>Aspergillus</i> (3 sp) <i>Syncephalastrum</i> .
A	7.46 × 10 <sup>3</sup>	Nil	<i>Penicillium</i> , <i>Geotrichum</i> <i>Aspergillus</i> , <i>Paecilomyces</i> .
A	7.78 × 10 <sup>3</sup>	Nil	<i>Aspergillus</i> , <i>Helicocephalum</i> <i>Cladosporium</i> , <i>Fusarium</i> , <i>Torula</i> .
B	5.34 × 10 <sup>3</sup>	Nil	<i>Aspergillus</i> .
B	5.92 × 10 <sup>3</sup>	3	<i>Rhizopus</i> , <i>Aspergillus</i> .
B	7.0 × 10 <sup>3</sup>	Nil	<i>Mucor</i> , <i>Curvularia</i> .
C	1.04 × 10 <sup>4</sup>	Nil	Not done.
C	2.4 × 10 <sup>3</sup>	Nil	<i>Aspergillus</i> .
C	2.7 × 10 <sup>3</sup>	88	<i>Syncephalastrum</i> .
C	6.1 × 10 <sup>1</sup>	Nil	Not done.
C	2.5 × 10 <sup>2</sup>	Nil	<i>Aspergillus</i> .
C	7.0 × 10 <sup>2</sup>	Nil	<i>Aspergillus</i> , <i>Curvularia</i> , <i>Gonatotryum</i> .
C	7.8 × 10 <sup>2</sup>	Nil	<i>Cladosporium</i> .
C	2.85 × 10 <sup>3</sup>	Nil	<i>Syncephalastrum</i> , <i>Aspergillus</i> .
D	8.75 × 10 <sup>2</sup>	Nil	<i>Penicillium</i> , <i>Syncephalastrum</i> .
D	7.6 × 10 <sup>2</sup>	Nil	Nil.
E	2.34 × 10 <sup>2</sup>	Nil	<i>Syncephalastrum</i> .
E	5.7 × 10 <sup>1</sup>	Nil	Nil.

presence of coliform bacteria in 0.1 g of the sample. It is worth noting that the sample with standard plate count above 50,000 (2.7 × 10<sup>5</sup>), had also coliform count of 88 per 0.1 g. Out of the 16 samples tested for the presence of fungi, 14 showed the presence of different types of fungi and 9 samples showed the presence of *Aspergillus* species. While *Syncephalastrum* was present in 5 samples. *Penicillium*, *Geotrichum*, *Paecilomyces*, *Helicocephalum*, *Cladosporium*, *Fusarium* and *Torula* were among the other fungi found.

Table 2 shows details of the samples of infant formulae that were analysed. None of the samples showed the presence of coliform bacteria and the highest plate count observed was 3.6 × 10<sup>3</sup>. Here also, the main fungus found was *Aspergillus* which was present in 5 out of 6 samples. Table 3 gives details of 6 samples of 2 manufacturers of milk cereal-based weaning

TABLE 2. MICROBIOLOGICAL STATUS OF INTANT FORMULAE

Manufacturer code	SPC/g	Fungi isolated
A	1.34 × 10 <sup>3</sup>	<i>Aspergillus</i> .
A	9.1 × 10 <sup>2</sup>	<i>Aspergillus</i>
A	8.6 × 10 <sup>1</sup>	<i>Penicillium</i>
A	3.6 × 10 <sup>3</sup>	<i>Rhizopus</i> <i>Syncephalastrum</i> .
B	1.1 × 10 <sup>3</sup>	<i>Aspergillus</i> .
B	2.8 × 10 <sup>3</sup>	<i>Syncephalastrum</i> , <i>Aspergillus</i> , <i>Penicillium</i> , <i>Spirosphaera</i> , <i>Rhizopus</i> .

Coliform count per 0.1 g sample was absent in all cases.

TABLE 3. MICROBIOLOGICAL STATUS OF INFANT MILK-CEREAL BASED WEANING FOODS

Manufacturer code	SPC/g	Coliform count/0.1g	Fungi isolated
A	$2.25 \times 10^1$	Nil	Nil
A	$2.5 \times 10^1$	Nil	Aspergillus
A	$2.9 \times 10^1$	Nil	Aspergillus
A	Not done	Nil	Aspergillus
B	$4.0 \times 10^2$	Nil	Aspergillus
B	$3.0 \times 10^2$	Nil	Nil

foods. Two samples did not show the presence of any fungi, while 4 samples showed the presence of *Aspergillus* as a sole contaminant. None of the samples showed the presence of coliform bacteria and the standard plate count was also well below the prescribed limit of P.F.A. Rules.

From the present study, it is concluded that the microbiological quality of infant foods moving in the market is satisfactory according to the standards given in P.F.A. Rules. However, the presence of various kinds of fungi points a finger towards lack of proper hygiene in the manufacture of these products. It is proposed that a further collaborative study may be undertaken to review the existing P.F.A. rules and standards. Microbiological standards for other types of infant foods may also be included in P.F.A. Rules.

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## BACTERIOLOGICAL QUALITY OF MILK AND MILK PRODUCTS WITH SPECIAL REFERENCE TO *SALMONELLA* AND ITS PUBLIC HEALTH SIGNIFICANCE

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Out of the 267 samples of milk and milk products examined, *Salmonella* spp. could be isolated from 5 samples of milk products. Among these, *S. Weltevreden* and *S. enteritidis* could be isolated from two samples each whereas *S. typhimurium* was isolated from one sample. Various other micro organisms isolated from these samples were *E. Coli*, *Klebsiella* spp., *Pseudomonas* spp., *Alkaligenes faecalis* and *Proteus* spp. The public health importance of these findings is reviewed.

Milk and its products provide an ideal medium for the growth of microorganisms and serves as a potential source of several diseases. In India, the chances of transmission of diseases through milk and milk products are much higher due to unsatisfactory milk hygiene, adulteration practices, poor health condition of animals, illiteracy and ignorance of dairy workers. The processing of dairy products requires a variety of heat treatments, handling by workers at various stages, drying, fermentation, etc. Frequently, substances such as salt, sugar, fruits, acids, eggs or other products of animal origin may be added to dairy ingredients in the manufacture of certain products. These additives may contribute to salmonellosis or other bacterial problems either by adding organisms or by creating conditions favourable for the growth and survival of bacteria<sup>1</sup>. The extent of *Salmonella* contamination in milk and its products is not properly known in our country. Keeping these facts in view, the present investigation was carried out to find out the presence of pathogenic organisms particularly *Salmonella* spp. and other enteropathogens in these products.

**Collection of samples:** In all, 69 samples of milk and 198 samples of milk products (Khoa, paneer, burfi, ice cream and kulfi) were collected from Ludhiana market under sterilized conditions. Samples were collected early in the morning.

Isolation and identification of *Salmonella* spp. was done according to the procedure recommended by Edwards and Ewing<sup>2</sup> and Cruickshank *et al.*<sup>3</sup> in which selenite cystine broth was used as enrichment medium and brilliant green agar and MacConkey's agar as selective media. Identification of various *Salmonella* isolates was done by various morpho-

logical, cultural and biochemical tests<sup>4</sup>. Agglutination with "Salmonella Poly-'O'-sera" was done to confirm the isolates before serotyping.

For serotyping, various isolates were sent to National *Salmonella* and *Escherichia* Centre, CRI, Kasauli, (HP). The isolation of other organisms was done on the blood agar, MacConkey's lactose agar and eosine methylene blue agar. These organisms were also identified according to Bergey's Manual<sup>4</sup>.

Examination of 198 samples of milk products resulted in the isolation of five strains of *Salmonellae*. Other bacteria isolated were *E.coli* (10 nos) *Klebsiella* spp. (59 nos), *Pseudomonas* spp. (27 nos), *Alkaligenes faecalis* (17 nos) *Proteus* spp. (9 nos) and unidentified Gram negative bacilli (36 nos). No organisms could be isolated from 35 samples (Table 1).

Excretion of *S. typhimurium* from cow's udder had been reported by Giles and King<sup>5</sup>. Such milk, if taken raw or cheese or ice cream, kulfi, etc. is prepared from that milk, may cause food poisoning. The presence of *Salmonella* in heat treated milk products is due to insufficient heat treatment or post contamination from infected persons or carriers coming in contact with these products.

No salmonellae were isolated from 69 milk samples while *E. coli*, *Kebsiella* spp., *Pseudomonas* spp. and unidentified Gram negative bacilli were found in 9,20,11 and 9 samples, respectively. Twenty samples had no organism (Table 1). The presence of these microorganisms in milk might be due to contamination of milk with contaminated containers, water or due to unhygienic handling.

The absence of *Salmonella* in milk samples in spite of the unhygienic practices employed in milk production could be due to the fact that in some cases *Salmonella* in milk might escape detection because of their usually small number, or possibly, the growth of *Salmonella* might have been inhibited by other fast growing organisms. The isolation of *Salmonella* from milk by other workers<sup>6,7</sup> might be due to the adulteration practices or due to *Salmonella* carriers.

All the five *Salmonella* cultures exhibited morphological, cultural and biochemical characteristics suggestive of *Salmonella* group. All the cultures were agglutinated with *Salmorella* "Poly-'O'-sera".

Table 2 shows the overall incidence of *Salmonella* in various milk products which was found to be 2.52 per cent. Maximum isolation was from paneer (4.54 per cent) followed by ice cream (3.92 per cent) whereas kulfi yielded least isolation (3.22 per cent). The presence of *Salmonella* in ice cream, kulfi and paneer could be due to the use of contaminated milk, water and other ingredients during their preparations as reported by Mathur<sup>8</sup> Patel and Vyas<sup>9</sup>, Barret<sup>10</sup>, and Das and Nag<sup>11</sup>.

No salmonellae could be recovered from burfi, khoa and raw milk. Sufficient heat treatment during the preparation

TABLE 1. BACTERIOLOGICAL QUALITY (NO. OF ISOLATES) OF VARIOUS MILK AND MILK PRODUCTS

Organisms isolated	Milk	Khoa	Burfi	Paneer	Ice cream	Kulfi	Total
<i>Salmonella</i> spp.	Nil	Nil	Nil	2 (4.54)*	2 (3.92)	1 (3.22)	5 (1.87)
<i>E. coli</i>	9 (13.04)	Nil	Nil	4 (9.09)	5 (9.80)	1 (3.22)	19 (7.2)
<i>Klebsiella</i> spp.	20 (28.98)	8 (26.67)	12 (28.57)	12 (27.27)	18 (34.29)	9 (29.03)	79 (29.59)
<i>Pseudomonas</i> spp.	11 (15.90)	7 (23.33)	8 (19.05)	5 (11.36)	5 (9.80)	2 (6.45)	38 (14.23)
<i>Alkaligenes faecalis</i>	Nil	3 (10.00)	4 (9.52)	5 (11.36)	2 (3.92)	3 (9.68)	17 (6.37)
<i>Proteus</i> spp.	Nil	Nil	1 (2.38)	2 (4.54)	5 (9.80)	1 (3.22)	9 (3.37)
Unidentified Gram-ve bacilli	9 (13.04)	6 (20.00)	9 (21.43)	8 (18.18)	5 (9.80)	8 (25.81)	45 (16.85)
Negative for any organism	20 (28.98)	6 (20.00)	8 (19.05)	6 (13.64)	9 (17.65)	6 (19.35)	55 (20.60)
Total samples examined	59	30	42	44	51	31	267

\*Figures in parentheses indicate % isolation

of khoa and burfi might be the reason that no salmonellae could be isolated from these products. The growth of *Salmonella* might be inhibited by other fast growing bacteria isolated in the present study. The presence of other enteric organisms might be due to the soiled hands of the persons handling these products or due to contaminated containers.

Out of the five confirmed *Salmonella* strains, three serotypes were identified: *S. typhimurium* (1), *S. enteritidis* (2) and *S. weltevreden* (2) (Table 2). *S. typhimurium* was isolated from one sample of paneer whereas one sample of ice cream and one sample of kulfi were positive for *S. enteritidis*. On the other hand, *S. weltevreden* was detected in one sample each of paneer and ice cream. All the three

serotypes of *Salmonella* isolated from milk products in the present study are important from public health point of view. Mathur<sup>8</sup> reported *S. weltevreden* as cause of food poisoning in humans is due to consumption of raw milk. The presence of these serotypes in cheese, ice cream and kulfi is of great public health concern as these products are consumed as such.

The occurrence of pathogenic *E. coli*, *Pseudomonas* spp., *Proteus* spp. and *Klebsiella* spp. in milk and milk products is dangerous because these often cause enteric disorders. *Klebsiella* spp. has been implicated in outbreaks of diarrhoea. Some strains have been implicated in food borne diseases in Sweden in which watery diarrhoea was the main symptom. The isolation of this organism from many foods suggest a role for *Klebsiella* in food borne illnesses.

The authors thank the Director, National *Salmonella* and *Escherichia* Centre, Kasauli for help in serotyping the *Salmonella* cultures.

TABLE 2. *SALMONELLA* ISOLATION FROM VARIOUS MILK PRODUCTS

Source	No. of samples screened	Salmonellae isolated (Nos.)	Serotype	Antigenic structure
Paneer	44	2 (4.54)	<i>S. typhimurium</i> <i>S. weltevreden</i>	4, 5, 12:1:1, 2 3, 10:r:z6
Ice cream	51	2 (3.92)	<i>S. weltevreden</i> <i>S. enteritidis</i>	3, 10:r:z6 9, 12:g, m:
Kulfi	31	1 (3.21)	<i>S. enteritidis</i>	9, 12, g, m:
Khoa	30	Nil	—	
Burfi	42	Nil	—	
Total	198	5 (2.52)	—	

No isolation could be made from 69 samples of milk  
Figures in parentheses indicate % isolation.

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## IRON AND COPPER UPTAKE DURING THE WET-MILLING OF SOME NIGERIAN FOODS

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Five food items, pepper, onion, tomato, fermented maize and dehulled cowpea, commonly wet-milled before further processing in Nigeria were milled with a commercially operated disc attrition mill for which the discs were made by local foundries. The pH, moisture, iron and copper contents of the milled samples were determined using atomic absorption spectrophotometer for metal determination. The iron content of the samples milled with a disc attrition mill was significantly higher than that of controls ( $p < 0.01$ ) which measured the actual metal content of the samples while there was no significant uptake of copper.

Iron is well known and studied for its role in preventing nutritional anaemia in human subjects<sup>1</sup>. However, copper though needed at trace levels may become toxic at higher levels. This is likely to happen if it is present in milling disc material as an impurity as a result of using different metal scraps in foundry work. This paper aims at investigating whether iron and copper are being contributed to the diet of Nigerians through milling pepper (*Capsicum annum*), onion, tomato fermented maize and dehulled cowpea with such discs.

