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(INDIA)

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Migration Studies on Some Selected Commercial Plastics Packaging Materials for Food Contact Applications

BALDEV RAJ, R.A.N. MURTHY*, N.S. VIJAYALAKSHMI, A.R. INDIRAMMA,
N. BALASUBRAHMANYAM AND P. VEERRAJU
Food Packaging and Distribution Engineering Discipline
Central Food Technological Research Institute, Mysore-570 013, India

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Commercial plastics materials used for food packaging applications were evaluated by migration tests to assess the suitability for food packaging. The tests were carried out as per the conditions prescribed by the Bureau of Indian Standards and the Food and Drug Administration (USA). Most of the tested materials had overall migration values within the specified limits. In case of some pigmented packaging materials, there was, however, the problem of leaching of colour. The PVC-based materials had the problem of odour. The uses of stretch blown polyester and PVC bottles appear to be limited to room temperature filling and storage due to appreciable shrinkage at temperatures greater than 60°C.

In recent years, plastics have come to be increasingly used in various forms such as films, laminates, rigid and semi-rigid containers in our country for food packaging, and are gradually substituting metal and glass containers. Besides, many plastic-based coatings are used on tin, aluminium, paper and paperboard containers to improve their surface properties. Although plastics (polymers) themselves are generally inert, the chemicals used as processing aids and adventitious impurities such as residual monomers, catalyst remnants and such others may also be present in the finished product and may leach out into the foods during direct contact with the food. Indiscriminate use of these plastics may endanger food hygiene, and in the long run, may lead to health hazards in view of leaching of additives. Hence, their safety evaluation is of great importance, and world over this has drawn considerable attention of health organizations, scientists, nutritionists and sociologists. Different countries like USA[1], UK[2], EEC[3], Japan[4], and India[5] have laid down specifications and Code of manufacture for use of plastics for food contact applications, and overall migration limits in food simulating solvents (FSL) have been specified for the finished plastics materials intended for food contact applications.

In India, government agencies like Bureau of Indian Standards (BIS) and Prevention of Food Adulteration (PFA) Act are formulating code of practice and specifications for a number of food grade plastics. In this regard, BIS has laid down positive list of constituents[6-10] and specifications for safe use of plastics[11-15] like polyethylene, polyvinyl chloride (PVC), polystyrene, polypropylene, nylon and ionomer polymers in contact with foodstuffs, pharmaceuticals and drinking water.

In the present study, various plastic packaging materials intended for/or used in various food packaging applications in our country are assessed for the extent of migration of additives by global migration tests and the results are reported in terms of overall migration values (OMV). Also a simple all-glass apparatus developed for this purpose is described.

Materials and Methods

Plastics packaging materials: Various plastics packaging materials used in the present study were supplied by different food packaging material manufacturers and some were procured from converters. These are classified into following groups: (a) Plain films: Polythenes [low density (LD), high density (HD), high molecular weight high density (HMHD)], polypropylene (PP), ethylene-acrylic acid (EAA), ethylene-methacrylic acid (EMAA), nylon (Ny), polyester (PET), plasticized poly vinyl chloride (PVC); (b) Multilayer films: Co-extruded films [LD-HD, HD-LD-HD (opaque and orange-coloured), PET-HD-LD, LD-Ny-LD]; Laminate films: [HD-Ionomer, Ny-EAA, Ny/ionomer, PET-LD-Ionomer and paper-LD-PET-LD; (c) Coated materials: Vinyl chloride-vinyl acetate coated aluminium foil (VC- VA/Al), PVDC-coated paper and PP wax-coated paper; (d) Rigid plastic containers: HD Jars and Jerry cans, PP bottles, polystyrene foamed tray (PSt tray), PVC blow molded and

*To whom all correspondence should be addressed.

stretch blown bottles (PVC-SB), and PET bottles; (e) Miscellaneous: Flowed-in PVC compound-lined crown caps (PVC- crown) and lug caps (PVC-lug).

Following Extraction Methods were followed

(A) *Extraction cell method:* This method is suitable for testing single, plain flexible films. A simple and convenient all glass extraction cell was developed for migration tests on plastic films in our laboratory. The new extraction cell is shown in Fig. 1 and 2. It consists of a glass (pyrex) beaker of 1 lt capacity provided with 2 pairs of grooves diametrically opposite to each other on the brim, and a glass hanger having two horizontal parallel rods, each 8 cm long and 2 cm apart and joined at both ends to clamp the test specimen in such a way that the specimen remains completely submerged in the simulating solvent when the hanger is placed in the grooves on the brim of the beaker, and a rectangular glass frame (8 × 6 cm) with leg of 2 cm height at each of the four corners of the frame for keeping it across the test specimen in the cell to prevent it from floating, and two pyrex glass pins (3 cm long) to secure the test specimen to the glass hanger, and a suitable glass lid to cover the extraction cell during extraction.

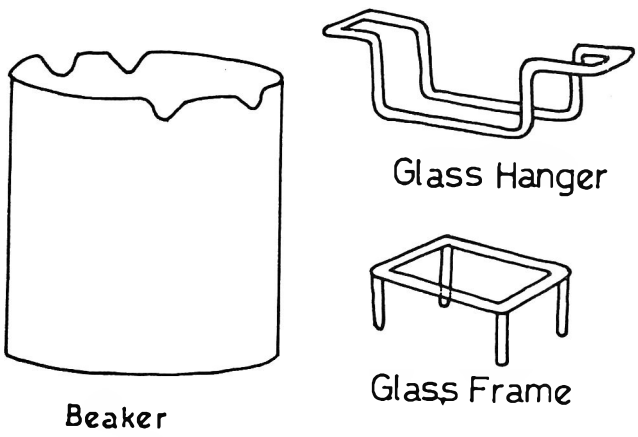

Fig.1. Extraction cell : Individual parts.

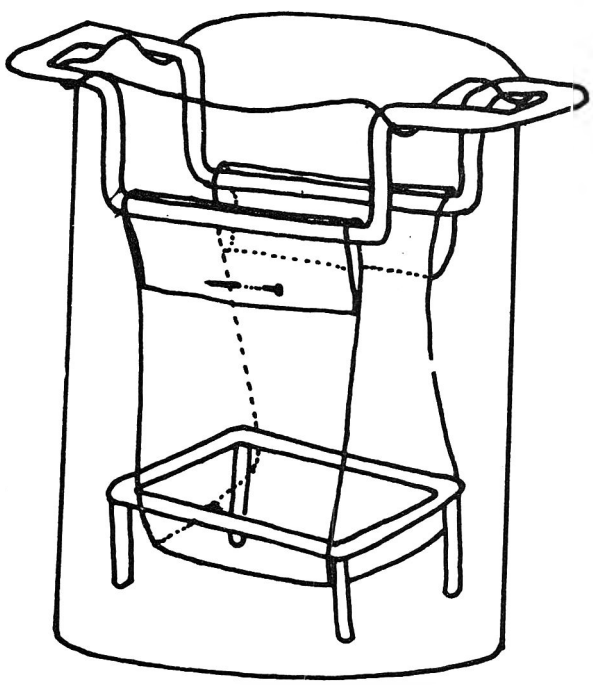

Fig.2. Extraction cell : When assembled.

With this cell, it is possible to expose, at a time, a plain film of size 33.4 × 7.5 cm (500 sq.cm. surface area of both sides) to 1000 ml of the FSL to maintain a ratio of 1:2 of surface area to volume leaving a head space of about 1 cm at the top.

(B) *Pouch method:* This method is suitable for testing co-extruded films and laminates where only one side needs to be exposed to FSL. In case of co-extruded and laminate film materials, since food contact occurs with a particular surface, pouches (500 ml capacity) with the food contact layer at the inner side of the pouch were made by heat sealing and filled with FSL to get a 1:1 ratio of volume to surface area. This was because in most of the market products, the volume to area ratio for 500 g capacity pouches was found to approach 1.

(C) *Container method:* Rigid containers were filled to their brimful capacity with FSL and tested.

Test procedure: The migration test was performed in quadruplicates exposing about 1000 sq. cm. surface area in each replicate. The sample to be tested (either in extraction cell or in the pouch or container form) was pre-conditioned for half an hour in a constant temperature chamber maintained at the test temperature. Simultaneously, the required amount of solvent was pre-heated to the test temperature and transferred to the extraction cell or pouch or container and kept at test temperature in a constant temperature chamber for the stipulated period. At the end of the test period, the sample was removed, and the extracted solutions in a replicate (i.e. from two cells) were pooled together, transferred to a suitable glass container for concentration.

Determination of amount of extractive: The extract solution in each replicate was concentrated to about 50 ml, by evaporation or distillation under low heat. The concentrated solution was transferred to a clean, tared platinum or stainless steel dish and further evaporated to dryness in an oven at 100±5°C. The dish was cooled for 30 min in a desiccator and the residue was weighed to nearest 0.1 mg. The amount of the extractive obtained was calculated and expressed as mg/dm^2 and p.p.m.

Results and Discussion

Methodology: Food simulants with categorization of foods as per BIS[5] and Food and Drug Administration (FDA) [USA][1,16,17] are shown in Table 1. Regarding the test conditions, mostly IS test conditions (40°C/24 hr, 60°C/2 hr for I-III food types, and 25°C/0.5 hr for n-heptane or 40°C/0.5 hr for n-hexane for food type IV. n-Hexane has since been discontinued as FSL, and occasionally FDA conditions

TABLE 1. FOOD CATEGORIZATION AND FOOD SIMULATING SOLVENTS

Specification	Food type		Food simulating liquid [FSL]
FDA[1]	I.	Acidic (pH<6), non-acidic (pH =>5) aqueous products	Water (W)
	II.	Alcoholic beverages (<8% alcohol content)	8% alcohol
	III.	Alcoholic beverages (>8% alcohol content)	50% alcohol
	IV.	Low moisture oils & fats	n-Heptane
BIS[5]	Same as FDA except for using 3% acetic acid also as a simulant for aqueous acidic products.		