Onion, tomato and pepper were milled as purchased after removal of the skin of onion and the stalk of pepper and tomato. Cowpea was soaked in distilled water for 15 min in a plastic bowl and manually dehulled. Maize was soaked in distilled water for 96 hr. During this period, it underwent mild fermentation as is the case during the preparation of 'Ogi'. One kg of each sample was taken to a local mill for milling and 200g of each sample was milled with a glass mortar and pestle in the laboratory for the determination of iron content.

The pH of a slurry of each milled food was measured with an EIL 7050 pH meter at 30°C (ambient temperature) immediately after milling.

All apparatus were soaked overnight in dilute HCl and rinsed with distilled water prior to use. This reduces the chances of metal contamination from apparatus.

Standard methods of food analysis were used<sup>2</sup>. The method of Reilly<sup>3</sup> was modified for the determination of moisture, iron and copper; moisture was determined by drying samples to a constant weight at 70°C in a forced convection oven. Samples were ashed at 425°C in a muffle furnace overnight as possible volatilisation of iron may occur above 450°C<sup>4</sup>.

TABLE 1. MEAN VALUES OF pH, MOISTURE, IRON AND COPPER CONTENTS OF FOOD SAMPLES

Sample	pH	Moisture (%)	Iron DAM	Actual (mg/100g)	Copper DAM	Actual (mg/100g)
Pepper( <i>C. annum</i> )	5.3	81.1	2.67	1.46	0.46	0.20
Onion	5.8	89.7	1.99	0.70	0.19	0.45
Tomato	4.1	90.9	3.07	2.10	0.55	0.16
Fermented maize	2.9	48.6	3.95	1.19	0.21	0.45
Dehulled cowpea	6.4	32.7	2.96	1.33	0.26	0.20

DAM = Milled with disc attrition mill.

The values of iron and copper were obtained on a Perkin Elmer 403 Atomic Absorption Spectrophotometer. The data obtained were subjected to ANOVA test<sup>5</sup>.

The results are shown in Table 1. All the samples milled in the disc attrition mill showed significantly higher iron content than the samples milled in the glass mortar and pestle (controls). There was no correlation between moisture content and iron content. The samples with the lowest pH i.e. fermented maize and tomato, had the highest iron uptake although a direct relationship between pH and iron uptake did not exist generally.

The results show that some iron is being ingested by those, particularly in the urban areas, who wet mill their food items with locally fabricated disc attrition mills. However, of the five samples analysed, only 'Ogi' from fermented maize and cowpea are consumed without much dilution. The others are diluted down during further processing like soup making. With such dilutions, the amount of iron ingested by an individual at a time is reduced significantly. Further work, especially clinical assessment will reveal the quantities taken in by individuals and the level of absorption in this form relating to nutritional requirement for iron in the diet.

Special thanks are due to the Analytical Laboratory of the International Institute of Tropical Agriculture, Ibadan for assistance in the use of their Atomic Absorption Spectrophotometer.

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## BIOCHEMICAL COMPOSITION AND NUTRITIONAL QUALITY OF TRITICALE

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Seven high yielding varieties of triticale, namely, 'Badger PM-118' 'UPT 72142', 'UPT 75182', 'UPT 76001', 'UPT 74304', 'UPT 7440' and 'UPT 75233' were analyzed for biochemical composition. PER of the highest yielding and highest protein containing variety, 'UPT 72142', was determined. The protein (N×5.7), iron, calcium, phosphorus and riboflavin contents of the triticales were higher than those of Indian wheats. The triticales had a lower proportion of phytic phosphorus than wheat. The PER of triticale chapati indicated that it was superior to wheat chapati.

Triticale promises higher yields of grain than the most productive wheat varieties. It can be grown in diverse locales and under varying climatic conditions and irrigations. Studies conducted at the G.B. Pant University of Agriculture and Technology revealed that triticale could yield at least 20 per cent more grain than commercial wheat. It had also been reported to be acceptable to farmers of U.P. hills as they grow local varieties of wheat having similar grain characteristics, i.e., dark colour and shrivelled grains<sup>1</sup>. In view of the possibility of cultivation and utilization of triticale at high altitudes, biochemical composition and nutritional quality of seven triticale varieties, namely, 'Badger PM-118', 'UPT 72142', 'UPT 75182', 'UPT 76001', 'UPT 74304', 'UPT 7440', and 'UPT 75233' were studied in order to ascertain their nutritional advantage. Samples were ground to pass 20 mesh sieve and were analyzed for protein (N×5.7) by the procedure

of AACC<sup>2</sup>. Iron, calcium, phosphorus and phytic varieties ranged from 13.55 to 15.03 per cent with a mean of 14.41 per cent; the highest being in variety 'UPT 72142'. phosphorus contents of the samples were estimated by the AOAC procedures<sup>3</sup>. Thiamine was extracted as described by Dawson and Martin<sup>4</sup> and measured fluorometrically by the AACC method<sup>2</sup>. Riboflavin was estimated by the procedure of Slater and Morell<sup>5</sup>. The protein efficiency ratio (PER) of the chapatis of one variety, viz, 'UPT 72142' having the highest yield and protein content was determined by the method of Campbell<sup>6</sup>.

It is evident from Table 1 that the protein contents of triticale A comparison with protein content of the wheat varieties<sup>7</sup> showed it to be higher in triticale. The average iron, calcium and phosphorus contents of the seven varieties of triticale were  $9.34 \pm 3.85$ ,  $135 \pm 46$  and  $534.98 \pm 185.46$  mg/100g, respectively. These were also higher in triticale varieties studied when compared to wheat. However, the phytic phosphorus content was lower in triticale as compared to wheat. The average thiamine content ( $3.45 \pm 2.24$  µg/g) was lower than in Indian wheat, while riboflavin content ( $2.99 \pm 0.35$  µg/g) was higher in triticale.

A comparison of the protein efficiency ratio at 10 per cent protein level obtained for triticale chapati diet and skim milk diet with wheat presented in Table 2 suggests that the protein quality of triticale is superior to that of wheat.

TABLE 2. PROTEIN EFFICIENCY RATIOS

Diet	PER (Mean ± S.E.)
Triticale chapati*	1.54 ± 0.09
Skim milk*	2.85 ± 0.09
Wheat <sup>7</sup>	1.50

\*Average of values from 10 rats.

TABLE I. BIOCHEMICAL COMPOSITION OF TRITICALE

Variety	Protein (N×5.7) (%)	Iron (mg/100g)	Ca (mg/100g)	P (mg/100g)	Phytic P (mg/100g)	Thiamine (µg/g)	Riboflavin (µg/g)
UPT 72142	15.0	8.30	103	476.8	199	3.59	3.20
UPT 75182	13.6	5.80	140	498.6	184	3.47	2.98
UPT 76001	14.7	8.56	140	621.5	184	5.45	3.18
UPT 74303	14.8	7.50	135	565.0	190	4.25	2.94
UPT 7440	13.7	7.76	166	576.3	177	3.95	2.84
UPT 75233	14.5	10.20	125	597.8	194	2.60	2.90
Badger PM 118	14.7	10.29	135	409.9	190	2.98	2.87
Mean ± S.D.	14.4 ± 1.37	8.34 ± 3.85	135 ± 46	534.98 ± 185.46	188 ± 18	3.75 ± 2.24	2.99 ± 0.35
Wheat	12.10	4.9	41	306	238	4.5	1.7

All values expressed as average of four replicates.

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**PROXIMATE AND MINERAL COMPOSITION OF SOYBEAN SEEDS GROWN IN NORTH EASTERN REGION**

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The proximate composition of the seeds of 12 varieties of soybean viz., 'Kalitul', 'TS-74-24-2', 'T-49', 'PK-416', 'PK-472', 'PK-453', 'PB-1', 'PK-327', 'MACS-13', 'TS-73-16', 'Bragg' and 'Ankur' suggests that they are good sources of protein (32.35-37.56%), fat (18.76-20.92%), carbohydrates (25.02-31.51%), minerals (4.50-5.46%), and energy (429-440 Kcal/100 g). On ash fractionation, mineral content was found to be in the range of 0.48 to 0.61, 1.56 to 1.92, 0.024 to 0.063, 0.094 to 0.281, 0.352 to 0.733 and 0.0044 to 0.0163% for sodium, potassium, calcium, magnesium, phosphorus and iron respectively. The soybean seeds are rich in sodium, potassium and phosphorus contents.

In recent years, great interest has been shown to cultivate soybean for various uses, mainly on account of its dietetic and industrial importance<sup>1</sup>. Among the grain legumes, soybean by virtue of its high and superior protein and oil contents offers as a potential source of protein for human consumption<sup>2,3</sup>. It is widely used in the preparation of bread, biscuits, cakes, pastries, soups, omelette, sprouts, milk, curd, paneer and other Indian tasty dishes like pulav, dal, idli, chevda, shev, etc<sup>1</sup>. But, it has not been cultivated in North Eastern part of India. An attempt has been made to cultivate different varieties of soybean at the Defence

Research Laboratory, Tezpur. In this report, physico-chemical characteristics of twelve varieties are presented.

Representative samples of the seeds from twelve varieties of soybean namely, 'Kalitul', 'TS-74-24-2', 'T-49', 'PK-416', 'PK-472', 'PK-453', 'PB-1', 'PK-327', 'MACS-13', 'TS-73-16', 'Bragg' and 'Ankur' were obtained from Agriculture Division (DRL, Tezpur). The seeds were washed with distilled water and sun-dried. Standard AOAC<sup>4</sup> methods were followed for the determination of moisture, protein, fat and total minerals. Total carbohydrate contents were determined by difference<sup>5</sup>. Energy was calculated by adding the calories contributed by protein, fat and carbohydrates<sup>6</sup>. Sodium and potassium were quantitated by flame photometry whereas phosphorous was estimated by Allan's<sup>7</sup> method and iron colorimetrically<sup>8</sup>. Calcium and magnesium were analysed according to NIN<sup>9</sup> laboratory techniques.

Proximate analysis of soybean seeds showed that they are good sources of protein and fat, the contents of which ranged from 32.35 to 37.56 and from 18.76 to 20.92 per cent respectively (Table 1). 'PK-472' contained highest protein of 37.56 per cent followed by 37.18, 36.57, 36.20, 35.81, 35.71, 35.45 and 35.36 per cent for 'PB-1', 'TS-73-16', 'PK-327', 'MACS-13', 'Bragg', 'Ankur' and 'T-49' respectively. 'PK-453' contained the lowest protein (32.35 per cent). 'TS-73-16' was found rich in oil content (20.92 per cent) followed by 'PK-416', 'Bragg', 'Ankur', 'PK-453', 'TS-74-24-2' and 'PK-327' containing 20.51, 20.50, 20.36, 20.19, 20.17 and 20.13 per cent respectively, while 'PK-472', contained the lowest of 18.76 per cent (Table 1.) Protein and fat contents of the soybean seeds were found in conformity with the observations of earlier workers reported from Haryana<sup>10</sup>, Madhya Pradesh<sup>11</sup>, Maharashtra<sup>12</sup> and Uttar Pradesh<sup>13</sup>, while high protein and

TABLE 1. PROXIMATE ANALYSIS OF SOYBEAN SEED

Variety	Moisture (%)	Crude fat (%)	Crude protein (%)	Ash (%)	Carbohydrates (%)	Energy (cal/100 g)
Kalitul	11.60 ± 0.79	19.27 ± 1.11	33.64 ± 2.08	4.50 ± 0.24	30.99 ± 0.41	432 ± 3.00
TS-74-24-2	11.19 ± 1.06	20.17 ± 1.81	34.33 ± 1.38	5.36 ± 0.38	28.94 ± 1.11	435 ± 6.50
T-49	11.01 ± 0.08	19.86 ± 1.44	35.36 ± 2.41	4.99 ± 0.18	28.79 ± 1.24	435 ± 8.50
PK-416	11.03 ± 0.42	20.51 ± 0.45	33.50 ± 2.90	4.58 ± 0.41	30.39 ± 1.57	440 ± 0.01
PK-472	11.19 ± 0.91	18.76 ± 2.01	37.56 ± 0.89	5.06 ± 0.00	27.45 ± 2.03	429 ± 6.21
PK-453	10.83 ± 0.47	20.19 ± 0.42	32.35 ± 0.62	5.11 ± 0.08	31.51 ± 0.64	437 ± 4.00
PB-1	10.59 ± 0.83	19.34 ± 0.04	37.18 ± 2.02	4.79 ± 0.35	28.07 ± 2.92	435 ± 5.00
PK-327	10.12 ± 0.55	20.13 ± 0.39	36.20 ± 0.48	5.46 ± 0.37	28.08 ± 1.05	438 ± 1.5
MACS-3	10.29 ± 0.36	19.26 ± 0.89	35.81 ± 2.65	5.14 ± 0.22	29.48 ± 1.62	434 ± 2.5
TS-73-16	12.32 ± 0.82	20.92 ± 0.09	36.57 ± 2.64	5.17 ± 0.11	25.02 ± 2.49	435 ± 4.50
Bragg	10.82 ± 0.38	20.50 ± 0.97	35.71 ± 3.13	4.88 ± 0.06	28.02 ± 3.61	440 ± 6.50
Ankur	10.79 ± 0.09	20.36 ± 0.49	35.45 ± 3.03	5.44 ± 0.13	27.95 ± 2.49	437 ± 2.00

Each value is the mean of three replications with ± SE.

TABLE 2. MINERAL COMPOSITION OF SOYBEAN SEEDS\*

Variety	Sodium (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Phosphorus (%)	Iron (%)
Kalitul	0.54	1.70	0.034	0.166	0.575	0.0064
TS-74-24-2	0.53	1.75	0.062	0.270	0.622	0.0070
T-49	0.48	1.56	0.043	0.255	0.634	0.0059
PK-416	0.53	1.69	0.036	0.214	0.586	0.0163
PK-472	0.49	1.92	0.039	0.281	0.733	0.0061
PK-453	0.48	1.85	0.047	0.189	0.532	0.0048
PB-1	0.54	1.75	0.035	0.094	0.512	0.0047
PK-327	0.55	1.81	0.063	0.136	0.621	0.0051
MACS-13	0.55	1.74	0.062	0.134	0.611	0.0049
TS-73-16	0.61	1.91	0.046	0.151	0.630	0.0067
Bragg	0.54	1.80	0.024	0.190	0.352	0.0044
Ankur	0.55	1.69	0.035	0.251	0.525	0.0068

\*Average of three replicates

fat contents were reported from Gujarat<sup>14</sup>. These workers also observed variations in protein and oil contents under different location and climatic conditions<sup>11</sup>. The highest mineral content was found for 'PK-327' and lowest for 'Kalitul' (Table 1). While carbohydrate content was found highest (31.57 per cent) for 'PK-453' and lowest for 'Kalitul' (Table 1), while carbohydrate content was found highest (31.51 per cent) for 'PK-453' and lowest (25.02 per cent) for 'TS-73-16', 'PK-416' and 'Bragg' provided the highest energy 440 Kcal/100 g of bean and 429 Kcal/100 g for 'PK-472' (Table 1).

The data on mineral composition are presented in Table 2. Mineral analysis of soybean seeds revealed that they are good sources of sodium, potassium, phosphorus and magnesium ranging from 0.48 to 0.61, 1.56 to 1.92, 0.352 to 0.753 and 0.094 to 0.281 per cent respectively. They are rich in sodium, potassium and phosphorus. The calcium and iron were found at low level, ranging from 0.024 to 0.063 and 0.0044 to 0.0163 per cent. However, the present results are slightly higher for sodium and iron but marginally low for potassium and calcium in comparison to the results of Sood *et al.*<sup>10</sup>. Low values for sodium, potassium, calcium, magnesium, phosphorus and iron were also reported by other workers<sup>1</sup>. These variations in the mineral composition might be due to variations in the variety, fertilizer treatment, soil quality and agroclimatic conditions, as all these factors are known to influence mineral composition quite considerably<sup>15</sup>.

Tables 1 and 2 show that there are considerable differences in the levels of all the biochemical constituents among all varieties. It may be due to varietal and agroclimatic factors<sup>11,15</sup>. Not a single variety was found with the highest level for both proteins and fat. However, 'PK-327', 'TS-73-16', 'Bragg' and 'Ankur' were found to be good sources of proteins and fat.

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## EFFECT OF STORAGE AND PRE-TREATMENTS ON POTATO CHIP COLOUR QUALITY

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A study was conducted, on potato varieties, 'K. Jyoti', 'K. Badshah' and 'K. Chandramukhi' to investigate the effect of storage temperature and various pre-treatments of potato slices on the chip colour quality. The varieties were stored at  $5 \pm 2^\circ\text{C}$ ,  $11 \pm 2^\circ\text{C}$  and  $23.5\text{--}37^\circ\text{C}$  (ambient conditions). Initial analysis showed that 'K. Jyoti' variety was best for chip colour. During storage under ambient conditions 'K. Chandramukhi' retained very good colour quality of fried chips upto 10 weeks while other varieties were assessed lower in colour quality. None of these varieties were found suitable for chips after storage at  $5 \pm 2^\circ\text{C}$  and  $11 \pm 2^\circ\text{C}$  due to poor colour quality even after reconditioning at  $20^\circ\text{C}$  for one month. Among the various pre-treatments given to slices of stored potato, blanching in water at  $80^\circ\text{C}$  for 1 min was found to be optimum and was very effective in improving the colour of chips while the chips obtained after treating the slices with absolute ethanol for 5 min followed by dipping in 0.2% potassium metabisulphite solution for 5 min was best among the chemical pre-treatments and assessed next in colour to that obtained after blanching.

Potato is the major vegetable crop of Punjab. Potato chip is the main snack food and many industries are going for production of chips in India. Being a perishable crop, tubers can be stored for short periods under ambient conditions in tropical countries like India. Visual colour is major quality criterion for potato chips which is also related with texture and flavour of the product. The maintenance of desirable colour is the most important problem<sup>1</sup> which is mainly dependent upon the environment of storage. A number of studies<sup>2,10</sup> have been conducted on the various factors affecting the chip colour quality. It is generally confirmed<sup>2,5,9</sup> that although the storage temperature is an important factor, and varietal characteristics are also of prime importance in changes in the bio-chemical properties of potatoes affecting their colour during storage, no such systematic report is available on the chip colour quality of Indian varieties. In the light of above, the present investigation was, therefore, undertaken to study the effect of storage temperature and pre-treatments of slices on the chip colour quality of potato varieties.

Three commercially grown varieties of potato namely, 'K. Jyoti', 'K. Badshah' and 'K. Chandramukhi' were selected for study and specific gravity was determined by the ratio

of weight in air and loss of weight in water immediately after harvest. The initial chip colour quality of the tubers was evaluated after one week of harvesting. For chipping, six tubers were selected (two replicates) for each analysis. The tubers were cut into halves and sliced longitudinally from stem to bud end to get four slices (1.4 mm thick) from each tuber, totalling 24 in number. The slices were washed for about one minute in tap water and excess water was drained off by wiping with tissue paper. The interval between slicing to frying did not exceed 5 min. The slices were fried in refined cotton seed oil (Ginni) in thermostatically controlled fryer (ITT-deluxe-2) to a moisture content of less than two per cent. The oil to slices ratio was 10:1 in fryer. The frying time varied between 1 min 45 sec and 2 min 30 sec at a temperature of  $170 \pm 10^\circ\text{C}$ . The initial oil content of fried chips was measured by Soxhlet extraction method. The colour of chips was compared visually with the Fry Colour Standards Chart<sup>11</sup>.

The storage of tubers was carried out at three different temperatures i.e.,  $5 \pm 2^\circ\text{C}$  (RH 60-85 per cent),  $11 \pm 2^\circ\text{C}$  (RH 60-85 per cent) and  $23.5\text{--}37^\circ\text{C}$  (RH 33-65 per cent). The variety 'K. Jyoti' stored at  $5 \pm 2^\circ\text{C}$  for 6 months was used to study the effect of pre-treatments on chip colour quality. The pre-treatments including blanching in water for different time-temperature combinations, different concentrations of absolute alcohol and potassium metabisulphite (KMS) solution in combination and alone, calcium chloride solution (0.25 per cent), sodium chloride solution (5.0 per cent) and a boiling solution containing 6.131 g sodium citrate, 1.230 g sodium bisulphite and 0.999 g phosphoric acid per litre for 1 min, were given to slices prior to frying. The tubers from all the varieties were oval in shape with white flesh.

The specific gravity of tubers, yield, oil content and initial chip colour index of the varieties are given in Table 1. The specific gravity of the 'K. Badshah' was highest and while that of 'K. Jyoti' was lowest. The yield of chips was highest in 'K. Badshah' showing its direct relation with specific gravity while oil uptake was maximum in 'K. Jyoti' which may be due to its lowest specific gravity. The variety 'K. Jyoti' had best initial chip colour while 'K. Chandramukhi' had lowest colour index.

It is evident from Table 2 that during storage under ambient conditions, 'K. Chandramukhi' improved in chip colour index showing its good stability. 'K. Jyoti' which had better

TABLE 1. SPECIFIC GRAVITY, YIELD, OIL CONTENT AND INITIAL CHIP COLOUR INDEX OF POTATO VARIETIES

Variety	Sp. gr.	Yield (%)	Oil (%)	Chip colour index
K. Jyoti	1.0575	26.04	53.39	64
K. Badshah	1.0665	31.05	42.21	50
K. Chandramukhi	1.0600	27.10	46.30	37

TABLE 2. EFFECT OF STORAGE FOR DIFFERENT WEEKS UNDER AMBIENT CONDITIONS ON THE CHIP COLOUR INDEX VALUES

Variety	Initial	2Wk*	4Wk	6Wk	8Wk	10Wk
K. Jyoti	64	68	54	50	54	50
K. Badshah	50	52	54	54	50	50
K. Chandramukhi	57	55	55	64	58	55

initial quality deteriorated in colour during storage, while 'K. Badshah' retained almost same colour score during storage of 10 weeks. The samples stored at  $11 \pm 2^\circ\text{C}$  (Table 3) were analysed at 1, 3 and 5 month intervals. There were slight changes in colour index of all the samples upto one month storage. The deterioration in quality was slow and continued upto the storage period of 5 months. This revealed that all these varieties were sensitive to this temperature and accumulated substances (reducing sugars) responsible for discolouration of chips as confirmed by earlier studies<sup>6,12</sup>. The samples stored at  $5 \pm 2^\circ\text{C}$  for 4 months resulted in very poor colour quality chips as indicated by the lowering of colour quality chips as indicated by the lowering of colour index from 64 to 30, 50 to 30 and 37 to 25, respectively in 'K. Jyoti', 'K. Badshah' and 'K. Chandramukhi'. The conditioning of samples at  $20^\circ\text{C}$  for 1 month after storage at  $5 \pm 2^\circ\text{C}$  for 4 months did not improve the colour index, showing the poor reconditioning ability of these varieties.

The effect of various pre-treatments of potato slices on the chip colour is given in Table 4. It was found that blanching greatly improved the chip colour. Blanching at  $80^\circ\text{C}$  for 1 min was found to be optimum for improving the colour of chips because beyond this time-temperature combination, there was no further improvement in the colour of fried chips. Among the chemical treatments, shaking of slices in absolute ethanol for 5 min followed by dipping in 0.2 per cent potassium metabisulphite solution for 5 min was found to be the best chemical treatment. Reducing the concentrations of ethanol with same concentration of potassium metabisulphite lowered the chip colour index. Ethanol alone was also effective but to lesser extent. The treatment with 0.25 per cent calcium chloride at  $90^\circ\text{C}$  for 3 min also improved the colour to some extent as reported by Patton<sup>13</sup>, while all the other treatments had negligible effect in improving the colour.

TABLE 3. EFFECT OF STORAGE FOR DIFFERENT MONTHS AT  $11 \pm 2^\circ\text{C}$ , RH 60-85 PER CENT ON THE CHIP COLOUR INDEX VALUES

Variety	Initial	1 Month	3 Months	5 Months
K. Jyoti	64	55	48	30
K. Badshah	50	45	44	35
K. Chandramukhi	37	35	35	32

TABLE 4. EFFECT OF PRE-TREATMENTS ON THE POTATO CHIP COLOUR (K. JYOTI.)

Treatment	Colour index
Control	25
Blanching $80^\circ\text{C}$ , 1 min (optimum)	70
Shaking with absolute ethanol (5 min) and dipping in 0.2% KMS solution (5 min).	65
Shaking with 50% ethanol (5 min) and dipping in 0.2% KMS solution (5 min).	60
Shaking with absolute ethanol (5 min)	52
Dipping in 0.25% calcium chloride solution at $90^\circ\text{C}$ (3 min)	54
Shaking with absolute ethanol for 5 min and dipping in 0.1% KMS solution (5 min).	54

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## QUALITY OF MOZZARELLA CHEESE PRODUCED BY USING DIFFERENT MILK CLOTTING ENZYMES

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**Mozzarella cheese was prepared from buffalo milk standardized to 4% fat using microbial Meito and Modilase rennets and calf rennet as control. Modilase produced better quality cheese than Meito rennet. Good quality Mozzarella cheese could be prepared by microbial rennet. Body and texture of the cheese made by using Modilase rennet were similar to the cheese made by using calf rennet. Fat and total solids recovery was more in the cheese made by using calf rennet whereas yield and melting were better in the cheese prepared by microbial rennet. Stretching was best in the cheese made by using calf rennet.**

Great attention has been paid in recent years to develop a suitable substitute for calf rennet. A number of proteolytic enzymes of plant, animal, bacterial and fungal origin have been investigated<sup>1</sup>. Coagulants obtained from fungi are mostly too proteolytic as compared to calf rennet and other clotting enzymes<sup>2</sup>. Some workers have found practically no difference between cheese made with microbial rennet and those made with calf rennet<sup>3</sup>. Others<sup>1,2</sup>, however, noted that cheese made with microbial enzymes often ripened differently from those made with calf rennet and had bitter flavour as well as body defects attributed to the type and extent of proteolytic activity during long ripening. These limitations would not be serious in a cheese like Mozzarella since it is used before its rheological characteristics are altered extensively by ripening agents.

The selection of a suitable microbial enzyme has become all the more important because of the ban on import of calf rennet to our country. Therefore, the relative merits of three coagulating enzymes, viz. Calf, Meito and Modilase rennets in the production of Mozzarella cheese were studied.

**Rennets:** Calf rennet powder was obtained from the Christen Hansen's Laboratory, Denmark. Microbial Meito rennet powder was procured from Meito Sangyo Co., Ltd., Tokyo, Japan. The rennet was derived from the fungus *Mucor pusillus* var. Lindt. Microbial Modilase liquid rennet, derived from *Mucor miehei* was obtained from Chr. Hansen's Laboratory, Wisconsin, USA.

**Cheese manufacture:** Standardized (4 per cent fat) pasteurised buffalo milk was cooled to  $34 \pm 1^\circ\text{C}$ . Two per cent starter culture consisting of *S. thermophilus* and *L. bulgaricus*

in the ratio of 1:1 was added. Mozzarella cheese was manufactured as per the method described by Ghosh and Singh<sup>4</sup> using the three enzymes. In order to coagulate 100 l milk in about 30 min, the quantity of rennets required were 2.5 g, 1.0 g and 15.0 ml in case of Calf, Meito and Modilase respectively.

**Analysis:** Fat, solids-not-fat (SNF), total solids (TS) and titratable acidity (TA) of milk were determined as per the Indian Standards Method<sup>5</sup>. Cheese samples were evaluated for appearance, body and texture and flavour by a selected panel of trained judges using an 18 point score card developed by Duthie *et al.*<sup>6</sup> The sensory scores obtained were analysed determined as per the method outlined in Laboratory Manual<sup>7</sup>. Titratable acidity was measured by the method recommended by AOAC<sup>8</sup>. Meltability and stretchability tests were carried out as described by Ghosh and Singh<sup>4</sup>.

The standardised milk was ripened for about 90 min after inoculation of starter culture prior to microbial renneting, though a ripening time of 1 hr after inoculation of starter culture prior to renneting was used by Demott<sup>9</sup> and Hutkin *et al.*<sup>10</sup>. Since the rate of acidity development in buffalo milk was considerably slower than in cow milk, a longer ripening period was used in this study. Further, the higher acidity attained due to longer ripening of milk, was found advantageous as the quantity of microbial rennets could be kept low. This reduces losses of milk solids during manufacture<sup>11</sup> and increases the shelf life of the product in addition to reducing the cost of production. As the microbial rennets have higher proteolytic activity, the quantity required could be lower than for calf rennet<sup>2</sup>. In addition, proteolytic degradation of the product during storage<sup>12</sup> would be less when lower levels of rennets are used.

**Sensory evaluation:** Calf, Meito and Modilase rennets had significant ( $P < 0.01$ ) effect on the body and texture score of the cheese (Table 1) while for flavour and appearance, it was not significant ( $P < 0.05$ ). The difference in the body and texture score between the cheese made by using calf and meito rennets was significant ( $CD = 0.26$ ). Similar was the case

TABLE 1. SENSORY QUALITY (SCORES) OF MOZZARELLA CHEESE

Enzymes	Flavour	Body & texture	Appearance
Calf	9.38	4.50	2.92
Meito	9.33	4.09	2.76
Modilase	9.35	4.50	2.83
C.D.	0.27	0.26	0.15

Maximum scores: Flavour-10, Body & texture-5, Appearance-3.