Limits: *FDA*: Variable and depends on the type of food packaging material.
BIS: 10 mg/dm^2 or 60 p.p.m. for all plastics.

(121°C/2 hr, 66°C/2 hr, 49°C/24 hr, 21°C/0.5 hr,) were used when relevant IS specifications were not available. In the Table, only the minimum and maximum OMV, irrespective of the test conditions, are shown. Unless indicated otherwise, test conditions are as per the IS. The studies reported in the present article were carried out during 1983-87.

The maximum limits specified by BIS[11-15] and FDA[1,16] for the extractive in overall migration tests with FSL are 10 mg/dm^2 or 60 mg/kg, and 7.75 mg/dm^2 (0.5 mg/sq.in.) or 50 p.p.m. respectively.

The all-glass extraction cell (Fig 1 and 2) maintains the area to volume ratio of 1:2 as specified by BIS and yields extractive in a replicate which can be conveniently weighed in an analytical balance of 0.1 mg accuracy whereas the cell specified by IS[5] exposes only 70 sq. cm area of the film micro specimen to 140 ml of solvent (1:2 ratio) and needs a microbalance with an accuracy of 10 μg which is not usually available in a general chemical laboratory. Even the FDA[16] specifies a testing area of about 180 sq. in. (1161 sq. cm.) in a replicate.

IS suggests platinum dish for weighing of the extractives where as the FDA mentions platinum or pyrex glass container (e.g. FDA 177.1330) for the same. In the present studies, apart from platinum and glass dishes, we also tried stainless steel (s.s.) dishes. The reproducibility of weighing in s.s dish equalled that in platinum dish whereas occasional weight-reproducibility problems which may perhaps be due to slow cooling rate of the glass because of low thermal conductivity and more time required to reach equilibrium were found in case of pyrex glass dishes. Therefore, in our experiments, s.s. dishes were used.

Migration results: About 40 per cent of the materials tested were intended for packaging of aqueous and alcoholic foods, and the remaining 60 per cent were meant for packaging of edible oils and fats. Accordingly, for convenience, the migration test results are grouped into two categories, namely, those for packaging of aqueous and alcoholic foods (Table 2) and others for edible oils and fats (Table 3).

1. *Packaging materials for aqueous (acidic and non-acidic) foods and alcoholic beverages:* Of the many polymeric materials shown in Table 2, all materials, except one PVC sample [No. 2(A)], had OMV within specified limits. Highest values, among those complying to limits, were observed for PET film [No. 3(B)], Nylon (No. 5) and Ny/ionomer laminate (No.8) in the range 0.9-2.4 mg/dm^2 which can be attributed to their highly polar nature and consequent better interaction with and penetration by the FSL (water, acetic acid, and alcohol). In fact, Ny/ionomer had highest p.p.m. values (8.9-44.8) among all packaging materials. On the other hand, PET bottles [No. 3(A)] and PVC-SB [No. 2(C)] had some of the lowest values which may be attributed to their highly oriented structures due to the stretch-blow moulding process. In fact, the OMV for PET bottles, though relatively low, were obtained under the extreme test condition of 121°C/2 hr. Interestingly, coloured materials were not offered for testing for this category of foods.

2. *Packaging materials for packaging of edible oils and fats (Table 3):* Majority of the packaging materials (about 60 per cent) tested was in this category, and the importance of plastics packaging materials for packaging oils and fats is increasing and easy to recognise. In this category, all the materials tested had OMV within specified limits, and any failure was due exclusively to migration of colour [No.1(B), 1(E)]. This is because IS:9833-1981[18] disqualifies a coloured packaging material if there is any visible bleeding or migration of colour into FSL.

Nylon and PET had very low OMV, whereas their laminates with polythenes and ionomer had relatively high OMV even, when the nylon or PET was sandwiched between polythenes [No. 12, 16], perhaps due to penetration of the FSL through the nylon or PET layer. Such penetration through the food contact layer in laminates was confirmed in case of the HD-LD-HD [No.9] film where migration of colour occurred eventhough the colour layer was behind the food contact layer LD. Accordingly, it is necessary, as also stipulated in FDA and EEC specifications, that all the layers including adhesives must also individually conform to the food grade composition.

HD cans still dominate in the high capacity region [1(D)] and till to-date the same trend continues. In case of bottles of upto 2 litre capacity, PVC and PET bottles seem to be the choice because crystal clear light weight bottles of superior characteristics can be produced by stretch blow moulding

TABLE 2. OMV FOR FOOD PACKAGING MATERIALS INTENDED FOR AQUEOUS (ACIDIC AND NON-ACIDIC) FOODS AND ALCOHOLIC BEVERAGES

No.		Material	Water	Food Simulating liquid AA	8 or 50% alcohol
1.	A)	LD film [80-5 μm] (2 grades)	—	—	0.7-0.8[b] (5.2-5.6)[c]
	B)	LD film [80-5 μm]	—	—	2.5-2.6[d] (18.7)
2.	A)	PVC film [27.5 μm] (5 grades)	0.7-12.8 (4-75.4)	—	—
	B)	PVC bottle [0.65-0.75 lt.] (2 grades)	0.4-0.74 (2.7-5.1)	0.34-0.7 (2.3-4.8)	0.8 (4.1)
	C)	PVC-SB bottle (1 lt.)	0.13-0.31 (0.8-1.85)	0.25-0.34 (1.5-2.04)	—
	D)	PVC crown caps	(22)[e,f]	(1.2)	(4.3)[g]
	E)	PVC lug caps	(5.4-6)[h,f]		
3.	A)	PET bottle [0.5-1 lt] (2 grades)	0.03-1.7[e] (2-11.9)	— —	0.5-0.6[d] (3.4-4.2)
	B)	PET film [12.5 μm]	0.9 (4.7)[e]	—	—
4.		PST tray	0.9 (4.9)	—	—
5.		Nylon film [30 μm]	1.4-1.5 (6.9-7.7)	1.5-1.9 (7.3-9.4)	1.4-2.5 (7.4-12.7)
6.		EAA [50 μm] and EMAA [70 μm]	0.3-0.8 (1.4-4.2)	0.6-0.9 (3.1-4.4)	0.4-1.0 (1.8-5.1)
7.		Ny/EAA [98 μm]	0.3-0.7 (2.4-4.6)	0.7 (4.6)	0.4-3.3 (3.0-23.4)
8.		Ny/Ionomer [87 μm]	1.3-2.4 (8.9-15.9)	4.0-5.6 (26-37.1)	3.3-6.8 (21.5-44.8)
9.		PP was-coated paper	0.9[d]	—	—
10.		VC-VA/Al	0.5[d]	—	—

[a]FSL abbreviations: AA-3% Acetic acid, [b]in mg/dm^2, [c]values in () are in ppm, [d]at 49°C/24 h, mg/dm^2, [e]at 121°C/2 h, [f]FDA limits are 50 ppm in water or alcohol [g]Lining distortion observed after migration test, [h]at 66°C/2 h.

process. As described earlier, there was problem of unpleasant odour in PVC materials in this category also. In case of PVC crown and lug caps [2(D) (E)], lining distortion was observed following migration test.

Among small size rigid containers of 1-2 l capacity, PET and PVC bottles continue to dominate oil packaging due to their superior characteristics. However, considerable shrinkage occurs in both PET and PVC-SB containers above 60°C, and as a result, their application seems limited to room temperature filling and storing.

In case of PVC bottles, the stretch blown (SB) bottles have almost replaced the conventional PVC bottles because of reduced material requirement, superior properties and appeal in the former. For example, for same filling volume, PVC-SB bottles were about 40-50 per cent lighter. Further, as described above, the OMV for PVC-SB bottles are also relatively low.

It has been a common observation that PVC materials show a characteristic unpleasant odour. In this study, such odour was found to persist even in extractive solutions, and the odour owes its origin most probably to the organotin sulphur compounds which are employed as thermal stabilizers during processing.

It is pertinent to stress here that compliance to migration limits is only one of the requirements in safety evaluation of food packaging materials to food contact. The other equally important factor is conformity to the requirement of food grade composition which, apart from listing the permitted adjuvants that can be used in making the packaging material, also prescribes the limits for residual monomers as well as limits for their migration into FSL and foods. The plastics which fall within the purview of such requirements are PVC and co-polymers, polystyrene and co-polymers, nylon and polyacrylonitrile.

Based on migration studies made on plastic packaging materials intended for/used in food contact applications, following inferences are made. In general, almost all the plastics materials tested had OMV within the specified limits.