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in the cheese made from meito and modilase rennets. No significant ( $P < 0.05$ ) differences on body and texture scores were observed in the cheese made by using calf and modilase rennets.

The body and texture of microbial rennet cheese was relatively softer than that of calf rennet though the difference between calf and modilase was statistically not significant. This softening effect could be due to differences in proteolytic property of microbial rennets and greater retention of moisture. Of the two microbial rennets used, modilase was found slightly superior to meito rennet on the basis of sensory properties of cheese.

**Physico-chemical characteristics:** The moisture content of the cheese varied significantly ( $P < 0.01$ ) when different coagulating enzymes were used for cheese making. Cheese made using modilase had maximum moisture retention (Table 2). The difference in moisture content among the three samples was significant ( $CD=1.10$ ). It has been reported that the moisture content of the cheese decreased with increase in the amount of rennet used. In the present study, yield of the cheese made from the three rennets varied slightly. The maximum yield was obtained by the use of modilase whereas the minimum was in case of meito rennet. However, the recovery of fat (88.76 per cent) and TS (53.35 per cent) was comparatively more in the cheese made by using calf rennet than meito or modilase. High recovery with calf rennet was also observed by Quarne *et al.*<sup>13</sup>. Though the recovery was more, the yield was maximum in cheese made by using modilase rennet. This was due to the higher moisture retention of the curd. The relatively greater loss of fat and TS in case of microbial rennets may be due to the relatively softer curd and higher proteolytic activity of the rennet. The acidity development was almost identical in all the cases. A slightly higher acidity in the cheese from calf rennet could be due to the lower moisture retention and higher casein retention in the cheese. The above observations indicate that modilase is better than meito in the production of Mozzarella cheese because of the better yield, fat and TS recovery.

TABLE 2. YIELD AND PHYSICO-CHEMICAL PROPERTIES OF CHEESE PREPARED FROM CALF, MEITO AND MODILASE ENZYMES

Attributes	Calf	Meito	Modilase
	Mean $\pm$ S.D	Mean $\pm$ S.D	Mean $\pm$ S.D
Yield (%)	15.40 $\pm$ 0.15	15.32 $\pm$ 0.11	16.05 $\pm$ 0.27
Fat recovery (%)	88.76 $\pm$ 0.45	86.60 $\pm$ 0.41	87.74 $\pm$ 0.72
TS recovery (%)	53.35 $\pm$ 0.12	51.75 $\pm$ 0.22	52.69 $\pm$ 0.17
Moisture (%)	50.96 $\pm$ 0.36	52.13 $\pm$ 0.25	53.49 $\pm$ 0.28
Fat (%)	23.05 $\pm$ 0.21	22.61 $\pm$ 0.22	21.88 $\pm$ 0.17
Total acidity (%)	0.56 $\pm$ 0.02	0.52 $\pm$ 0.03	0.54 $\pm$ 0.01
Melting (ratio)	2.87 $\pm$ 0.38	3.28 $\pm$ 0.04	3.39 $\pm$ 0.37
Stretching (score)	5/5	4.5/5	4.8/5

The microbial rennets had significantly ( $P < 0.01$ ) higher melting than calf rennet. Of the two microbial rennets, modilase was rated high in this respect, though the difference was not statistically significant ( $CD=0.225$ ). Based on melting characteristics, the rating is modilase  $>$  meito  $>$  calf rennet. Higher melting in the cheese from microbial rennets could be due to higher proteolysis and higher moisture content.

The stretching characteristics of the cheese made by using meito rennet were slightly inferior to the other cheeses. The best stretching was observed in the cheese made by using calf rennet. This may be due to better rennet action on calcium and full conversion of di-calcium paracaseinate to monocalcium paracaseinate<sup>14</sup>.

The study shows that a good quality Mozzarella cheese can be prepared from buffalo milk by using microbial rennet.

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## STUDIES ON QUALITY OF PANEER

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Yield (18.24%) and total milk solids (53.16%) recovered were found to be highest in paneer prepared from buffalo milk. Buffalo milk paneer was graded as "Excellent" and scored highest (93.33) for its overall sensory qualities. In the case of skim milk paneer, moisture, protein and ash were found to be maximum. The values obtained for hardness, gumminess and chewiness in case of skim milk paneer were highest while lowest values were obtained for cohesiveness and springiness. The highest and significant correlation coefficient of 0.85 was between springiness and fat whereas the lowest and non-significant correlation coefficient of 0.11 was between cohesiveness and fat.

The studies related to sensory quality and chemical composition of paneer have been reported by several workers<sup>1,4</sup>, but only limited data have been published on textural quality of paneer. Efforts were made to study the various quality aspects of paneer related to the types of milk used in its preparation.

Cow milk standardised to 4.5 per cent fat and 8.5 per cent solids-not-fat and buffalo milk to 6.0 per cent fat and 9.5 per cent solids-not-fat were used. Paneer was also prepared from skim milk obtained from standardised cow milk, adjusted to 0.1 per cent fat. The method suggested by

Rao *et al.*<sup>5</sup> was followed for preparation of paneer. Samples of paneer prepared from different types of milk were compared with respect to sensory and textural qualities.

The fat contents of milk, skim milk and cream were determined by the Gerber's method and total solids of milk were determined by standard gravimetric method<sup>6</sup>. The paneer samples were analysed for total solids and protein as per the method described in AOAC<sup>7</sup>. The fat and ash contents of paneer were determined as per BSI<sup>8</sup>.

Sensory evaluation and grading of paneer were done by using modified score cards as suggested by Patil and Gupta<sup>9</sup>. The textural profile analysis of paneer was done by using Universal Testing Machine (Instron Model 1000) as per the method suggested by Bourne *et al.*<sup>10</sup>.

Comparative attributes of paneer prepared from different types of milk are given in Table 1.

*Sensory evaluation:* The paneer prepared from buffalo milk secured maximum score and paneer from skim milk had minimum score with respect to all the parameters of sensory qualities (Table 2). The maximum sensory score awarded to buffalo milk paneer might be due to its higher fat and low protein contents. These results are in agreement with those of Bhattacharya *et al.*<sup>1</sup>. On the basis of sensory score, the paneer samples were graded. Excellent grade was awarded to all the quality attributes of paneer prepared from buffalo milk whereas cow milk paneer and skim milk paneer were graded 'good'.

*Textural qualities:* In comparison to paneer prepared from cow and buffalo milk, skim milk paneer showed higher values for hardness (3.00 kg) gumminess (0.86 kg) and chewiness (1.36 kg) and lower values for cohesiveness (0.27), and springiness (1.50 cm). The maximum hardness in case of skim

TABLE 1. CHEMICAL COMPOSITION OF PANEER PREPARED FROM DIFFERENT SOURCES OF MILK\*

Source of paneer	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Yield (%)	Total solids recovered (%)
Skim milk	62.14	4.00	27.48	1.80	9.85	42.17
Cow milk	55.26	24.15	18.43	1.60	14.14	46.77
Buffalo milk	53.00	28.22	16.42	1.50	18.24	53.16

\*Means from 4 trials.

TABLE 2. SENSORY SCORE AND TEXTURAL CONDITION OF THE PANEER PREPARED FROM DIFFERENT SOURCES OF MILK\*

Source of paneer	Sensory attributes					Textural attributes				
	Colour & appearance	Body & texture	Flavour	Overall score	Grading	Hardness (kg)	Cohesiveness	Gumminess (kg)	Springiness (cm)	Chewiness (kg-cm)
Skim milk	12.50	29.70	42.62	84.87	Good	3.00	0.27	0.86	1.50	1.36
Cow milk	13.10	31.50	44.34	88.97	Good	2.11	0.28	0.58	2.10	1.25
Buffalo milk	14.30	33.00	46.03	93.33	Excellent	1.93	0.29	0.52	2.05	1.07

\*Means from 4 trials

milk paneer may be due to low fat, high protein and ash contents.

*Correlation studies:* Statistical analysis of relevant data by using multiple regression correlation was done. Protein and ash were found to have positive correlation with gumminess and chewiness. Positive correlation was also observed of cohesiveness and springiness related to fat. The remaining correlations were found to be negative.

The correlation coefficients were significant in case of hardness, gumminess and springiness related to protein and fat whereas all other correlations were found to be non-significant. Higher and significant correlation coefficient of 0.85 was obtained between fat and springiness of paneer.

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## EFFECT OF BLENDING SOYMILK WITH BUFFALO MILK ON QUALITIES OF PANEER

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The possibility of blending soymilk with buffalo milk for obtaining good quality paneer has been examined. Soaking soy dhal in sodium bicarbonate was preferred by consumer panel over other treatments. Addition of soymilk to buffalo milk up to 20% had no adverse effect on quality of paneer and resembled that of milk paneer in taste, colour and springiness. However, the paneer prepared by blending soymilk showed higher protein content.

Paneer is obtained by the acid precipitation of cow milk, buffalo milk or combination thereof at higher temperatures. Precipitation involves the formation of large structural aggregates of proteins, in which milk fat, other colloidal and the soluble solids are entrained with whey.

Coagulation of soymilk, results into a white, soft gelatinous mass. The product has bland taste, unique body and texture resembling paneer obtained from milk<sup>1</sup>. Thus, it can serve as a substitute for milk paneer and can also be a cheaper source of quality proteins. Vijayalakshmi and Vaidehi<sup>2</sup> prepared an acceptable product from the coagulum obtained by precipitation of soymilk or its combination with other milk. Consequently, the present investigation was undertaken to see the effect of blending soymilk with buffalo milk on qualities of paneer.

Buffalo milk and soybean (var. 'PK-472') were obtained from the Dept. of Animal Husbandry and Dairying and Soybean Research Centre, Marathwada Agricultural University, Parbhani respectively, for the present investigation.

Soy milk was prepared by soaking soy dhal in 0.06 M sodium bicarbonate solution (1:3 w/v) for 14–16 hr. Dhal was washed with fresh water and blanched for 40–45 min in boiling water, then ground in a mixer with hot water. One hundred g of dhal per litre of water was used for grinding. The resulting suspension was filtered through double layered muslin cloth and filtrate was boiled for 10 min with continuous stirring to prevent sticking of solids and scorching. The soymilk of 2.6 per cent fat and 10.20 per cent total solids, so obtained was blended with standardised buffalo milk of 6.0 per cent fat and 9.0 per cent SNF at the levels of 0.0, 20, 30, 40 and 50 per cent. Using this blend as a base, the paneer was prepared as per the procedure given by Bhattacharya *et al.*<sup>3</sup>. The paneer, so obtained was used to assess its

qualities. The yield of paneer was recorded by weighing the paneer block after pressing, but prior to dipping in chilled water. Sensory evaluation of paneer was carried out using 9-point hedonic scale. Textural qualities of paneer were determined by Instron Universal Testing Machine (Model-1000) as per the method suggested by Bourne<sup>4</sup>.

Fat content of milk (soya and buffalo) was determined by ISI method<sup>5</sup>. The moisture content was determined as per the method cited by Atherton and Newlander<sup>6</sup>. Protein and ash contents of paneer were estimated by A.O.A.C. method<sup>7</sup>. pH was determined by digital pH meter using the method of Arora<sup>8</sup>.

Paneer was cut into cubes of approximately 2.54×2.54×1.27 cm size and was presented to the judges for sensory evaluation with respect to colour and appearance, body and texture, flavour and taste.

*Soaking treatment of soybean:* The effect of soaking of soybean on yield and sensory qualities of paneer is given in Table 1. The highest yield of paneer was found in case of T<sub>3</sub> treatment followed by T<sub>1</sub> and T<sub>2</sub>. The increase in yield of final product by incorporation of calcium chloride might be due to higher precipitation of proteins and phosphates<sup>9</sup>. The colour and appearance score was highest (7.13) in treatment T<sub>2</sub> followed by T<sub>1</sub> and T<sub>3</sub>, whereas the score for body and texture flavour and taste was found to be maximum in treatment T<sub>2</sub> and minimum in treatment T<sub>3</sub>. The increase in score of flavour and taste might be due to reduction of beany flavour of soy milk when soaked in 0.06 M sodium bicarbonate solution. The overall acceptability of the product by the consumer panel was highest for T<sub>3</sub> treatment. Therefore, the product obtained by T<sub>3</sub> treatment was selected for further studies.

*Effect of levels of soymilk:* The yield of paneer decreased with increase in levels of soymilk in the blend<sup>10</sup>. It decreased from 19.04 per cent in control to 14.30 per cent in paneer samples containing 50 per cent soymilk (Table 2). There is decrease in the score of all the parameters of sensory qualities

TABLE 1. EFFECT OF SOAKING TREATMENT ON YIELD AND SENSORY SCORE OF PANEER

Soaking treat	Yield (%)	Colour & appearance	Body & texture	Flavour	Taste
T <sub>1</sub>	16.21	7.04	6.53	6.12	6.80
T <sub>2</sub>	14.4	7.13	7.72	6.60	6.96
T <sub>3</sub>	18.13	6.72	6.72	6.56	6.52
SE	± 0.061	± 0.112	± 0.053	± 0.080	± 0.084
CD at 5%	0.177	0.325	0.153	0.233	0.245

T<sub>1</sub>: Soybeans soaked in plain water T<sub>2</sub>: Soybeans soaked in sodium bicarbonate solution of 0.06M and T<sub>3</sub>: Soybeans soaked in 0.06 M sodium bicarbonate solution + addition of 0.02% calcium chloride during precipitation.

TABLE 2. EFFECT OF BLENDING SOYMILK WITH BUFFALO MILK ON YIELD &amp; SENSORY SCORE OF PANEER

Buffalo milk (%)	Soy milk (%)	Yield (%)	Score			
			Colour & appearance	Body & texture	Flavour	Taste
100	0	19.04	8.43	7.77	2.61	7.85
80	20	17.05	7.50	7.37	7.21	7.38
70	30	15.48	6.93	6.90	6.84	6.77
60	40	14.94	6.28	6.64	6.38	6.24
50	50	14.30	5.08	6.27	5.57	5.54
SE		+0.079	+0.144	+0.068	+0.104	+0.109
CD at 5%		0.229	0.419	0.198	0.301	0.316

such as colour, appearance, body and texture, flavour and taste with the increase in soymilk in blend. The highest score was obtained in case of paneer prepared from buffalo milk alone and the lowest was in case of paneer prepared from blend containing 50 per cent soymilk. Abou and Ella<sup>11</sup> have also reported similar trend with 5.0 per cent soymilk which improved the sensory qualities of Domiati cheese, but further increase in levels of soymilk decreased the sensory qualities. However, our product was quite acceptable to the consumer panel up to 20 per cent of soymilk in blend.

**Textural qualities:** The average values for textural qualities of paneer by Instron Universal Testing Machine were calculated from graphical representation for hardness, cohesiveness, springiness and chewiness (Table 3). The increase in levels of soymilk in blend (from 0.0 to 20 per cent) decreased the values of hardness from 1.74 to 1.56 kg. But decrease was found to be statistically non-significant. Thus, it indicates that fortification of soymilk up to 20.00 per cent did not affect the hardness of final product, as well as other textural qualities.

**Chemical qualities:** Table 4 indicates that there is an increase in moisture content of paneer with increase in per cent of soymilk in blend. Increase in soymilk ratio increases the moisture content also<sup>12</sup>. The fat per cent of paneer

TABLE 3. EFFECT OF BLENDING SOYMILK WITH BUFFALO MILK ON TEXTURAL QUALITIES OF PANEER

Buffalo milk (%)	Soy milk (%)	Hardness (kg)	Cohesiveness	Springiness (cm)	Chewiness (kg-cm)
100	0	1.74	0.24	2.64	1.10
80	20	1.56	0.26	2.73	1.08
70	30	1.60	0.25	2.64	1.06
60	40	1.60	0.24	2.87	1.08
50	50	1.68	0.25	2.79	1.10
SE		+0.098	+0.019	+0.072	+0.070
CD at 5%		NS	NS	NS	NS

TABLE 4. EFFECT OF BLENDING SOYMILK WITH BUFFALO MILK ON PROXIMATE COMPOSITION OF PANEER

Buffalo milk (%)	Soy milk (%)	Moisture (%)	Total solids (%)	Fat (%)	Protein (%)	Ash (%)	pH
100	0	49.67	50.33	25.50	16.15	1.56	6.00
80	20	50.93	49.07	23.67	16.71	1.58	5.91
70	30	53.00	47.00	22.18	17.46	1.66	5.87
60	40	53.60	46.40	20.01	19.37	1.75	5.82
50	50	54.60	45.40	18.33	19.81	1.68	5.78
SE		+0.457	+0.457	+0.318	+0.211	+0.021	+0.010
		1.329	1.324	0.921	0.611	0.060	0.029
			Fat		TS		
	Soy milk		2.6%		10.20%		
	Buffalo milk		6.0%		15%		

samples decreased from 25.50 to 23.67, when blended with 20 per cent soymilk due to dilution of buffalo milk. The effect on chemical qualities of final product was found to be statistically significant.

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## CHEMICAL QUALITY OF SOME MARKETED INDIGENOUS MILK PRODUCTS: MAJOR CONSTITUENTS AND MINERAL COMPOSITION OF SHRIKHAND

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Shrikhand collected from five shops of Anand (Gujarat) showed wide variations in total solids, fat, protein, carbohydrates, ash and pH values. The fat varied from 2.0 to 5.0%. All the samples, except one under organised dairy undertaking, did not conform to the minimum limit of 8.5% as prescribed by Bureau of Indian Standards. Wide variations were also observed for different minerals in Shrikhand from various shops. The market samples also showed wide variations in citrate, copper and iron levels.

Shrikhand is a fermented, self-stabilized sugar containing delicacy having low pH. Despite regional preference, this product, in recent years, is becoming more and more popular. The chemical quality of Shrikhand largely depends on the type of milk, recovery of solids in Chakka, moisture retention and level of addition of sugar. There are no mandatory or prescribed standards as yet formulated. The information on major constituents of Shrikhand from market as well as laboratory sample is reported by many workers<sup>1,3</sup>. However, information on minerals either from market or laboratory product is not available. The data on chemical composition will be useful while standardising the product for commercial production.

The market samples of Shrikhand were collected in manufacturer's package/polyethylene bags from places nearby Anand in Gujarat State. One sample was from a commercial organised dairy. The samples were transported in an ice box and stored at 5°C until analysed. The entire 250 g product collected from each five shops, after thorough mixing with stainless steel spatula was used for chemical analysis.

The moisture and total solids were determined by standard gravimetric method with minor modification which involved addition of 5 ml of hot distilled water to disperse the curd particles uniformly. The fat content was determined as modified Gerber test<sup>4</sup> after Shrikhand was diluted with distilled water (1:2.5 w/v) and using 80 per cent sulphuric acid (sp.gr. 1.73 at 20°C).

The protein content was determined by micro-Kjeldahl method<sup>5</sup>. Carbohydrate content was calculated by difference. Calcium content was determined by the method of Bureau of Indian Standards<sup>5</sup>, ash content was estimated by the method used for Chhana<sup>6</sup>, phosphorus by the method of Fiske and Subba Row<sup>7</sup>, citrate by modified colorimetric

method of White and Davies<sup>8</sup> and chloride by the semi-micro mercurimetric titration method of Roy and Yadav<sup>9</sup>, with minor modification. Sodium and potassium concentrations were determined on ash solution in a systronic digital flame photometer (with sodium and potassium filter) Model 121 at constant air pressure of 0.5 kg/cm<sup>2</sup> using regulated indane gas as a source of fuel. Magnesium, copper, iron, and zinc in ash solution were determined with the help of Pye-unichem SP-191 Atomic Absorption Spectrophotometer.

pH of Shrikhand was determined by the method of Kosikowaski<sup>10</sup> for soft cheeses. The data were analysed by employing statistical design.

The average values for major constituents of market samples of Shrikhand are shown in Table 1. The moisture retention was less in all samples except those from an organised dairy. However, the total solids in all cases were above the minimum limit of 58 per cent prescribed by Bureau of Indian Standards. It is a common practice in market trade to prepare Shrikhand from Chakka obtained from buffalo skim milk dahi, which retains less moisture. The range of values observed in present study are in close agreement within ranges in market sample of Shrikhand observed by different workers<sup>2,3</sup>.

The average fat contents in market samples varied from 1.97 to 5.08 per cent. There is no prescribed standard for fat in the product. Low fat content in market samples could be attributed to the differences in the fat content of milk used and fat losses in whey during Shrikhand preparation.

The protein contents varied from 6.82 to 7.20 per cent. A wide range from 3.4 to 15.7 per cent for proteins in market samples of Shrikhand was observed by Sharma and Zariwala<sup>3</sup>.

The carbohydrate levels of market samples ranged from 47.3 to 57.4 per cent. The use of Shrikhand milk for dahi attains higher acidity values than whole milk dahi which permits high levels of addition of sugar in market trade. The ash contents ranged from 0.34 to 0.41 per cent. Sharma and Zariwala<sup>3</sup> also reported similar values for ash contents in market samples of Shrikhand.

The pH values ranged from 3.97 to 4.18. The mineral composition of market samples of Shrikhand is given in Table 2.

Information on major minerals either in market or laboratory made Shrikhand is not available for comparison. Among major minerals under present investigation, the concentration of phosphorus was found to be maximum. It varied from 86.13 to 100.94 mg per 100 g Shrikhand. The calcium and potassium contents ranged from 55.33 to 75.13 mg and 48.71 to 60.69 mg per 100 g respectively. The chloride content showed a narrow variation from 42.90 to 49.56 mg per 100 g. The concentrations of sodium and citrate were found to vary from 23.48 to 32.02 and 15.69 to 32.19 per 100 g

TABLE 1. MAJOR MILK CONSTITUENTS AND pH OF MARKET SHRIKHAND

Shops	Moisture (%)	TS (%)	Fat (%)	Protein (%)	Carbo-hydrates (%)	Ash (%)	pH
1	33.2 ± 0.3	66.7 ± 0.3	1.9 ± 0.4 (2.9)	7.1 ± 0.1 (10.7)	57.2 ± 0.6 (85.7)	0.39 ± 0.01 (0.57)	4.06 ± 0.06
2	32.5 ± 0.6	67.4 ± 0.6	2.4 ± 0.5 (3.6)	7.2 ± 0.01 (10.6)	57.4 ± 0.9 (85.0)	0.40 ± 0.01 (0.60)	4.06 ± 0.04
3	35.2 ± 0.7	64.7 ± 0.7	3.8 ± 0.5 (6.0)	7.1 ± 0.1 (11.0)	53.3 ± 0.9 (82.3)	0.41 ± 0.01 (0.63)	3.97 ± 0.06
4	32.2 ± 1.4	67.7 ± 1.4	4.8 ± 0.3 (7.2)	6.8 ± 0.1 (10.0)	55.4 ± 1.3 (81.8)	0.34 ± 0.01 (0.51)	3.99 ± 0.06
5	40.2 ± 0.5	59.7 ± 0.5	5.0 ± 0.1 (8.5)	6.9 ± 0.1 (11.6)	47.3 ± 0.5 (78.8)	0.41 ± 0.01 (0.68)	4.18 ± 0.03
Mean	34.70 ± 0.70	65.3 ± 0.7	3.6 ± 0.4 (5.65)	7.0 ± 0.1 (10.84)	54.1 ± 0.7 (82.7)	0.39 ± 0.01 (0.60)	4.05 ± 0.05

Figures in parantheses indicate values on dry matter basis.

Mean ± S.E. of 8 samples.

TABLE 2. MINERAL COMPOSITION (MG PER 100 G) OF MARKET SHRIKHAND

Shops	Calcium	Magnesium	Phos-phorus	Citrate	Sodium	Potassium	Chloride	Copper	Iron	Zinc
1	63.5 ± 5.4 (95.3)	12.7 ± 0.8 (19.0)	95.6 ± 4.6 (143.3)	15.6 ± 2.4 (23.5)	31.5 ± 1.1 (50.6)	57.1 ± 2.3 (85.5)	46.6 ± 1.8 (69.8)	0.53 ± 0.14 (0.80)	3.41 ± 0.43 (5.10)	0.73 ± 0.07 (1.09)
2	68.06 ± 2.40 (101.0)	14.5 ± 1.4 (21.5)	100.9 ± 4.4 (150.4)	30.0 ± 3.5 (44.6)	32.0 ± 2.5 (47.4)	60.6 ± 1.7 (90.01)	46.0 ± 1.2 (68.3)	0.48 ± 0.12 (0.71)	1.47 ± 0.23 (2.26)	0.73 ± 0.08 (1.07)
3	66.3 ± 3.2 (102.7)	14.2 ± 1.3 (21.9)	100.1 ± 3.5 (155.1)	32.1 ± 3.3 (50.3)	30.3 ± 1.4 (47.0)	59.7 ± 1.5 (92.24)	42.9 ± 2.4 (66.3)	0.53 ± 0.10 (0.80)	2.03 ± 0.30 (3.11)	0.69 ± 0.03 (1.00)
4	55.3 ± 3.3 (78.2)	11.9 ± 1.1 (17.6)	86.1 ± 4.8 (127.9)	20.4 ± 2.0 (30.4)	23.4 ± 2.0 (34.7)	48.7 ± 2.8 (71.9)	49.5 ± 2.3 (73.1)	0.39 ± 0.58 (0.58)	1.56 ± 0.16 (2.29)	0.67 ± 0.04 (1.00)
5	75.1 ± 3.3 (125.6)	16.8 ± 1.1 (28.1)	94.6 ± 3.2 (158.3)	26.2 ± 1.7 (43.9)	26.5 ± 1.7 (44.5)	59.5 ± 2.9 (99.7)	44.7 ± 2.6 (70.01)	0.17 ± 0.03 (0.28)	0.27 ± 0.02 (0.45)	0.64 ± 0.07 (1.06)
Mean	65.6 ± 3.5 (100.6)	14.0 ± 1.0 (21.6)	95.5 ± 3.4 (147.1)	24.9 ± 3.2 (38.5)	28.8 ± 1.8 (44.8)	57.1 ± 2.3 (87.8)	45.9 ± 1.8 (69.6)	0.42 ± 0.09 (0.63)	1.75 ± 0.28 (2.64)	0.69 ± 0.05 (1.05)

Figures in parantheses indicate values on dry matter basis

Mean ± S.E. of 8 samples.

respectively. Cream addition to skim milk Chakka at the time of Shrikhand preparation showed presence of citrate in market samples. Cream contains a minute quantity of citrate also.

Among the major minerals, the concentration of magnesium was observed to be the lowest being in the range of 11.95 to 16.87 per 100 g of the product.

Among the trace elements, iron was found to be leading from 0.27 to 3.41 mg per 100 g. Zinc and copper ranged from 0.64 to 0.73 and 0.17 to 0.53 mg per 100 g respectively. The values for iron and copper in samples from organised dairy were 0.24 and 0.14 mg/100 g respectively, which are also the values reported by Sharma and Zariwalla<sup>11</sup>.

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**PREPARATION AND SHELF LIFE OF SEMI-DRIED FISH CAKE FROM DHOMA (*OTOLITHUS* SPP.)**

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Semi-dried fish cakes from *Otolithus* spp. prepared at 7 and 10% salt levels were assessed chemically and microbiologically. The shelf life for the two batches were 18 and 24 days respectively. High water activity ( $a_w$ ) and subsequent growth of fungi were found to be the reason for decreased shelf life.

Consequent to urbanisation, the preference of the people shifted from raw fish to ready-to-cook fishery products. Delvalle *et al.*<sup>1</sup> studied the pilot plant production of quick salted fish cakes from different fishes. *Haplochromis* spp.<sup>2</sup>, sea bream<sup>3</sup> and ribbon fish<sup>4</sup> have been used for the preparation of quick salted fish cakes. In this study, an attempt has been made to produce low salted fish cake from *Otolithus* spp. to reduce desalting time and increase rehydration capacity, so that it nearly resembles fresh fish.

Fish mince from the fish was prepared after scaling, beheading and gutting using a Baader 694 deboning machine. The dressed fish was thoroughly washed as the process involved no further washing. The mince was made into two batches, one with 7 and the other with 10 per cent salt and 0.01 per cent sodium propionate was added as a preservative.

After thoroughly mixing in a Habort mixer for 10 min, the mince was placed in a tray, pressed and dried for an initial period of one hour. The set blocks were cut into pieces (2×2½×3 cm), dried in the sun light for 12-14 hr and packed in polythene bags (200 gauge) and stored at room temperature (27-29°C and RH > 75 per cent).

Samples were analysed for chemical composition, microbiological and sensory characteristics during storage. Moisture, fat, ash, total nitrogen and salt (as NaCl) were determined in triplicate by the methods of A.O.A.C.<sup>5</sup>. The water activity calculations were made by the method of Doe<sup>6</sup>. The total bacterial count (TBC) was carried out by the standard plate count method. The method described by Ramachandran and Solanki<sup>7</sup> was followed for rehydration and desalting studies. The storage studies were discontinued when the samples were found to be unacceptable by visual examination.

Fish mince with a moisture content of 80.19 and protein content (N×6.25) of 17.99 per cent was used for the preparation of cakes salted to 7 and 10 per cent levels and drying.

The moisture content decreased to 47.35 and 43.56 per cent with consequent increase in protein content to 65.3 and 59.14 per cent respectively. The final salt contents in dried cakes were 24.37 and 29.94 per cent respectively for 7 and 10 per cent levels of salting.