TABLE 3. OMV FOR FOOD PACKAGING MATERIALS INTENDED FOR EDIBLE OILS AND FATS

No.	Material	*FSL*[a] H [Hx]
1.	A) LD [125 μm]	1.2[b] (14.1)[c]
	B) HMHD [50-100 μm]	0.7-2.1 (5-16.1)[d]
	C) Yellow HMHD [87.5]	0.5 (4.0)[d]
	D) HD Jerry can [5 lt.]	0.3 (1.0)[d]
	E) Yellow HD Jar [1 lt.]	0.04 (0.1)[d]
2.	Yellow PP bottle [1 lt.]	1.4 (7.6)[d]
3.	A) PVC bottle [0.75 lt.] (2 grades)	0.1-0.39 (0.8-2.7)
	B) PVC-SB bottle [1-2 lt.]	0.05-0.1 (0.26-0.53)
	C) PVC-SB bottle [1 lt.]	0.39 (2.3)
	D) PVC crown and lug caps	(120-153)[d]
4.	A) PET film (12.5)	0.2 (1.1)[e]
	B) PET bottles [0.5-1 lt.] (2 grades)	0.2-0.3 (0.6-1.5)[e]
5.	EAA [50 μm] and EMAA [70 μm] films	1-1.3 (5-6.4) 1.8 (9.2)
6.	Nylon [30]	0.1 (0.5); 0.2 (0.8)[f]
7.	Ny/EAA [98 μm]	2.5 (17.3); 2.6 (18.5)[f]
8.	Ny/Ionomer [87 μm]	1.1 (7.1); 3.2 (21.1)[f]
9.	HD-LD-HD [120-137 μm]	0.12-0.13 (2.1-5.6)[g]
10.	PET-HD-LD [132 μm] (4 grades)	0.8-2.2 (5.9-17.1)
11.	PET-LD-Ionomer [92 μm]	2.2 (17.1)
12.	LD-Ny-LD [75 μm]	2.1 (17.1)
13.	HD-Ionomer [100]	1.7 (10)
14.	PP wax-coated paper	8.5
15.	PVDC-coated paper	0.3 (2.1)
16.	Paper-LD-PET-LD [100 μm]	3.7 (25.8)
17.	VC-VA/AI	0.3
18.	PSt tray	2.2 (10.8)

[a]FSL: H-heptane, Hx-hexane, [b]values in mg/dm^2, [c]values in () are in ppm, [d]at 38°C/0.5 h, [e]at 66°C/2 h, [f]Results using Hx as FSL, [g]Colour leaching from one orange-red coloured sample.

In some yellow and orange-coloured materials, migration of colour into oils and n-heptane (fat simulant) was observed. In case of multi-layer films, leaching of colour from outer (i.e. other than the food contact) layers also occurred. Therefore, coloured materials must be tested for colour migration before they are considered for food contact applications. Although most of the PVC-based materials had lower OMV than the specified limits, there was, in general, unpleasant odour. The use of stretch-blow moulded bottles of PVC and PET, with better functional properties and appeal, seems limited to room temperature filling and storing due to considerable shrinkage above 60°C.

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Interaction of Plastic Films with Foods. II. Effect of Polyethylene and Polypropylene Films on the Stability of Vegetable Oils

G K Sharma, C V Madhura and S S Arya
Defence Food Research Laboratory, Mysore - 570 011, India.

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Effect of plastic films contact (polyethylene, polypropylene and butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) incorporated polyethylene) on the storage stability of refined sunflower oil and groundnut oil was studied at 37°C. The changes in peroxide value (PV), thiobarbituric acid (TBA) and total carbonyls (Tc) were significantly lesser in the presence of plastic films than in control samples. Relatively, reduction in the rate of autoxidation was more pronounced in the presence of antioxidant incorporated film. Both butylated hydroxy anisole and butylated hydroxy toluene were found to leach out in vegetable oils during storage.

Plastic films and bottles are extensively used for the packaging of vegetable oils, fruit juices and dehydrated foods. The quality and shelf life of the packaged foods are mainly determined by the barrier properties of the package against moisture, oxygen and the interaction of food constituents with the packaging material. Though most of the plastic films have been found to be almost inert towards the food constituents, a small amount of monomeric and oligomeric constituents or additives used in their manufacture to provide them stability, plasticity and other desirable functional characteristics are known to migrate into foods[1,2]. Though maximum limits of leached out substances for various plastic materials for food packaging applications have been laid down[3,4], the factors determining the rate of migration and the influence of these migrant residues on sensory quality and stability of foods are not known. In a previous communication, the role of polyethylene and polypropylene film contact on the quality and stability of fruit beverages was reported[5]. In the present study, effect of plastic film contact *per se* on the stability of sunflower and groundnut oils has been reported.

Materials and Methods

Thiobarbituric acid and butylated hydroxy anisole (BHA) were from Loba Chemie while butylated hydroxy toluene (BHT) and trichloroacetic acid were from Robert Johnson and Sisco Research Lab respectively. HPLC grade solvents, methanol, chloroform and hexane were procured from M/s Spectrochem, Bombay. Sunflower (Flora brand) and groundnut oils (Postman brand) were procured from the local market and used for storage studies. Polyethylene granules (10 kg) were treated with 1 per cent (w/w) antioxidant by immersing in hexane solution (3 per cent w/w, 3 l) and subsequently hexane was allowed to evaporate by spreading in trays. The treated granules were extruded into films (75 μm thickness). The concentration of BHT and BHA in antioxidants treated films were 0.81 and 0.72 per cent respectively.

Storage tests: Samples of refined sunflower and groundnut oils (400 g) were stored in loosely stoppered flasks (500 ml) both with and without plastic films at 37 ± 1°C. The ratio of oil to film area was 1:5. Initially and periodically, the oil samples were analysed for peroxide value (PV), thiobarbituric acid (TBA) value[6], total carbonyl (Tc)[7], leached out BHA and BHT and sensory acceptance of oil by a panel of ten judges on a nine point Hedonic scale (9 for excellent and 1 for totally spoiled) for any change in colour, smell and consistency during storage.

Antioxidant analysis in films and oil: Treated polyethylene film (2.0 g) was dissolved in xylene (60 ml) and polymer was precipitated by adding methanol (40 ml). The precipitate was filtered and the filtrate was evaporated to about 40 μl and the volume was made to 50 ml with methanol. Ten ml of this solution was injected into HPLC for determining antioxidant concentration using chromatographic conditions described below.

The concentration of antioxidants migrated from film to oil was determined by the method of Niebergall and Hartmann[8]. Ten g oil was dissolved in 10 ml chloroform and this sample was directly injected into Shimadzu LC-6A HPLC mobile phase, methanol-H_2O (90:10); column, Zorbax ODS (25 cm × 4.6 mm); detector, variable wavelength Detector Monitoring Wavelength 277 nm; flow rate, 2 ml/min.

Concentration of antioxidants was determined by the standard graph drawn with known concentrations of standard BHT and BHA against the area of the peaks.

Results and Discussion

Rancidity resulting from autoxidation of unsaturated fatty acid glycerides is the major cause of quality deterioration in vegetable oils during storage. The effect of plastic film contact on the quality and stability of sunflower and groundnut oils

in terms of changes in PV, TBA, Tc and sensory evaluation by a panel of judges are given in Tables 1 and 2. It is interesting to observe that plastic film contact slightly but significantly reduced the rate of autoxidation in both sunflower and groundnut oil samples during storage. The reduction in the rate of autoxidation was slightly more with polypropylene (PP) than polyethylene (PE) and highest with antioxidant treated PE. Among the two antioxidants tried, the reduction in rate of autoxidation was more pronounced with BHT than BHA treated film. After 105 days storage at 37°C, the PV of stored sunflower oil were 318.0, 246.2, 165.8 and 250.7 with PE, PE (BHA), PE (BHT) and PP films respectively as compared to 384.0 of the control sample stored without plastic film contact. The changes in TBA and total carbonyls also followed the same pattern.

The changes in groundnut oil also followed similar pattern but as expected the rate of increase was considerably smaller as compared to sunflower oil. After 105 days storage, the PV of control groundnut oil sample was 98.7 as compared to 82.9, 69.9, 54.8 and 76.5 of samples stored with PE, PE (BHA), PE (BHT) and PP films respectively which clearly indicate the retardatory effect of plastic films on the autoxidative degradation of vegetable oils. After 105 days of storage, the odour scores of sunflower oil samples treated with BHA and BHT films were 5.8 ± 0.5 and 6.5 ± 0.4 as compared to 4.1 ± 0.6 for control sample. In case of stored groundnut oil after 105 days storage, the corresponding odour scores were 6.1 ± 0.3 and 6.9 ± 0.5 for BHA and BHT film treated samples and 5.0 ± 0.3 for control sample.

The concentrations of leached out antioxidants from antioxidant treated films into oil are given in Tables 1 and 2. It is interesting to observe that the rate of extraction of antioxidants in vegetable oils depended both on the nature of oil and the antioxidant. The rate of extraction of both BHA and BHT was considerably greater in sunflower oil than in groundnut oil and BHT migrated slightly more than BHA. Figge[9] has reported that migration of antioxidants in vegetable oils depended on the nature and thickness of films, method of fabrication, storage temperature and duration and the nature of oils and antioxidants. The oils having lower viscosity and lower solidification points are known to extract higher concentration of antioxidants and other plastic additives. A decrease in viscosity is known to increase the solvating action of organic solvents. Slightly higher extraction of BHA and BHT in sunflower oil than groundnut oil is therefore in conformity with the published results.

Also, migration of antioxidants from the films to oils continued during the entire storage period though at a slightly lower rate during the later stages of storage. In the oil samples stored with commercially produced PE and PP films, no antioxidant could be detected by the analytical method followed in the present study. Commercially Irganox and Ionox, complex high molecular weight compounds, are employed as antioxidants for stabilization of PE and PP films.