The storage characteristics of the fish cakes are given in Table 1. The  $a_w$  of 7 per cent salted samples increased during storage, thereby creating a conducive atmosphere for bacterial multiplication which is reflected by the TBC values.

TABLE 1. STORAGE CHARACTERISTICS OF LOW SALTED FISH CAKES

Storage period (days)	Salt (%)	$a_w$ *	Total bacterial count*	Colour/appearance	Odour	Texture (raw)
0	7	0.76	$5.54 \times 10^4$ ( $1.37 \times 10^4$ )	Good (Meaty)	Characteristic	Soft rubbery
0	10	0.79	$3.9 \times 10^2$ ( $1.53 \times 10^3$ )	Good (Meaty)	Characteristic	Soft rubbery
18	7	0.81	Crowded	Fungal colonies seen	Slight loss of characteristics	Soft rubbery
24	10	0.77	$2.87 \times 10^3$	Salty ppt. fungal colonies seen	Characteristic	Slight hard
35	7	0.82	—	Fungal colonies	—	—
35	10	0.77	Crowded	Salt ppt. fungal colonies seen	—	—

Figures in parentheses indicate TBC values before salting

\*Mean of two experiments

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The samples became crowded with bacterial colonies in 18 days. In the case of 10 per cent salted cakes, crowding with bacterial colonies was noticed at the end of 35 days. Fungal colonies were noticed on 18 and 24 days respectively in the two batches.

In spite of the shorter shelf life of 7 per cent salted cakes, they were preferred to those with 10 per cent salt (Table 1) due to their better appearance and texture. Desalting studies with the 7 per cent salted cakes showed that within 3 hours, salt content was reduced to 5 to 6 per cent and a maximum rehydration capacity of 57 per cent was achieved during the same period. The products retained their shape even after cooking.

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## EFFECT OF WHEAT GERM SUPPLEMENT TO FLOUR MIXTURES ON GROWTH, SERUM AND LIVER PROTEINS OF WEANLING ALBINO RATS

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Cereal flour mixtures with the addition of wheat germ were fed to weanling albino rats at 10% protein level. Diets with 1:1 ratio of flour to wheat germ promoted maximum weight gain and P.E.R. No significant difference was observed in the total serum protein level of animals in experimental groups as compared to the casein fed group. The serum albumin level as well as the liver protein reflected a significant increase. It is concluded that wheat germ can be used as an effective supplement for improving the nutritive value of cereal flour mixtures.

Commercial milling of wheat into flour aims at maximum extraction of the endosperm with the minimum possible contamination by bran and germ, and the latter are generally used in animal feed formulations. There is a special interest in wheat germ from the nutritional stand point, because it is a rich source of certain vitamins, especially vitamin E.

Early investigations have reported<sup>1-5</sup> that quantitatively cereals are poor sources of proteins and have poor protein efficiency ratio (PER). Interestingly enough, wheat germ proteins have been classed with superior animal proteins.

Among vegetable proteins<sup>6</sup>, wheat germs have probably the best essential amino acid make-up, which compares well with that of egg protein. It is a rich source of lysine, B-group vitamins and tocopherols, which enhance the value of the germ as a food supplement. In view of this, it was thought worthwhile to assess the protein quality by conducting an animal experimentation. The present experiment was conducted on 21-day old female weanling rats (Wistar strain). The animals were divided into 7 groups of 8 rats in each group on the basis of their body weight by the process of restricted randomization, such that there was equal distribution of weight in all the groups. The animals were housed group-wise in cages and marked differently for identification as shown below:

- C – 10 per cent protein from casein.
- W – 10 per cent protein from wheat flour
- WG<sub>1</sub> – 5 per cent protein from wheat flour + 5 per cent protein from wheat germ.
- WG<sub>2</sub> – 7 per cent protein from wheat flour + 3 per cent protein from wheat germ.
- RF – 10 per cent protein from refined flour.

RF<sub>1</sub> – 5 per cent protein from refined flour + 5 per cent protein from wheat germ.

RF<sub>2</sub> – 7 per cent protein from refined flour + 3 per cent protein from wheat germ.

The level of protein was fixed at 10 per cent in all the diets. Starch was added to make the diets isocaloric. The composition of the diets is shown in Table 1. The experiment was continued for a period of 28 days and the animals in all the groups were weighed every week. At the end of the experimental period, all the animals were sacrificed by decapitation. Blood was collected and centrifuged for analysis. Liver was removed, cleaned, blotted, weighed and preserved for further analysis. Estimation of total serum proteins and liver proteins was done by Biuret method. The results were subjected to statistical analysis using 't' test of significance.

The protein content of wheat germ, wheat flour and refined flour was 19.55, 10.79 and 8.47 per cent respectively. The moisture, fat and ash contents of the germ were 10.0, 6.98 and 4.28 per cent respectively. The germ has been reported to be rich in fat, protein, iron and B-vitamins<sup>7</sup>.

The average food consumed, gain in weight of animals and the protein efficiency ratio of the control and experimental diets fed to albino rats are shown in Table 2. It is observed from Table 2 that the average gain in weight of animals recorded at the end of the experimental period varied widely. Animals in group RF<sub>1</sub> recorded highest average gain in weight (62.25 g) followed by animals in group WG<sub>1</sub> (58.00 g). Drastic reduction (18.37 g) in average gain in weight in animals fed exclusively on protein from refined wheat flour (RF) was observed. Statistical analysis revealed that the average gain in weight recorded by animals in groups WG<sub>1</sub>, RF and RF<sub>2</sub> were significantly higher than the control group (C).

TABLE 1. COMPOSITION OF CONTROL AND EXPERIMENTAL DIETS

Ingredients	Gp. I	Gp. II	Gp. III	Gp. IV	Gp. V	Gp. VI	Gp. VII
Casein (g)	10.00	–	–	–	–	–	–
Wheat flour (g)	–	92.70	46.30	64.90	–	–	–
Wheat germ* (g)	–	–	25.57	15.30	–	25.57	15.30
Refined flour (g)	–	–	–	–	118.00	54.50	74.50
Oil (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Vitamin and mineral mix (g)	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Starch (g)	108.95	26.30	47.08	39.75	0.95	38.88	29.15

\*Procured from Wallace Flour Mills, Bombay;

Vitamin and mineral mix as in *Manual of Laboratory Techniques*, by Reghuramalu N. Madhavan Nair, and Kalyanasundaram S, (Eds), National Institute of Nutrition, Hyderabad, 1983.

TABLE 2. AVERAGE FOOD CONSUMPTION, GAIN IN WEIGHT AND PER OF CONTROL AND EXPERIMENTAL DIETS FED TO ALBINO RATS

Group	Food consumed (g/day/rat)	Initial wt. (g)	Final wt. (g)	Gain in wt. (g)	P E R
C	9.385	45.38	85.13	39.75	1.49
W	10.840	46.63	88.13	41.20	1.38
WG <sub>1</sub>	10.760	42.63	101.63	58.99	1.96
				3.42*	2.54*
WG <sub>2</sub>	10.920	44.00	93.88	49.87	1.62
				1.81**	0.70**
RF	11.380	41.00	59.37	18.37	0.58
				4.0*	5.06*
RF <sub>1</sub>	11.320	39.25	101.50	62.25	1.98
				4.01*	2.66*
RF <sub>2</sub>	11.320	41.125	93.37	52.25	1.65
				2.22**	0.86**

\* 't' values significant at 0.05% level.

\*\* 't' values not significant at 0.05% level.

The protein efficiency ratios of the control and experimental diets ranged between 0.58 and 1.98. Animals in groups RF<sub>1</sub> and WG<sub>1</sub> which had recorded higher gain in weight also showed higher P E R values (1.98 and 1.96). A low P E R value (0.58) was observed in group RF, fed exclusively on 10 per cent protein from refined wheat flour. Statistical analysis revealed that the PER values of experimental diets fed to animals in groups RF<sub>1</sub> and WG<sub>1</sub> were significantly higher than those fed on control diet (Group C). Significantly low P E R values were observed in diets fed to groups W and RF as compared to the control diet. All the animals fed on 5 and 3 per cent wheat germ supplemented diet showed more average gain in weight than the control diet group fed on casein. The lowest weight gain was in group RF on 10 per cent protein diet from refined flour.

The average serum and liver proteins of animals are shown in Table 3. No statistical significance in total serum proteins was observed in the control and experimental groups of animals. Earlier studies<sup>8</sup> have pointed out that the plasma proteins are well maintained both in concentration and in total circulating quantity, in simple undernutrition, atleast for a period of many months.

The average liver weights of animals in group W and RF were significantly lower when compared to those of the control group. The average liver protein levels of animals in groups WG and RF<sub>1</sub> were found to be higher (19.90 and 19.50 g per cent) than those observed in the animals in group C (17.83 per cent). Statistically this difference was, however, found to be insignificant. Animals in groups W, WG<sub>2</sub>, RF and RF<sub>2</sub> showed significantly the lower liver protein levels as compared to those of normal group (C).

TABLE 3. AVERAGE TOTAL SERUM PROTEIN, LIVER WEIGHT AND LIVER PROTEIN OF CONTROL AND EXPERIMENTAL ANIMALS

Group	Total serum protein (mg/100 ml)	Albumin (mg/100 ml)	Globulin (mg/100 ml)	Liver wt (g)	Liver protein (mg/10 ml)
C	4.740	2.73	2.01	4.07	17.83
W	4.230	2.65	1.59	3.08	16.40
	1.214**	1.053**	1.240**		
WG <sub>1</sub>	5.195	3.02	2.17	4.06	
	1.323**	2.870*	0.421**		
WG <sub>2</sub>	4.615	2.86	1.75	3.94	18.31
	0.318**	1.383**	0.65**		
RF	3.940	2.36	1.61	2.43	15.56
	2.040*	1.610*	1.670**		
RF <sub>1</sub>	4.246	2.99	1.32	3.72	19.50
	0.448**	2.490*	1.770**		
RF <sub>2</sub>	4.440	2.75	1.62	3.58	17.30
	0.944**	0.400**	1.220**		

\* 't' value significant at 0.05% level.

\*\* 't' value not significant at 0.05% level.

It was thus observed in the present experiment that albino rats fed on protein from wheat germ and wheat flour/refined flour in the ratio of 1:1 promoted maximum growth. Though no significant difference was observed in the total serum protein levels of animals in the control and experimental groups, the serum albumin level as well as the liver protein reflected a significant increase.

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## EFFECT OF COOKING PROCEDURE AND VARIETY ON ACCEPTABILITY OF UNRIPE MANGO BEVERAGE

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Five cooking procedures were tried with 'Dushehari' variety. Pressure cooking whole fruits under 15 p.s.i. steam pressure for 20 min, removing peels and stones manually, and passing through a blender gave highest pulp yield (67.8%) without adversely affecting TSS and acidity of pulp, or sensory qualities of the beverage. Pulp yields for 'Deshi', 'Safeda' and 'Chousa' varieties were 35.0, 64.8 and 68.4%, respectively. 'Dushehari' beverage was rated best, followed by 'Deshi', 'Safeda' and 'Chousa' beverages in that order.

More than 75 per cent of mango fruits set in a tree are knocked-off starting from the flowering stage<sup>1,2</sup>. About 20 per cent of these drops occur after 5 weeks of fruit set<sup>3</sup>. By this time, they gain considerable size and develop good edible qualities. But due to injuries caused, they are generally converted into 'amchur'<sup>4</sup> and do not find full utilization. Saltish unripe mango drinks are very popular in India during summer. Kaushik<sup>5</sup> developed a beverage base from unripe 'Dushehari' mangoes which could be diluted before use. But pulping procedure and variety may influence the quality of the beverage. Therefore, effect of these two parameters on acceptability of the beverage was determined.

Unripe fruits of 'Deshi', 'Dushehari', 'Safeda' and 'Chousa' varieties, dropped by wind with slight physical injuries but otherwise sound, were procured from the Horticultural Research Centre of this University. They were washed well in warm water of about 50°C, cooked and pulped. Cooking procedure was standardized using 'Dushehari' mangoes only because of their early availability. Cooking procedures tried were (A) whole mangoes boiled for 25 min in twice their amount of water, peeled and destoned, (B) mangoes peeled, sliced, destoned and slices boiled for 15 min in 1.5 times water, (C) whole mangoes autoclaved for 20 min at 15 p.s.i., peeled and destoned, (D) slices obtained as in procedure (B) were cooked in an autoclave for 15 min at 15 p.s.i., and (E) whole mangoes oven-baked at 215°C for 25 min, peeled and destoned. Ratio of mango or its slices to boiling water and/or cooking times were standardized by trial and error to ensure

softening of the pulp without scorching. Pulp was blended in a blender. Cooking procedures were compared on the basis of pulp yield, T.S.S. and acidity of pulp<sup>6</sup>, and sensory characteristics<sup>7</sup> of their beverages. Since, recipe was not standardized at this stage, beverage was prepared using 20.0 per cent pulp, 1.0 per cent NaCl, 0.1 per cent citric acid and 5.0 per cent sugar. Pulp obtained by the above methods had a tendency to settle down due to inadequate size reduction in blender. Therefore, three methods of pulping pressure-cooked whole mangoes were tried – (i) manual separation of pulp and blending in a blender, (ii) passing whole fruits through a pulper, and (iii) passing the pulp from the pulper through a colloid mill. Pulp (1 part) was mixed well with water (5 parts), 25 ml of this suspension was centrifuged for 10 min at 3500 r.p.m. and the volume of supernatant was measured. Larger supernatant volume was taken as an index of larger pulp particles. All the four varieties were pulped separately by procedure 'C' and beverages were prepared using 16.7 per cent pulp, 1.5 per cent NaCl, 0.2 per cent citric acid, 5.0 per cent sugar and 1.0 per cent of a commercial spice-mix. Sensory evaluation was done on a 5-point scale with score of 3.0 indicating neither liked nor disliked<sup>7</sup>. Beverages were evaluated for their colour, consistency, aroma and taste separately, and these scores were averaged to determine their overall acceptability. Sensory data were statistically analyzed by applying analysis of variance to determine the significance of difference at 5 per cent level. Pulp yield ranged from 53.0 to 67.8 per cent depending upon the cooking procedure (Table 1). Uncooked mangoes yielded minimum pulp because of greater losses during peeling and destoning. Pressure-cooking whole mangoes gave highest yield as it softened the pulp and facilitated peeling, destoning and pulping. TSS of pulp remained unchanged (10 per cent), but acidity decreased slightly. During baking, some liquid oozed out from panicle and some pulp was lost alongwith the charred peel. It reduced the pulp yield to 61.8 per cent, but increased the TSS to 11.0 per cent due to loss of moisture during baking. TSS (7-8 per cent) and acidity (1.47-1.66 per cent) of pulp from water boiled fruits/slices were lowest due to leaching of the soluble constituents (Table 1). Leaching losses were more during boiling of slices due to their greater surface to volume ratio. For evaluating the effect of pulping procedure on sensory qualities, beverages containing more pulp but without spice-mix were prepared. There was no significant difference in taste of these beverages (Table 2) but their colour, consistency and aroma differed significantly ( $P < 0.05$ ). Beverage from boiled slices scored lowest for all the quality attributes and since its score was below 3.00, the product was unacceptable. The difference between sensory scores of various attributes

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TABLE 1. EFFECT OF COOKING PROCEDURES AND MANGO VARIETY ON THE YIELD, TSS AND ACIDITY OF PULP

Variety	Cooking method*	Cooking duration (min)	Part used	Yield (%)	TSS (%)	Acidity (as % anhyd. citric acid)
Dashehri	Pressure	20	Whole	67.8	10.0	1.79
	Pressure	15	Peeled slices	67.0	10.0	1.78
	Boiling	25	Whole	61.5	8.0	1.47
	Boiling	15	Peeled slices	66.6	7.0	1.66
	Baking	25	—	61.8	11.0	1.79
			(215°C)			
	Uncooked	—	—	53.0	10.0	1.90
Deshi <sup>a</sup>	Pressure	20	Whole	35.0	11.4	1.73
Safeda <sup>a</sup>	Pressure	20	Whole	64.4	10.0	1.28
Chousa <sup>a</sup>	Pressure	20	Whole	68.4	11.0	1.12

\*Pressure cooking was done at 15 p.s.i. in all cases.

<sup>a</sup>TSS (%) and acidity (% anhyd. citric acid) of Deshi, Safeda and Chousa varieties were 12 and 1.88, 10 and 1.28 and 11.0, and 1.20, respectively.

and overall acceptability for beverages from pressure-cooked whole mangoes and baked mangoes were non-significant, but the former scored higher. The former method yielded maximum pulp (Table 1). Control of cooking conditions is easier than baking and it is very well adaptable to large scale processing. Therefore, only this procedure was used subsequently for pulping. However, this is contrary to the observations of Hoang *et al.*<sup>8</sup> who found baking yielded more pulp and better squash and nectar.

Supernatant obtained from 25 ml of blender-pulp, pulper-pulp and pulper+colloid mill-pulp were 21 ml, 18 ml and 18 ml, respectively. Small differences between the supernatant volumes indicated that all the three methods are equally

suitable. Blenders are easily available and would be suitable for home/small scale production while pulper will be suited for large scale production. In this study, blender was used for pulping pressure-cooked mangoes.

Beverages were prepared from 4 commercial mango varieties of North India— 'Deshi', 'Dashehari', 'Safeda' and 'Chousa'. Pulp yield was lowest (35.0 per cent) for 'Deshi' mangoes due to their thick peel and large stones (Table 1). Pressure cooking reduced its TSS and acidity slightly. Yield of pulp from other varieties ranged between 64.4 and 68.4 per cent which is in the range of 45-75 per cent reported by Roy and Singh<sup>1</sup>. Sensory qualities of beverages from these four varieties were compared (Table 2). 'Dashehari' beverage got

TABLE 2. EFFECT OF COOKING PROCEDURES AND VARIETY ON SENSORY SCORE OF BEVERAGE

Pulping procedure/variety	Colour	Consistency	Taste	Aroma	Overall
<b>Cooking procedure for Dashehari mangoes</b>					
Whole fruits water boiled	2.80	2.70	3.10	3.30	3.00
Slices water boiled	2.50	2.50	3.00	2.40	2.70
Whole fruits pressure cooked	3.40	3.70	3.80	3.40	3.70
Whole fruits baked	3.10	2.90	3.30	3.10	3.10
F (cal). at 5% level <sup>a</sup>	3.06*	4.71*	1.40	2.90*	3.90*
C.D.	0.86	0.96		1.00	0.84
<b>Variety</b>					
Deshi	3.70	3.30	3.30	3.50	3.40
Dashehari	3.90	3.30	4.00	4.20	4.00
Safeda	2.90	3.30	2.90	3.10	3.20
Chousa	3.20	3.00	3.00	2.90	2.90
F (cal). at 5% <sup>a</sup>	3.10*	0.28	4.20*	4.20*	14.09*
C.D.	1.04		0.94	1.10	0.4

Table value of F at 5% level 2.9<sup>a</sup> and 3.01<sup>b</sup>



the highest sensory score (4.0) and was rated significantly superior (Table 2). It was followed by 'Deshi', and 'Safeda', while 'Chousa' beverage was liked least. This trend was observed in all the quality attributes except for consistency. The panelists gave almost equal marks for consistency of all the 4 beverages. This was also reflected by the narrow range of their viscosity (11-14 cp).

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## EFFECT OF MANGO GINGER (*CURCUMA AMADA* ROXB.) ON LIPID STATUS IN NORMAL AND HYPERTRIGLYCERIDEMIC RATS

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When 10% mango ginger or a comparable level of 10 mg % curcumin was fed with a normal diet or with a sucrose based hypertriglyceridemic diet to adult female albino rats, significant decreases were observed in liver and serum triglycerides. Both mango ginger and curcumin caused significant decreases in liver total lipids and free fatty acids in rats on the normal diet and in liver weight and serum total lipids in rats on the hypertriglyceridemic diet. The curcumin-free lipid fraction from mango ginger when fed at 0.3% level (comparable to 10% mango ginger) with either diet similarly decreased the liver triglycerides on both diets but the liver weight, serum total lipids and triglycerides were decreased only in rats on the hypertriglyceridemic diet. Mango ginger, curcumin or the curcumin-free lipid fraction did not affect fat absorption.

Mango ginger (*Curcuma amada*) is an exotic flavourant spice possessing excellent medicinal properties and is used as a carminative and stomachic. Because of its typical 'raw mango-like' flavour, it is popularly known as mango ginger and is commonly used as a basic ingredient in pickles and salads<sup>1</sup>. Besides, it is also used in making preserves, candy and sauce<sup>2</sup>. The pale yellow colour of mango ginger is due to curcumin (diferuloyl methane) and the content ranges between 0.1 and 0.25 per cent. Curcumin has been shown to possess hypo-cholesterolemic activity<sup>3</sup> and stimulate bile secretion and composition in rats<sup>4</sup>. Sucrose-induced hypertriglyceridemia in rats has also been shown to be countered by curcumin<sup>5</sup>. So far, there are no reports on the nutritional influence of mango ginger. In view of the wide use of mango ginger, studies were conducted to evaluate whether the spice has any beneficial effect on lipid status in either normal or experimentally induced hypertriglyceridemic rats and if so, whether any other component besides curcumin would be responsible for such effects.

Freshly harvested mango ginger purchased locally was thoroughly washed with water free of mud and mechanically sliced. It was then dried in a cross-flow drier ( $60 \pm 2^\circ\text{C}$ , 8 hr) and powdered to pass through 30-40 mesh sieve. Two kg mango ginger powder was obtained from sixteen kg of the fresh rhizome. The 'lipid extract' was prepared from mango ginger powder by Soxhlet extraction with petroleum

ether ( $40-60^\circ\text{C}$ ) for 24 hr and the lipids recovered by evaporating-off the solvent in vacuo. This lipid fraction was three per cent of the dry mango ginger. The spent residue left over after the extraction of lipids was vacuum oven-dried ( $40 \pm 2^\circ\text{C}$ , 8 hr) and then tested. The curcumin contents in the mango ginger powder and the dry lipid-free residue were determined<sup>6</sup> and found to be 0.1 and 0.11 per cent of dry weight respectively.

Groups of six adult female albino rats each weighing 150-160 g were used for experiments. Mango ginger (10 per cent) and lipid fraction (0.3 per cent) were tested by addition to both normal and the high sucrose hypertriglyceridemic semi-synthetic diets<sup>5</sup>. Additional groups fed the respective diets supplemented with 10 mg per cent of curcumin were also included for purposes of comparison. Diet and water were provided *ad libitum*. The duration of feeding was 4 weeks in the case of normal and 10 days in the case of hypertriglyceridemic diets. The experimental animals were then sacrificed and blood and liver collected for analyses<sup>7</sup>. The effect of mango ginger, its lipid fraction or curcumin on fat absorption from the intestine in rats maintained on the different diets was evaluated by instilling peanut oil into ligated-loops of small intestine. Two hours after instilling, the left over oil was recovered and measured as described<sup>8</sup>. Analytical data of the various lipid parameters were evaluated for statistical significance by using Student's 't' test<sup>9</sup>.

As could be seen from data presented in Table 1, significant decreases in liver total lipids (TL), triglycerides (TG), free fatty acids (FFA) and serum TG were observed in rats fed the normal diet to which mango ginger or curcumin had been supplemented. The mango ginger fed group of animals showed significant increases in serum FFA unlike those fed curcumin. The hypertriglyceridemic (HTG) diet led to increases in liver and serum TL and TG and decrease in liver FFA in conformity with our earlier studies<sup>5</sup>. When mango ginger or curcumin was added to HTG diet, the increases in liver and serum TG and serum TL were very much less. In contrast, mango ginger led to an increase in liver FFA. The decreases in liver TL, TG and FFA and serum TG brought about by incorporating the lipid-free residue of mango ginger into the normal diet were very much similar to the effects of a comparable dose of curcumin. Further, serum FFA changes were also similar to those caused by curcumin. Since the lipid-free residue contains all the curcumin present in mango ginger it was inferred that the lipid-free residue did not probably have any component other than curcumin which would influence lipid changes. On incorporation into the normal or HTG diet, the lipid fraction isolated from mango ginger caused decreases in liver TG and serum TL and TG. However, only the changes in serum were significant in animals fed the HTG diet. As in the group fed mango ginger, the liver FFA was also elevated by lipid fraction feeding on

TABLE 1. EFFECT OF FEEDING MANGO GINGER AND ITS FRACTIONS ON LIVER AND BLOOD LIPIDS IN NORMAL AND HYPERTRIGLYCERIDEMIC (HTG) DIETS

Parameters	Additions to normal diet*					Additions to HTG diet**			
	Nil	10% Mango ginger	10 mg % Curcu- min	0.3% Lipid fraction	10% LF-R	Nil	10% Mango ginger	10 mg % Curcu- min	0.3% Lipid fraction
<b>Liver wt (g/100 g BW):</b>									
Liver (mg/g)	2.94±0.16	3.03±0.07	2.96 ± 0.07	2.71±0.05	2.95±0.07	3.17±0.09	2.73*±0.08	2.80 ± 0.08	2.71* ± 0.07
Total lipids	8.52±0.35	6.63*±0.27	7.41* ± 0.30	8.00 ± 0.67	6.34*±0.27	9.23±0.23	9.41±0.61	8.45±0.38	8.85±0.13
Triglycerides	2.50±0.05	2.07*±0.04	2.18*±0.05	1.97*±0.03	1.95*±0.03	3.64±0.05	3.33*±0.02	3.18*±0.07	3.31*±0.06
Free fatty acids	0.42±0.03	0.24*±0.04	0.22*±0.04	0.40±0.01	0.32*±0.02	0.24±0.02	0.31*±0.02	0.30±0.03	0.34*±0.03
<b>Serum (mg/dl):</b>									
Total lipids	211.0±10.9	232.0±4.5	207.0±4.4	187.4±8.1	190.0±13.3	324.0±6.0	265.0*±10.0	292.0*±5.6	260.8*±7.6
Triglycerides	71.5±4.2	59.0*±1.3	58.1*±0.9	66.8±5.0	48.6*±1.4	136.5±2.7	113.7*±2.9	107.3*±2.3	109.2*±2.4
Free fatty acids	20.0±5.0	35.7*±1.7	27.7±2.4	32.4*±1.6	17.5±1.6	21.2±3.1	25.0±3.6	28.6±5.9	16.4±3.3

\*Diet fed for 4 wk. \*\*Diet fed for 10 days. Values are Mean ± SEM of 6 rats per group.

\*Significant ( $p < 0.05$ ) against nil addition on respective diets. LFR = Lipid-free residue.

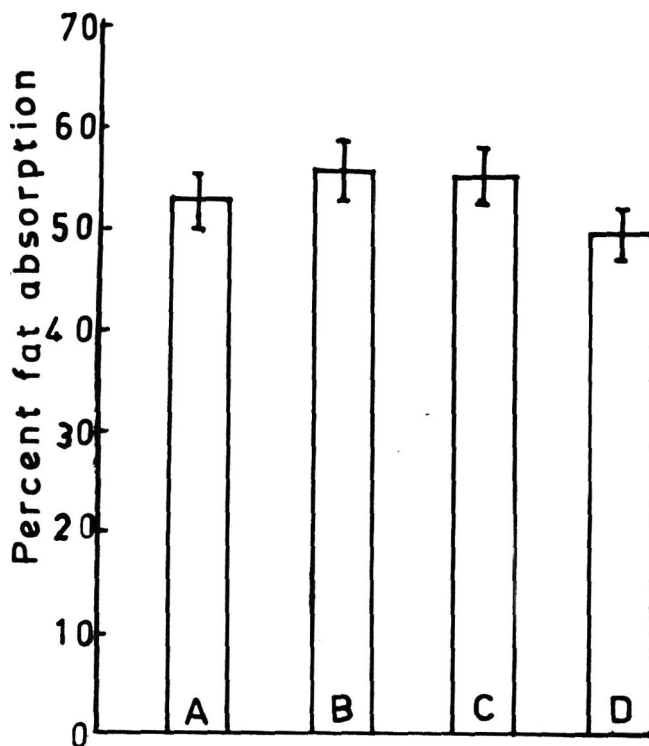


Fig. 1. Two hr fat absorption expressed as Mean ± SEM per cent of peanut oil dose instilled into ligated intestinal loops of rats (6 per gp) prefed, normal (A), 10% mango ginger (B), 10 mg % curcumin (C) and 0.3% lipid fraction (D) for 4 weeks.

the HTG diet. It is known<sup>10</sup> that the hypertriglyceridemia induced by high sucrose diet is due to the fructose moiety which not only causes increased lipid biogenesis but also causes hyperplasia of liver. It is noteworthy that addition of mango ginger or its lipid fraction to the HTG diet significantly lowers the hyperplasia (Table 1, column 6-9).

Liver is the main organ that regulates the provision of lipids into blood. FFA in blood arises through the lipolysis of stored glycerides. Absorption of dietary lipids from the intestine would be another regulatory factor which influences blood lipids. *In situ* fat absorption study showed that fat absorption was not adversely affected in rats which had been fed mango ginger, its lipid fraction or curcumin (Fig. 1). The mean per cent absorption was around 50 in 2 hr when a fat load of 3 g/kg body weight was instilled into the intestine.