TABLE 1. EFFECT OF PLASTIC FILM (WITH AND WITHOUT ANTIOXIDANT TREATMENT) ON THE STORAGE STABILITY OF SUNFLOWER OIL AT 37°C

Storage period (days)	Storage system	PV (meq O_2/kg fat)	TBA (mg malonaldehyde/ kg fat)	Total carbonyl (mg hexanal/ 100 g oil)	Antioxidant present in oil (ppm)
0		14.4 ± 0.07	0.19 ± 0.01	2.06 ± 0.01	—
21	Oil alone	39.9 ± 0.1	0.39 ± 0.00	13.76 ± 0.75	—
	Oil + PE	34.4 ± 2.7	0.31 ± 0.01	12.81 ± 0.39	—
	Oil + PE (BHA)	34.1 ± 2.5	0.33 ± 0.04	12.27 ± 0.03	27.0
	Oil + PE (BHT)	31.1 ± 2.1	0.28 ± 0.01	12.18 ± 0.10	37.0
	Oil + PP	36.7 ± 1.2	0.31 ± 0.00	13.11 ± 0.08	—
42	Oil alone	93.7 ± 2.1	0.52 ± 0.01	30.88 ± 0.28	—
	Oil + PE	91.1 ± 0.8	0.49 ± 0.01	30.56 ± 0.77	—
	Oil + PE (BHA)	75.3 ± 0.2	0.39 ± 0.00	31.56 ± 0.08	75.0
	Oil + PE (BHT)	70.5 ± 0.4	0.30 ± 0.01	24.36 ± 0.07	78.0
	Oil + PP	90.7 ± 1.2	0.40 ± 0.00	26.32 ± 0.34	—
70	Oil alone	142.9 ± 0.6	2.03 ± 0.02	44.90 ± 1.06	—
	Oil + PE	142.9 ± 0.8	2.24 ± 0.06	46.07 ± 0.69	—
	Oil + PE (BHA)	129.4 ± 0.8	2.04 ± 0.05	41.44 ± 0.59	88.0
	Oil + PE (BHT)	106.8 ± 3.4	1.82 ± 0.06	37.58 ± 0.78	92.0
	Oil + PP	127.4 ± 0.6	2.11 ± 0.03	47.86 ± 0.03	—
105	Oil alone	383.9 ± 13.7	9.15 ± 0.07	98.08 ± 1.03	—
	Oil + PE	318.0 ± 22.5	5.31 ± 0.01	63.16 ± 3.33	—
	Oil + PE (BHA)	246.2 ± 2.8	3.91 ± 0.03	49.45 ± 0.64	90.0
	Oil + PE (BHT)	165.3 ± 0.6	2.11 ± 0.01	39.31 ± 0.52	96.0
	Oil + PP	250.7 ± 10.4	4.80 ± 0.07	64.73 ± 0.04	—

TABLE 2. EFFECT OF PLASTIC FILM (WITH AND WITHOUT ANTIOXIDANT TREATMENT) ON THE STORAGE STABILITY OF GROUNDNUT OIL AT 37°C

Storage period (days)	Storage system	PV (meq O_2/kg fat)	TBA (mg malonaldehyde/ kg fat)	Total carbonyls (mg hexanal/ 100 g oil)	Antioxidant in oil (ppm)
0		7.1 ± 0.9	0.13 ± 0.01	0.83 ± 0.04	—
21	Oil alone	24.7 ± 0.0	0.28 ± 0.01	2.03 ± 0.04	—
	Oil + PE	21.2 ± 1.0	0.26 ± 0.00	2.06 ± 0.04	—
	Oil + PE (BHA)	18.7 ± 2.9	0.19 ± 0.01	1.64 ± 0.06	20.0
	Oil + PE (BHT)	19.4 ± 0.3	0.17 ± 0.02	1.07 ± 0.01	21.0
	Oil + PP	20.5 ± 0.4	0.19 ± 0.00	2.03 ± 0.07	—
42	Oil alone	36.3 ± 3.6	0.42 ± 0.01	8.72 ± 0.12	—
	Oil + PE	35.8 ± 0.6	0.45 ± 0.01	8.70 ± 0.06	—
	Oil + PE (BHA)	30.4 ± 1.5	0.40 ± 0.00	7.78 ± 0.21	47.0
	Oil + PE (BHT)	28.4 ± 1.0	0.40 ± 0.01	6.98 ± 0.08	50.0
	Oil + PP	34.9 ± 1.6	0.45 ± 0.03	8.01 ± 0.37	—
70	Oil alone	75.7 ± 0.1	1.41 ± 0.01	15.17 ± 0.26	—
	Oil + PE	65.9 ± 0.4	1.24 ± 0.07	16.22 ± 0.33	—
	Oil + PE (BHA)	70.8 ± 2.3	1.21 ± 0.01	17.26 ± 0.05	61.0
	Oil + PE (BHT)	56.7 ± 3.1	0.91 ± 0.09	12.37 ± 0.71	66.0
	Oil + PP	69.2 ± 1.4	1.10 ± 0.01	17.18 ± 0.26	—
105	Oil alone	98.7 ± 5.1	2.49 ± 0.01	26.33 ± 0.48	—
	Oil + PE	82.9 ± 3.7	2.02 ±0.07	21.50 ± 0.71	—
	Oil + PE (BHA)	69.9 ± 1.4	1.64 ± 0.01	19.98 ± 0.06	68.0
	Oil + PE (BHT)	54.8 ± 1.8	1.42 ± 0.35	13.28 ± 1.09	70.0
	Oil + PP	76.3 ± 0.1	1.98 ± 0.07	20.10 ± 0.37	—

Schwapo *et al.*[10] have reported considerably less migration of Irganox as compared to BHT in corn oil from low density PE films. Negligible concentrations of leached out antioxidants from commercially available PE and PP films are therefore in conformity with the previous studies[10].

It has been reported[11] that the rate of migration is quite high from PP and PE films which swell rapidly in contact with vegetable oils and organic solvents as compared to rigid PVC films which exhibit negligible swelling. Niebergall and Hartmann[8] have also reported very rapid migration of antioxidants in vegetable oils and ground spices having large percentage of aromatic oils. Though no systematic study has been reported in the literature on the effect of plastic films leached out substances on the stability of vegetable oils, the beneficial effects of incorporating antioxidants in the film on the stability of potato chips and quacker oats have been reported previously[12]. Experiments conducted earlier have revealed loss of BHA as a result of migration to atmosphere as well as to food [13]. In the present study also, the concentration of BHA and BHT in the film tended to decrease during storage. Also, the BHT treated films tended to become yellow indicating further interaction of BHT with film constituents or with oxygen.

It is evident from the above study that stability of vegetable oils packed in plastic films is governed by not only the barrier properties of the film (oxygen transmission rate of the films) but also by the nature and extent of leached out antioxidants from the films which seem to exhibit stabilizing effect on the stored oil against autoxidative degradation of vegetable oils and consequently the rancidity development which is the major cause of spoilage during storage.

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Studies on Stabilization of Wheat Bran

R. VETRIMANI AND P. HARIDAS RAO
International School of Milling Technology and Baking Technology
Central Food Technological Research Institute, Mysore-570 013, India.

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Studies were carried out to determine the shelf-life of bran and its possible improvement by suitable processing. Inactivation of the lipolytic enzyme system in bran was achieved when bran was toasted at 175°C for 40 min. Toasting resulted in inactivation of lipase by 40%, of lipoxidase by 100% and of protease by 50%. Storage trials were conducted with samples of untoasted and toasted bran packed in polypropylene pouches (37 μ thick film). The fat acidity increased from 35 to 175.0 mg KOH/100 g in raw bran stored at 27°C for 105 days whereas the increase was negligible in heat stabilised bran. The peroxide value increased from 0 to 63 and 132 meq/kg oil in toasted and raw bran respectively, during the storage period. Untoasted raw bran developed rancid taste within 20 days, whereas a rancid flavour became perceptible in toasted bran only after 90 days.

Wheat bran forms an important by-product of flour milling industry and about 15-20 per cent of the wheat is normally recovered as bran, during commercial milling of wheat. It is mainly used as a cattle feed either as such or in formulations. However, with the recent information on the beneficial effects of dietary fibre in preventing certain diet related diseases[1,2], its use in food products, particularly in bakery products, has increased considerably[3,4]. Wheat bran is likely to have a poor shelf life as it is rich in enzymes[5] which are present in the aleurone layer, which forms a part of commercial wheat bran. This necessitates a study of shelf life of wheat bran, as there may be a time gap between separation of bran and its utilization in food products. Published information on the storage life of commercial bran is scanty. Therefore, studies were undertaken on the shelf life of bran and its possible improvement, the results of which are presented in this paper.

Materials and Methods

Coarse bran used in this study was obtained from a commercial roller flour mill. Bran was sieved through 60 mesh sieve to remove finer endosperm and bran particles. Toasting of bran was carried out by spreading 500 g of bran in an enamel tray of size 40 × 60 cm and heating in an air circulation oven set at different temperatures (130-200°C) for varying periods (15-120 min.). The optimum period, that is the time required to heat the bran before the colour changes to dark brown was determined by preliminary trials. The toasted bran was then cooled and stored in an air-tight tin container for use in different studies.

Raw as well as stabilized bran samples were packed in 37 microgauge polypropylene pouches of size 12 × 18 cm and stored in two incubators maintained at 27°C and 40°C. The stored samples were withdrawn at suitable intervals and analysed for moisture, free fatty acids, and peroxide value. The organoleptic quality of stored bran was assessed by a panel of six judges.