The metabolic basis for changes brought about by mango ginger such as increase of FFA in serum with the normal diet, and that of liver with HTG diet and the underlying influences on lipolysis and lipid biogenesis remains to be elucidated. However, since the isolated lipid fraction of mango ginger also showed similar responses on these diets, it underscores the potential activity due to this fraction. Apart from what constituents of lipid fraction are responsible for such effects, it is concluded from the present studies that the beneficial effects on lipid metabolism by mango ginger is probably due both to the curcumin and the non-curcumin lipid components.

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#### FORM IV

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I, Dr. M.S. Prasad hereby declare that the particulars given are true to the best of my knowledge and belief.

(Sd)

**M.S. Prasad**

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#### VIII CARBOHYDRATE CONFERENCE

The Eighth Carbohydrate Conference will be held during 18-20 November 1992 at Regional Research Laboratory, Trivandrum. Topics include (1) Carbohydrate chemistry and structure, (2) Synthesis of carbohydrates, (3) Fermentation/Bio-transformation of carbohydrates, and (4) Polysaccharides.

Further information may be obtained from Dr. Ashok Pandey, Convener or Dr. K.C. Raja, Organizing Secretary, VIII Carbohydrate Conference, Regional Research Laboratory, Trivandrum-695 019, Kerala, India.

## BOOK REVIEWS

*Selected Mycotoxins: Ochratoxins, Trichothecenes, Ergot*: Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation and the World Health Organisation (WHO), W.H.O., Geneva, 1990; pp: 263; Price: Sw.fr. 29 and Sw.fr. 20.30, in developing countries.

The book is one of the Environmental Health Criteria series (105) published under a joint venture called 'The International Programme on Chemical Safety' (IPCS) of the above International Organizations. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. The IPCS has supporting activities such as development of methods that could produce internationally comparable results and development of manpower in the field of toxicology. The other activities include the development of know-how for coping with the chemical accidents, co-ordination of the laboratory testing and epidemiological studies, and promotion of research on the mechanisms of the biological action of chemicals.

The book is a kind of monograph which contains information of the currently available literature on three groups of mycotoxins, namely Ochratoxins, Trichothecenes and Ergot alkaloids, along with summary and recommendations for future research. Each group of mycotoxins is dealt very systematically under various sections such as properties and analytical methods, sources and occurrence, metabolism, effects on animals, effects on man and evaluation of human health risks.

It serves as a good reference book for finding out standard AOAC methodology for the estimation of the above mycotoxins in both foodstuffs and biological samples. It provides important information on the correlation between the feed level of toxins and the residue level in different animal tissues, blood, egg and milk, as well as on binding of toxins with various macromolecules. Metabolic transformation of toxins and their excretion, toxic effects such as teratogenicity, mutagenicity and carcinogenicity, biological effects and mode of action have been reviewed in some detail. The aspects of prevention and therapy of toxicosis have also been indicated and well presented in the book.

The book has a vast bibliography containing about 700 references which will be useful to those researchers working on mycotoxicology, food microbiology and environmental health. The public health analysts, food analysts involved in mycotoxins regulation and other analytical chemists would find the book most useful in their work. And it will be a

valuable addition to the already existing food science and technology literature.

S.C. BASAPPA  
CFTRI, MYSORE

*Evaluation of Certain Food Additives and Contaminants*: 37th report of the Joint FAO/WHO Expert Committee on Food Additives, Technical Report Series 806, Geneva 1991, pp: 49, Price: Sw.fr. 8.

The report deals broadly on three aspects namely: 1) General considerations 2) Comments on specific food additives and contaminants and 3) Revision of certain specifications followed by future work and recommendations. The report is also supplemented with five annexures about 1) Reports and documents resulting from previous meetings of the Joint FAO/WHO Expert Committee of Food Additives 2) Acceptability of daily intakes, other toxicological information and information on specifications 3) Further toxicological studies and other information required or desired 4) Matters of interest arising from meetings of the Codex Alimentarius commission and the codex committee on Food Additives and Contaminants and 5) Matters of interest arising from the 43<sup>rd</sup> World Health Assembly.

Based on the available toxicological data, the ADI limits of some of the antioxidants, flavouring agents, food colours, sweetening agents and miscellaneous food additives have been recommended for revision. They are: Butylated hydroxy toluene (BHT) 0-125 mg., tert-Butyl hydroquinone (TBHQ) 0-0.2 mg., (+)- Carvone 0-1 mg., Erythrosine 0-0.1 mg., Trichlorogalactosucrose 0-15 mg., and Dioctyl sodium sulfosuccinate 0-0.25 mg.

The committee has done a commendable job of going through in detail about all specific food additives and their recommendations are presented in this 37th report.

The report provides a flood of information on the specifications for those who are engaged in the field of specification formulations, quality control and food additives.

K. VIDYASAGAR  
DFRL, MYSORE.

*Purification and Analysis of Recombinant Proteins*: Edited by R. Seetharam and S.K. Sharma, Published by Marcel Dekker Inc. U.S.A. 1991; Price: US \$ 99.75 (US \$ Canada), \$ 114.50 (All other Countries).

On glancing through the book, it appears at the first instance that it is a commercial production. The word

'commercial' is not used in the sense that it will accrue any commercial benefit. The addresses of most of the authors are from commercial houses. That is however, no wonder. Recombinant DNA techniques are being equally exploited by the basic scientists as well as the industrial organisations. The objects of the two groups are quite different although a good deal of liaison is visible today (and even it was earlier) between the two in the present day scenario of science. This does not undermine the basic reason for publication of a book. There is however, an inherent difficulty in such publication and the present editors have faced the same problem as mentioned below.

Purification and analysis of proteins are the primary concern of those involved in biochemistry since the birth of biochemistry. If DNA is the backbone of molecular biology, protein is that of biochemistry. Their discovery, isolation, purification, analysis, etc. were of major concern during past few decades. Recombinant proteins may be basically looked upon as the same proteins we come across in nature. So, the old strategies are equally valid for handling recombinant proteins although new challenges peculiar to recombinant proteins have to be met. This has been properly reflected in the leading article 'Purification and characterisation of recombinant proteins: opportunities and challenges'. But the main question that arises is whether these have been met in this book? Let us take the extreme example in the last article. The age-old technique of X-ray crystallography has been re-described. The authors are not to blame for the simple reasons 'What else to do?'

The article (Chapter 10) on 'Purification of monoclonal antibodies' is well-written and quite informative. It will be useful to those who want to exploit the technique. But the same question arises 'How much of this write-up is under the purview of the book', perhaps very little. The Editors definitely had great difficulty in grouping the articles under four sub-heads (I-IV). Obviously, it has been rather arbitrary.

Two chapters need special comment. The write up on 'Properties of recombinant protein containing inclusion bodies in *E. coli*' is of special importance. The association of proteins with inclusion bodies is usually helpful but sometimes it can be a nuisance. Both the aspects have been dealt with nicely in this chapter. The other one describes the methods for removing the N-terminal methionine from recombinant proteins. It is directly concerned with a recombinant problem as the trick of fusion through methionine of a protein of interest to a well-translated one, is usually taken resort to. A few others like those dealing with secreted proteins, methods of engineering proteins to enable their isolation in biologically active form etc. deal specifically with some challenges in the area.

As it happens in most edited books, the style of writing in this compilation varies from article to article, text book style from one end to research publication style to the other end. Therefore, the question can justifiably be raised 'To

whom the books is addressed?' Or, 'To whom it will be of maximum help?' For that, we have to go back to the statement made in the introduction. May be, it will be of some use to scientists of commercial concerns or to some extent to the basic scientists as a partial guide to search for original publications. The treatment of the subject is in proper perspective and naturally, the proper choice of topics would have greatly improved the quality and consequently the use of the book by general readership.

Last but not the least, the price \$ 99.75 for U.S.A. and Canada is quite justified but that of \$ 114.50 for all other countries, specially the developing ones, is prohibitive and not commensurate with the utility of the book.

D.P. BURMA  
BANARAS HINDU UNIVERSITY  
VARANASI

*Fish Quality Control by Computer Vision:* Ed. by L.F. Pau and R. Olafsson, Marcel Dekker Inc., New York 1991; pp: 312; Price: \$ 115.00 (US and Canada), \$ 132.25 (All other Countries).

Quality control in the fish processing industry remains to be a challenging problem due to the inherent characteristics of the raw material. These include compositional variations of fish due to differences in species, age and growth environments, varying delays in processing the catch and sensitivity of fish to rapid microbial spoilage. Availability of high quality fishery products to the consumer demands fast movement of catch through the processing line to the distribution centres associated with rapid and efficient quality evaluation at various stages of production and supply. Currently, sensory evaluation is the most reliable method for quality assessment. However, it has several limitations like slow turnover rate, chances of human error and fatigue. Automation has, therefore, been felt as an immediate necessity. In this context, the present book will be received well by the seafood industry. The applications of automation include sorting and grading, making use of length, weight of characteristics of the fish species, quality inspection for spoilage and other defects and detection of undesirable components in fish fillets such as worms, bones or blood clots. The technique could also be used for survey and monitoring of fishing zones as well as study of age-dependent behaviour of fish. The components of automation include camera sensors, lighting and mechanical handling equipments, time-temperature integrators, algorithms, software and user interfaces.

The book is the result of a co-operative project undertaken in 1988 and 1989 by about 15 individual researchers mostly from Europe. Chapters 1 and 2 survey the scope for application of vision techniques for quality control at various stages in the fish processing industry, as mentioned earlier.

Vision techniques make use of differential optical properties of fish muscle, skin and bone which could be measured using appropriate sensors. These aspects have been lucidly discussed in Chapters 2, 3 and 4. Requirements of different types of sensors and light sources are also presented in these chapters. Since presence of parasites is a major problem, particularly in the fillets, a whole chapter is devoted for a discussion on nematodes with respect to their biology, presence in fish and public health aspects. Chapters 6 and 7 are devoted to experimental approaches to vision data. The problem in getting ideal vision data to help quality control lies in the fact that fish are shiny three-dimensional objects producing strong specular components of reflection. These difficulties have been overcome to a great extent by approaches (Chapter 7) for spatial and temporal distribution of light, spectrum of the radiation illuminating the object and spectral responses of the lenses. These efforts might lead to practical applications of image analysis for quality evaluation of fish fillets. Sorting of fish by measurements of length and width could be facilitated by the use of structured light (Chapter 8). Basic principles of structured lighting, methods of generation, its applications and limitations have been well

described in this chapter. Chapter 10 is devoted to efforts at Torry Research Station, Aberdeen, Scotland to use image analysis for fish inspection.

Although image analysis systems are being used for a number of purposes in fruit, vegetable or meat industries, their use in fish industry is yet to come to commercial levels of application. In view of the concerted efforts in several institutes, such machines could become a reality in the next ten years, at least for some limited applications. These include remote sensing of fish location, study of fish behaviour and aquaculture as pointed out in Chapters 9 and 13. A chapter is also included as a way of instruction for practical application of computer vision to fish inspection.

The book is well written and can be understood even by fishery technologists who are not familiar with this field. Although, the technology is presently in the developmental stage, its success will be greatly welcomed by fishing, aquaculture and processing industries who look for modernisation, high quality and productivity.

V. VENUGOPAL

B.A.R.C., BOMBAY 400 085.

## AFST(I) News

### Jabalpur Chapter

The Annual General Body Meeting was held and the following office-bearers were unanimously elected for the year 1992-93.

President	:	Dr. Y. K. Sharma
Vice-President	:	Dr. A. Mishra
Hony. Jt. Secretary	:	Dr. P. K. Jain
Joint Secretary	:	Dr. Suman Kumar
Hony. Treasurer	:	Ms. A. Verma

### Kharagpur Chapter

The Annual General Body Meeting was held on 28th January 1992. The following office bearers were elected for the year 1992-93.

President	:	Prof. R. K. Mukherjee
Vice-President	:	Prof. H. Das
Hony. Secretary	:	Prof. Suresh Prasad
Hony. Jt. Secretary	:	Dr. P. P. Srivastava
Hony. Treasurer	:	Dr. H. N. Mishra

### IFCON-93

Dates of IFCON-93 at CFTRI, Mysore, India have been changed to July 5 to 9, 1993. Further communication will follow in due course.

### NEW PUBLICATION

**POST-HARVEST TECHNOLOGY OF OILSEEDS** by Jaswant Singh and B. D. Shukla, published by Central Institute of Agricultural Engineering (ICAR), Nabibagh, Barasia Road, Bhopal-462 018 (MP); Pages: 342; Price: Rs. 125/- (Postage extra). Contains information on post-harvest technology of oilseeds, processes and equipment for primary processing and village level oil extraction. The book will be useful to entrepreneurs of small scale industries, planners, research and extension workers.

### NEW PUBLICATIONS OF NICFOS

The National Information Centre for Food Science and Technology (NICFOS) at Central Food Technological Research Institute, Mysore, has just brought out the following two publications:

1. **DIRECTORY OF ONGOING PROJECTS IN FOOD SCIENCE AND TECHNOLOGY IN INDIA** : This is the 3rd edition covering the period 1986-90 and includes information on 409 projects collected from 54 institutions. Nominally priced at Rs. 70/- + Postage, the Directory aims at serving the needs of R&D workers and management personnel in Food Science and Technology. The Directory can also be supplied in floppy, the rate for which will be intimated on request.
2. **CFTRI SCIENTIFIC AND TECHNICAL PAPERS : A BIBLIOGRAPHY - 1950-1990** . Lists nearly 4600 papers published in periodicals by R&D personnel of CFTRI from its inception. Arranged yearwise and author alphabetical within the year; also provided a consolidated Author Index. Aimed to highlight work already done in specific subject areas in Food Science and Technology and helpful in understanding gaps for planning future work. Material also available in floppy. Price details on request.

Please address enquiries to the Area Co-ordinator, FOSTIS, CFTRI, Mysore - 570 013.



# 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 Calicicolous 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*.

# JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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## Research Papers

EFFECT OF DIFFERENT COOKING METHODS ON  $\beta$ -CAROTENE CONTENT OF VEGETABLES by *K. Padmavati, S.A. Udipi and M. Rao.*

RESPONSE SURFACE MODELLING OF EXTRUSION TEXTURING OF DEFATTED SOYA GRITS by *Himadri K. Das, A. Lambrev, Jen Hi Te, S. Akterian and S. Tantchev.*

EFFECT OF CONVENTIONAL AND NON-CONVENTIONAL GREEN LEAFY VEGETABLES ON HAEMATOLOGICAL INDICES AND BLOOD CONSTITUENTS OF RATS by *Kaushalya Gupta, G.K. Barat, D.S. Wagle and Kuldip Singh Dhindsa.*

STUDIES ON THE USE OF GARLIC IN BREAD by *G. Venkateswara Rao, G.D. Savithri and D. Indrani.*

EFFECT OF ONION EXTRACT ON THE RHEOLOGICAL AND BREAD MAKING CHARACTERISTICS OF WHEAT FLOUR by *D. Indrani, G.D. Savithri and G. Venkateswara Rao.*

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