The moisture, ether extractives, crude fibre, total ash and free fatty acids were determined using standard AOAC methods[6]. Crude protein (N × 6.25) was determined by the micro-Kjeldahl method. Lipase activity of bran was determined by the method of Kantharaj Urs *et al.*[7] and the protease activity was determined by a standard AACC procedure[8]. All the experiments were carried out in triplicate.

Lipoxydase activity was determined by the method described by Tappel[9], with modification as suggested by Axelrod[10]. Five g sample was extracted with 25 ml of 50 mM sodium phosphate buffer (pH 6.8) for 30 min in a cold room (4°C). The extract obtained by centrifuging at 10,000 r.p.m. was used in this assay.

The hydroperoxide formed by reacting 20 ml of the dilute enzyme (1 in 5 dilution) with the substrate viz., linoleic acid of concentrations 20 mM in a buffer solution (0.96 ml of pH 6.8) is measured at a wave length of 234 nm on a spectrophotometer. The increase in the absorbance ratio in one minute of reaction time was calculated from the straight line obtained from the initial velocity of the curve. Then the activity for 100 g sample was calculated and expressed as units/100 g sample.

Results and Discussion

The proximate composition of commercial bran used in this study (Table 1) is within the range of values reported[11]. Toasting of bran as expected only reduced the moisture to 1.2 per cent and the other values remained almost similar when expressed on equi-moisture basis. The fat acidity of fresh bran was found to be 34.9 mg KOH/100 g sample which

TABLE 1. PROXIMATE COMPOSITION OF COMMERCIAL BRAN

Constituent	Level (%)
Moisture	9.85
Total ash	5.78
Crude protein (N × 6.25)	13.12
Ether extractives	3.26
Crude fibre	12.15
Total carbohydrates (by diff.)	55.84
Fat acidity (mg KOH)	34.90

slightly increased to 37.1 mg KOH/100 g sample on toasting at 175°C for 40 min.

The optimum period of toasting evidently decreased from 120 min to just 15 min when the toasting temperature was increased from 130°C to 200°C. The optimum period of toasting was 120, 60, 40 and 15 min at temperatures of 130, 150, 175 and 200°C respectively.

Effect of toasting bran on the enzyme activity: Lipase and proteolytic activities of bran toasted at different temperatures (Fig. 1) indicated that maximum inactivition of these enzymes occurred when the toasting was done at 175°C. However, the extent of inactivation was found to be only 40 and 50 per cent respectively of lipase and protease (Table 2). This partial inactivation even at these higher temperatures might be

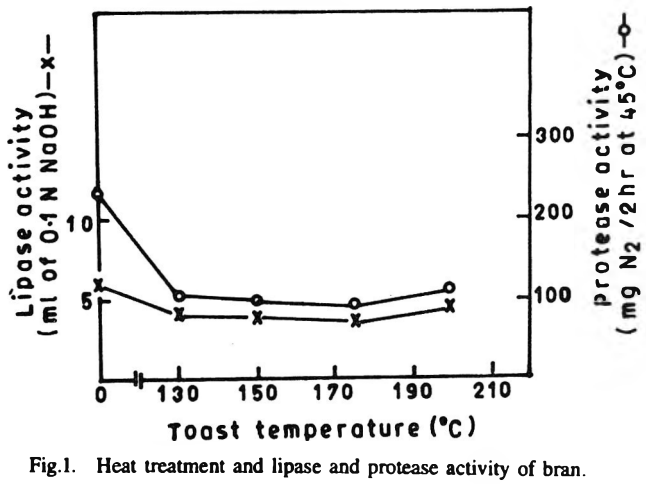

Fig.1. Heat treatment and lipase and protease activity of bran.

TABLE 2. EFFECT OF TOASTING BRAN AT OPTIMUM CONDITIONS* ON THE ENZYME ACTIVITY AND MICROBIAL LOAD

Enzyme	Level**		Inactivation (%)
	Raw	Toasted	
Lipase (ml of 0.1 N NaOH)	6.0	3.6	40
Lipoxidase (units/ml)	750	0	100
Protease (mg N_2O/2 hr at 45°C)	240	120	50
Microbial count	2.1×10^3	1.0×10^3	52.4

*at 175°C for 40 min., **expressed on 14% moisture basis.

possibly due to dry nature of heat treatment. Similar partial and lower inactivation of lipase and protease on toasting of wheat germ was reported by Haridas Rao *et al.*[12]. Toasting at higher temperatures of 200°C resulted in a lesser degree of inactivation, since optimum toasting time was only 15 min. Further, increase in time of toasting to enhance the enzyme inactivation darkened the colour of bran. Lipoxidase activity, however, was completely inactivated during toasting at any temperature studied, except that at highest temperature of 200°C (Fig. 2) at which there remained some residual activity of 250 units/100 g as compared to 750 units/100 g observed for untreated bran (Table 2).

Considering the duration of toasting and the extent of enzyme inactivation, toasting of bran at 175°C for 35-40 min was considered as optimum for stabilizing wheat bran. During the heat treatment, it was observed that total microbial count was reduced to 1.0×10^3 to 2.1×10^3 colonies/g in untreated bran.

Changes in the quality characteristics during storage of bran moisture: The moisture content of stored bran (Fig. 3) indicated 2 to 3 per cent increase, when toasted bran was stored for 140 days. The extent of increase evidently was more

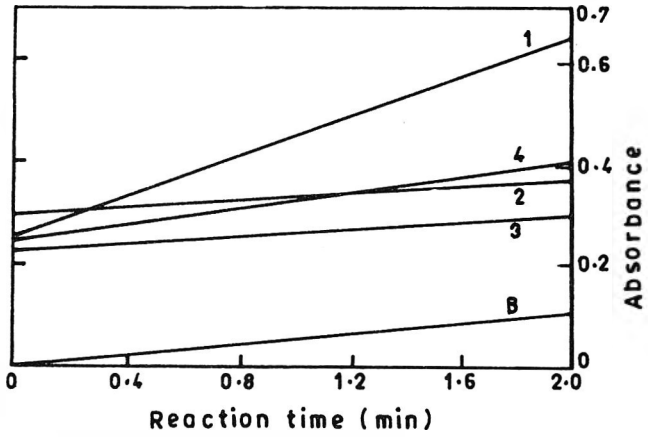

Fig.2. Heat treatment on the lipoxidase activity.
Toasting conditions 1-130°C for 120 min. 2-150°C for 60 min. 3-175°C for 40 min. 4-200°C for 15 min.

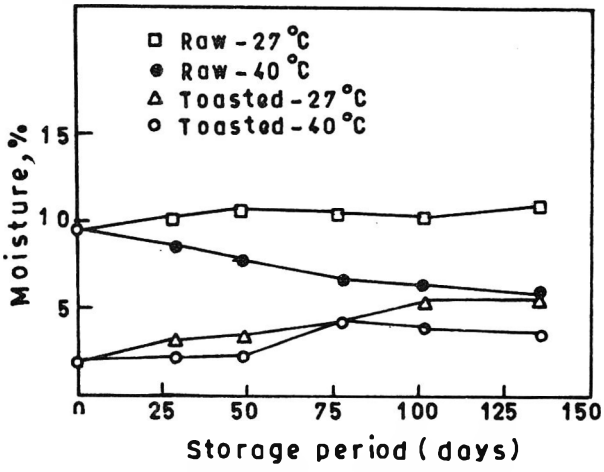

Fig.3. Heat treatment of bran on the moisture pick up during storage.

in bran stored at room temperature (27°C) as compared to that stored at higher temperature (40°C), due to lower RH in the later case. Since the initial moisture in untreated bran was higher (9.9 per cent), a loss of about 4 per cent moisture was observed when the same was stored at 40°C. However, there was hardly any change in moisture when raw bran was stored at 27°C. This indicated that the raw bran was in equilibrium with atmospheric RH ranging from 65-70 per cent.

The changes in the fat acidity during storage of bran for 140 days are depicted in Fig. 4. There was only a marginal increase in the acidity (3-10 mg KOH/100 g) in toasted bran when stored either at 27°C or 40°C. However, in the case of untreated bran, fat acidity increased considerably from 35 to 168.75 mg KOH/100 g when bran was stored at 40°C, while it increased to 142.5 mg KOH/100 g in bran stored at 27°C. However, the maximum values for acidity (180 mg KOH/100 g) were observed much earlier. In the case of bran stored at 27°C. it was observed at a storage period of 105 days while that stored at 40°C, peak value was observed at 90 days of storage.

In toasted bran, the peroxide value increased gradually with storage while in untreated raw bran, it increased rapidly upto a certain period and then decreased with further storage (Fig. 5). Similar to acidity, the increase in peroxide value was also much greater in untreated raw bran as compared to toasted bran. In untreated bran, the peroxide value increased from 0 to over 145 meq/kg oil during 125 days of storage, while it increased only to 52 meq/kg oil for toasted bran during the same period of storage. The increase in peroxide value was (as expected) higher when stored at 40°C than when stored in 27°C in both raw and toasted bran. The maximum value of peroxide was reached in 90 days of storage at 40°C in untreated bran whereas in toasted bran it continued to increase over a storage period of 140 days.

Sensory quality: The organoleptic quality of stored bran is given in Table 3. At 27°C, the raw untreated bran developed a slightly rancid taste at 25 days of storage while the toasted bran did not show any rancid taste upto 90 days of storage. Rancid taste became perceptible in toasted bran stored for 120 days.

Off-flavour was developed much earlier when either toasted or untreated bran was stored at higher temperature of 40°C

The studies indicate that the shelf life of wheat bran could be improved by toasting. Toasting at 175°C for 25 min was found to be the optimum for stabilization of bran and it resulted in the inactivation of lipase by 40 per cent, of protease by 50 per cent and of lipoxidase by 100 per cent. The extent of inactivation of these enzymes was less at the highest toasting temperature (200°C) employed. Stabilised bran could be stored for 90 days at 27°C, while the safe storage period for untreated raw bran at that temperature was only 20 days. as compared to that stored at 27°C. The safe storage life at 40°C was only 15 days and 60 days for raw and toasted bran respectively. The delayed development of rancidity in toasted bran was also indicated by the slower rate of increase of fat acidity as well as peroxide value during storage.

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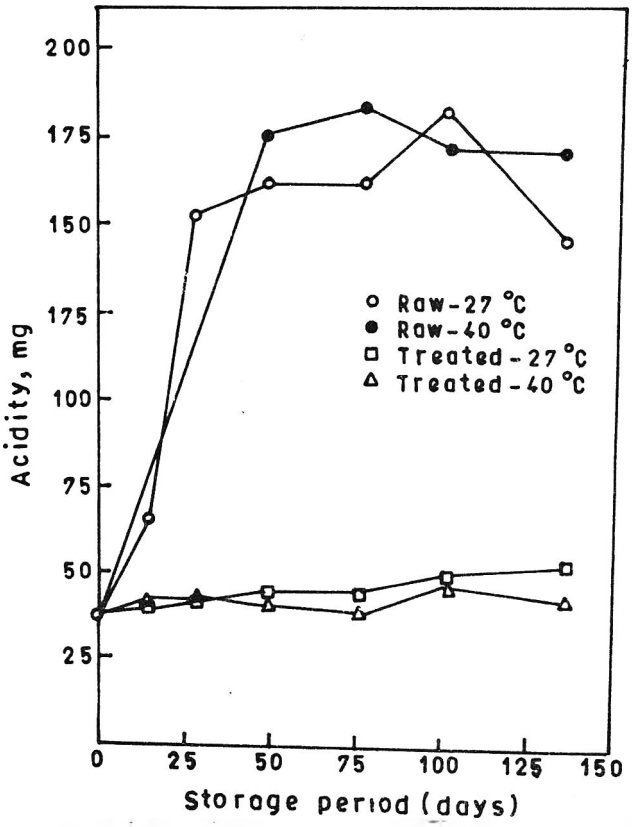

Fig.4. Changes in acidity during storage of heat treated bran.

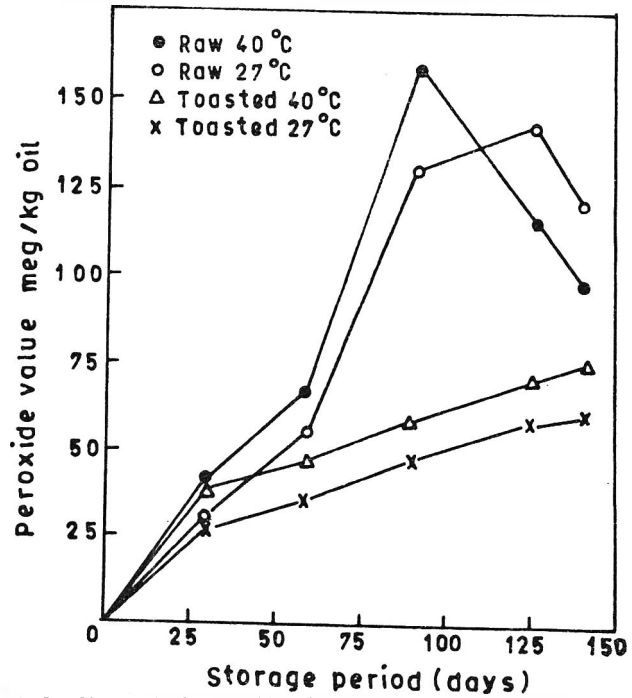

Fig.5. Changes in the peroxide value in heat treated bran during storage.

TABLE 3. ORGANOLEPTIC QUALITY OF STORED BRAN

Treatment	Storage temp (°C)	Quality at indicated storage period (days)								
		10	15	20	25	30	60	90	120	150
Nil	27	N	N	N	+	+	++	+++	++++	—
	40	N	N	+	++	+++	++++	—	—	—
Toasted*	27	N	N	N	N	N	N	N	+	+
	40	N	N	N	N	N	N	+	+	++

N — Normal, + — Slightly rancid, ++ — Rancid, +++ — highly rancid, ++++ — highly rancid and bitter.

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Studies on Different Methods of Extraction of Betalaines from Red Beet *(Beta vulgaris)*

M. ABEYSEKERE*, S.R. SAMPATHU AND M.L. SHANKARANARAYANA
Central Food Technological Research Institute, Mysore - 13, India.

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In the search for natural pigments to replace artificial dyes in food preparations, the pigments of red beet *(Beta vulgaris)* appear to be very promising. The principal red pigment betanine might be considered as a food colourant and accounts for about 75 - 90% of the total betacyanine content of beet. In this study, the pigment recovery efficiencies by different methods of extraction like diffusion extraction in single column and multiple columns and by hydraulic pressing at the laboratory level are compared.

Recent restrictions in the use of artificial food dyes have prompted extensive research into natural pigments as suitable substitutes. The red beet *(Beta vulgaris)* is of interest as a natural red pigment since dehydrated beet (beet powder) and vegetable juice/concentrates are permanently listed as colour additives for food use under the FDA 1960 Colour Additive Amendment and thus need not go through certification procedures[1]. Beet root colour consists of red and yellow quaternary ammonium amino acid pigments of the betalaine class[2,3,4]. The betalaines are water soluble and preparation of crude pigment extracts is, therefore, primarily based on solubility. Purification and separation of individual pigments can be accomplished by electrophoretic[5], chromatographic[6,7] and extractive fractionation[8] techniques at the laboratory level. Since the yield of purified pigment is low, suitable methods have to be found for large-scale extraction and purification of these pigments.

In this paper, a comparison has been made of the presently used methods of extraction.

Beet pigments: The betalaines consist of both red and yellow pigments. The red pigments are betacyanines and the yellow pigments are betaxanthines. The major betacyanine and betaxanthine pigments of beet root are betanine (λ max 535 - 540 nm) and vulgaxanthine-I (λ max 476 - 478 nm) respectively. Of the betacyanine content of beet root, 75 - 95 per cent consists of betanine. The ratio between red and yellow pigments determines the hue of the pigment extract. High values of pigment concentration as compared to anthocyanin containing plants and high yields per acre make the beet a valuable source of food colourant eventhough its chemical properties restrict application to some foods[9-10].

Analysis of pigments: The spectrophotometric method of Nilsson[11] directly determines the betacyanine and betaxanthine pigments in beet root without initial separation. The extinction values ($E^{1\,cm}_{1\%}$) of betanine and vulgaxanthine-I are 1120 and 750 at their respective absorption maxima.

For estimation of the pigments in beet pulp, 25 g of a representative sample was blended with the extracting solvent (citric acid - ascorbic acid mixture) and made up to 250 ml. One ml of the extract was diluted to 10 ml and absorbance was measured at 540 nm and 480 nm in one cm cells.

$$\text{Per cent betacyanines} = \frac{X}{1120} \times \frac{250}{25} \times \frac{100}{10} = X/11.2$$

$$\text{Per cent vulgaxanthines} = \frac{Y}{750} \times \frac{250}{25} \times \frac{100}{10} = Y/7.5$$

Where X and Y are corrected absorptions of betanine and vulgaxanthine respectively as described by Wiley *et al*[12].

For estimation of the pigments in various extracts, appropriate dilution factors were used.

Optimising of processing conditions: It was observed that the red colour of raw beet extracts was unstable. This may be attributed mainly to the polyphenolase enzymes present in raw beet[13,14]. Steam-blanching was carried out to destroy the enzymes. In order to ensure maximum stability of the product, it was necessary to optimise the blanching conditions, since excessive steaming would result in degradation of the pigment. Betalaines are most stable between pH 4.0 and 5.0[15]. Hence a solution containing 0.05 per cent citric acid and 0.01 per cent ascorbic acid in water was used as the extracting solvent and compared against distilled water control.

Materials and Methods

Red beet *(Beta vulgaris)* obtained locally and known in the trade as 'Bangalore' variety was used in the studies.

Blanching studies: Four kg separate lots of whole and cut beet were blanched at 100°C using open steam in a retort for 5, 10 or 20 min, cooled and immediately pulped using a fruit mill. Pigment content in representative samples was estimated using separately distilled water and citric acid - ascorbic acid

**Present address:* Ceylon Institute of Scientific and Industrial Research, 363, Baudhaloka Mawatha, Colombo 7, Sri Lanka.

mixture as solvents. Pigment stability in the extracts was monitored at 25°C (room temperature) and 6°C.

Extraction studies: Pulped beet from the 10 min blanched (cut) lot and room temperature extraction with citric acid — ascorbic acid mixture were employed in all the extraction trials. The following methods of extraction were evaluated in laboratory experiments. Progress of recovery of pigments and soluble solids (°Brix) in drained/filtered extracts was monitored at intervals till equilibrium was reached.

1. *Effect of contact time with stirring:* Beet pulp (300 g) was separately mixed with 300 and 600 ml solvent and kept gently stirred.
2. *Effect of contact time without stirring:* Beet pulp (300 g) was separately mixed withh 300 and 600 ml solvent and allowed to soak.
3. *Extraction in single columns:* Beet pulp (150 g each) was packed in 5 columns (4 cm diameter and 30 cm long) and contact time with solvent (150 ml) was varied. Drained extracts were analysed.
4. *Multiple column extraction:* Beet pulp (150 g each) was packed in 5 columns as above. A 150 ml of solvent was added to column 1 and allowed to soak for 30 min. The extract was drained off, its volume adjusted to 150 ml with fresh solvent, and added to column 2. The extract was again drained off after 30 min soaking, adjusted to 150 ml volume with fresh solvent and added to column 3. This procedure was repeated for 5 columns. Brix and pigment content were monitored at every stage. This may be considered as the first cycle of extraction of building up solids and pigment from column 1 through column 5. Three more such cycles were repeated using fresh solvent for the first column.
5. *Extraction by pressing:* Beet pulp (1 kg) was pressed out using a laboratory hydraulic press. The press cake (300 g) was mixed with an equal quantity of fresh solvent, allowed to equilibrate for 30 min and pressed again. A third press was also similarly made.

Results and Discussion

Blanching studies: Beet with treatment of 10 min blanching (cut) showed the highest pigment content. Samples of the pulp extracts (at an effective concentration of 1 g pulp/100 ml extract) from whole beet 5 and 10 min blanched, and, cut beet 10 and 20 min blanched and whole control (unblanched) were stored at 6°C and at 25°C and the absorbance determined after 3 and 6 days. It was observed that all the extracts in distilled water had completely decolourised in 3 days at 25°C while at 6°C the pigment was lost at a considerable rate of 12.2, 12.5, 18.7, 2.1 and 16.3 per cent, for whole beet 5 and 10 min blanching, and cut beet 10 and 20 min blanching and whole unblanched respectively. However in citric acid - ascorbic acid mixture, the loss of pigment was smaller for 25°C (by 60-78 per cent) and 6°C (by 2-11 per cent) storage. The retentions of red pigment for the five treatments (as in the above order) in citric acid — ascorbic acid solution were 56.0, 82.5, 81.3, 87.5 and 51.0 per cent for 6°C and 51.0, 70.0, 56.0, 60.4 and 36.7 per cent for 25°C storage, respectively. It can be concluded that citric acid-ascorbic acid mixture was a better solvent for extraction and pigment stability and that the optimum blanching time was 10 min, with the beet being cut before blanching. Beet should be blanched immediately after cutting as nearly 20 per cent of the pigments had got destroyed in one hour from cut unblanched beets.

Extraction studies: For extraction by soaking in solvent, constant Brix values (6° and 4° Brix) were attained after 30 min of soaking both with and without stirring (Table 1). Pigment recoveries were better without stirring (58 and 78 per cent) as against stirred extraction (55.6 and 66.7 per cent) for material to solvent ratios of 1:1 and 1:2 respectively. The lower pigment recovery in stirred extraction can possibly be attributed to the oxygen sensitivity of the betalaines.

In the case of single column extraction also, equilibrium in brix value (7°) was attained in 30 min and 50 per cent of betacyanines could be extracted with pulp to solvent ratio of 1:1.

The progressive build-up of pigments and soluble solids (brix value) in multiple column extraction is presented in Table 2. Maximum levels reached were 0.6 mg betacyanines per ml of the extract and 10° Brix for the first extracts from column 3 and 4 respectively. The values remained steady thereafter.

As it is desirable to achieve maximum pigment recovery with the minimum use of solvent, it would be interesting to compare the cumulative pigment recoveries at similar material to solvent ratios at various stages of multiple column extraction (Table 3). Column 1 extract 1 (C_1E_1), C_2E_2, C_3E_3 and C_4E_4 can be considered to have an effectively similar material to solvent ratio of 1:1; the respective pigment

TABLE 1. EFFECT OF STIRRING ON EXTRACTION OF PIGMENTS FROM RED BEET

Extraction method	Pulp to solvent ratio	Final °Brix	Betacyanines in beetroot (g/100 g)	% betacyanines extracted	Red to yellow ratio
Soaking with stirring	1 : 1	6.0	0.045	55.6	1.17
	1 : 2	4.0	0.045	66.7	1.02
Soaking without stirring	1 : 1	6.0	0.045	58.0	1.16
	1 : 2	4.0	0.045	78.0	1.13

TABLE 2. PROGRESSIVE BUILD-UP OF PIGMENTS AND SOLUBLE SOLIDS DURING MULTIPLE COLUMN EXTRACTION OF RED BEET

Column number	Extract 1		Extract 2		Extract 3		Extract 4	
	Beta-cyanines concn. (mg/ml)	°Brix	Beta-cyanines concn. (mg/ml)	°Brix	Beta-cyanines concn. (mg/ml)	°Brix	Beta-cyanines concn. (mg/ml)	°Brix
1	0.32	6.5	0.16	5.0	0.17	4.0	0.09	3.0
2	0.47	9.0	0.32	7.0	0.21	5.0	0.10	4.0
3	0.49	10.0	0.44	9.0	0.30	7.0	0.22	5.0
4	0.60	10.0	0.58	10.0	0.37	8.0	0.32	6.0
5	0.60	10.0	0.58	10.0	0.40	8.0	0.32	6.0

TABLE 3. CUMULATIVE EXTRACTION OF PIGMENTS DURING MULTIPLE COLUMN EXTRACTION OF RED BEET

Column/s	Betacyanines in charged material (g)	% betacyanine recovery (cumulative)			
		Extract 1	Extract 2	Extract 3	Extract 4
1	0.1125	42.7 (1:1)	64.0 (1:2)	87.1 (1:3)	98.4 (1:4)
1 + 2	0.2250	31.1 (2:1)	52.4 (2:2)	66.3 (2:3)	73.1 (2:4)
1 + 2 + 3	0.3375	21.9 (3:1)	41.5 (3:2)	54.8 (3:3)	64.6 (3:4)
1 + 2 + 3 + 4	0.4500	20.0 (4:1)	39.3 (4:2)	51.7 (4:3)	62.3 (4:4)
1 + 2 + 3 + 4 + 5	0.5625	16.0 (5:1)	31.5 (5:2)	42.1 (5:3)	50.7 (5:4)

Figures in parenthesis indicate effective ratio of material to solvent for extraction.

recoveries were of the order of 43, 53, 55 and 62 per cent. In the case of C_1E_2 and C_2E_4 having effective ratios of 1:2, the pigment recovery was 64 and 73 per cent respectively. However, these values are quite low in comparison with C_1E_4 at 98.4 per cent recovery but requiring 1:4 material to solvent ratio.

The betacyanines/vulgaxanthines ratios for the final extracts from the four cycles of extraction were 1.00, 1.03, 1.05, and 1.10 respectively, indicating red pigment stability during the course of multiple column extraction.

Extraction by hydraulic pressing yielded 61.9, 16.5 and 9.5 per cent recovery of red pigments for the first, second and third pressings respectively, totalling 88 per cent at an effective material to solvent ratio 1 : 1.25.

Adams *et al.*[16] used a screw-type continuous press to extract juice from blanched, peeled, diced and comminuted beet; the pulp was wetted and pressed again. Wiley and Lee[12] studied the efficiency of a continuous counter-current diffusion apparatus. They obtained a recovery of about 71 per cent betanine using unblanched sliced beet, solvent (acidified water) to material ratio of about 1.5/1, and elevated temperatures (70-74°C) for extraction. Per cent betalaine lost during processing was 34.4 and 26.4 for plain water (pH 6.3) and acidified water (0.05 per cent citric acid, pH 5.2) respectively for the Dark Red Detroit variety of beet. Wiley *et al.*[17] studied this diffusion extraction in greater detail and showed that an initial fill temperature of 85°C, followed by a lower temperature (75°C) of extraction and juice pH of 5.2 were best conditions if extraction of both red and yellow

TABLE 4. EXTRACTABILITY OF BETACYANINES FROM RED BEET BY DIFFERENT METHODS.

Extraction method	Material to solvent ratio	% betacyanines extracted
(i) Soaking with stirring	1 : 1	55.6
	1 : 2	66.7
(ii) Soaking without stirring	1 : 1	58.0
	1 : 2	78.0
(iii) Extraction in single column	1 : 1	50.0
	1 : 2	64.0
	1 : 4	98.4
(iv) Extraction in multiple columns	1 : 1	62.3*
	1 : 2	73.1**
(v) Hydraulic pressing	1 : 1.25	88.0

*4 columns. **2 columns.

components of beet colour were required. By a proper selection of conditions, a maximum recovery of 90 per cent for betacyanines or 80 per cent for betaxanthines could be obtained. Block *et al.*[18] while studying the energy requirements for beet colourant production used a centrifugal separator to separate the pulp from supernate.

Table 4 gives a comparative data of betacyanines recovery obtained by the various methods of extraction considered in the present study. Hydraulic pressing gave relatively the highest pigment yield (88 per cent) for a given material to solvent ratio (1:1.25). Next in order for a ratio of 1:1 was multiple column (4 nos.) extraction giving an yield of 62.3 per cent. At the ratio of 1:2, extraction by soaking with stirring (78 per cent) was slightly superior to multiple column (2 nos.) extraction (73 per cent). Single column multiple extraction (ratio 1:4) gave the highest pigment yield (98.4 per cent) but obviously required more solvent.

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A Study on the Behaviour of Air Microflora in Food Industries

SHABBIR A. SAYEED AND R. SANKARAN
Defence Food Research Laboratory, Mysore - 570 011, India.

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Qualitative composition of the air microflora in convenience food factories was appreciably similar both in the day-long monitoring and spot sampling. Bacteria constituted a dominant fraction with Gram positive cocci being the most prevalent. Species of *Aspergillus* were more abundant than other moulds. Microbial load in terms of bacteria and moulds monitored as a function of time exhibited an oscillatory behaviour during the day with peak counts coinciding with hectic processing or cleaning operations in the factories. The composition of microflora analysed by spot sampling during the manufacture of different food items did not differ significantly from that observed on day-long sampling. The qualitative and quantitative variations in air microflora observed in the food industries seem to reflect a complex pattern of interactions between the numerous intrinsic and extrinsic parameters that govern the prevalence of microorganisms in the air.

The potential hazards of airborne microbial contamination of food products resulting in food spoilage and foodborne illnesses have drawn the attention of scientists. Currently, the importance of indoor air hygiene in food processing industry is being emphasised as much as in the medical sciences. Downgraded product quality or reduced product shelf life have often been related to airborne contamination of the products by microorganisms from sources within the processing areas of the food industries. While air has no natural microflora of its own, contamination of clean air occurs through a number of sources such as soil, water, raw materials of both plant and animal origin and human beings[1,2]. The magnitude of airborne contamination is known to be determined by not only the microbial load in air but also by the period of exposure[3].

Aflatoxin induced cancer in the workers of corn and groundnut processing plants has been related to high levels of Aspergillus spores in the airborne dust samples[4,5]. Reports are also available on the microbial content of air in food production and distribution areas[6], carcass storage under refrigeration[7], citrus packing houses[8] and in corn processing facilities[9]. However, information on the microbial profile of air in the enclosed critical hygiene areas of food industries at various stages of processing and packaging of the products is scanty.

Knowledge of the load and composition of air microflora in the indoor atmosphere of industries at different times of a normal working day as well as the influence of various interacting parameters such as weather conditions, product range and quantity, types of processing operations involved etc., will be of immense value in evolving suitable methods to control airborne pollution and reduce the risk of product contamination. The present study is an attempt to generate information on these lines in order to gain a better insight into the problem of airborne contamination and its control in food industries. We present data on qualitative and quantitative assessment of the distribution and composition of microorganisms in the enclosed atmosphere of food industries manufacturing several popular Indian convenience foods.

Materials and Methods

Sampling sites: Two convenience food industries situated at Mysore, South India, were chosen for the purpose of this investigation. The range of convenience food items manufactured include *Jamoon mix* (milk based sweet), *Idli mix* (steamed rice cake), *Vada mix*, Vegetable *pulav* (fried rice), *Puliogare mix* (rice with tamarind preparation) and spice mix. While both the factories operate round the year manufacturing similar products, one of them caters to the needs of the local market (designated as site A) and the other has a larger market spread over the country with a higher production capacity (designated as site B). Processing as well as packaging of the products are carried out in the same hall in these factories and therefore, samples of air were always collected from various parts of the premises in triplicates.

Air sample collection: Representative air samples from the processing-cum-packaging halls of the factories were collected at different times during a normal working day and on several such days spread over a period of five months. Quantitative samples for the estimation of airborne microbial count per unit volume of air were drawn using a Biotest RCS centrifugal air sampler (Biotest, West Germany) as described earlier[10]. Before every series of sampling, the impeller drum

assembly was sterilised at 121°C for 20 min. After running the impeller for a predetermined time (30 sec) the exposed agar strips were removed and incubated. Total counts were made on TSA (pancreatic digest of casein, soy bean peptone, sodium chloride, agar) strips after 48 hr incubation at 37°C and yeast and mould counts were obtained on Rose Bengal Agar (Peptone, Dextrose, Salts, Rosa Bengal, Streptomycin, Agar) strips incubated for 120 hr at 30°C.

The colony forming units (cfu) per unit volume of air were calculated as given below:

$$\text{cfu } m^{-3} = \frac{\text{colonies on agar strip} \times 25}{\text{sampling time (min)}}$$

Results and Discussion

Population density and composition of the indoor air microflora per unit volume of air were monitored at regular time intervals during normal working days at site B. A representative pattern of the mean composition of microbes in the air on any given day of sampling is shown in Fig.1. Total bacterial counts accounted for 83.5 per cent of the mean microbial load for the day, while yeast and mould cfus were only 16.5 per cent of the total. Among the bacterial population, the most predominant were gram positive cocci which represented 59.4 per cent of the total load and 71.1 per cent

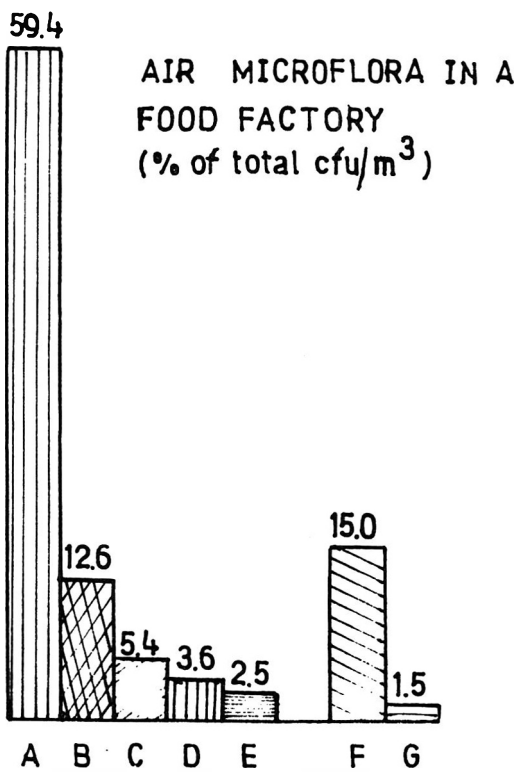

Fig.1. Qualitative and quantitative composition of microorganisms present in the indoor air of a food factory. A, Gram positive cocci; B, Gram-positive rods (non-spore forming); C, Gram-positive rods (spore forming); D, Actinomycetes; E, Gram-negative rods; F, Moulds; G, yeasts.

of the bacterial counts. Other significant fraction was that of gram positive rods (both spore formers and non-spore formers) accounting for 18 per cent of the total air flora. *Aspergillus sp.* including *A. niger* and *A. flavus* represented 42.8 per cent of the total mould counts amounting to 7.1 per cent of the day's mean microbial load. Although the total number of microorganisms *per se* varied on different sampling days, qualitative and quantitative composition of the sample did not differ significantly. Table 1 presents data on the content of microbes in the air samples at site A. Unlike the day-long monitoring of air quality at site B, here the sampling was restricted to a fixed time once a day but repeated on several days, also exhibited a remarkable similarity in the qualitative composition of the microflora.

The high incidence of bacteria in air may be partly due to the nature of raw materials used in these factories such as spices, pulses, grains and milk powder which can contribute to the air microflora during cleaning and processing operations through dust formation. However, Singh *et al.*[10] reported an almost equiproportional distribution of bacteria and fungi (45 and 55 per cent respectively) in the air of a closed room. Similarly, another study in a confectionery factory[11] also reported a predominance of moulds. While in these reports, the Gram positive cocci constituted only a small fraction of the bacterial counts, our results show a significantly high coccal cfus in the air at both the sample sites (Fig.1 and Table 1). Prevalence of Gram positive cocci in the processing and packaging areas of food industries can be attributed partly to personnel location and movement in the critical operation areas as human beings are known to be a major source of these organisms[12,13]. More recent observations of Someya and Chihara[14] on the predominance of Gram positive cocci and Gram positive non-sporeforming rods constituting 81 per cent of the bacterial cfus in grocery

TABLE 1. COMPOSITION OF THE ATMOSPHERIC MICROFLORA SAMPLED AT A FOOD FACTORY (SITE A).

Type of microorganism	cfu/m^3	% total load
Bacteria		
Gram-positive cocci	4288	55.00
Gram-positive non-spore forming rods	1280	16.40
Gram-positive spore forming rods	256	3.30
Gram-negative rods	448	5.70
Gram-negative cocci	Nil	Nil
Actinomycetes	128	1.65
Total	6400	82.05
Moulds		
Aspergillus sp	752	9.64
Mucorales	187	2.46
Unidentified fungi	300	3.85
Yeasts	161	2.00
Total	1400	17.95

shops and hospital waiting rooms in an university campus lend support to our findings in the food industries. Extremely small numbers of Gram negative rods observed in our study are also consistent with the fact that the sample sites did not handle any raw materials of animal origin with which these organisms are more commonly linked.

Changes in the microbial load and composition at site B were monitored as a function of time of the day. Fig. 2 shows a representative pattern of time dependent changes in the bacterial content of air at site B. From an initial low level, at the beginning of the working hours, bacterial cfus peaked to the maximum value within 45 min, by which time the processing activity in the factory had started in full swing. The activities such as raw material cleaning and processing, grinding and blending operations were carried out in the same hall. However, over the next three hours the counts fell rapidly reaching the minimum level of the day. During this time, the production activity had stabilized and was at a steady state. Soon after lunch break, bacterial load in the air increased parallel to the changes in the intensity of activity, though the second maximum was far short of the initial peak. Towards the end of the day, as a consequence of cleaning operations sharp rise in the bacterial population in air was noticed. This dusting and sweeping mediated increase in the load rapidly settled to a steady state value which constituted the initial reading of the following morning. A pattern closely comparable with that of the bacterial profile was also observed in the case of yeast and mould colony forming units (Fig.3). The maximal and minimal counts were observed at similar time intervals as in the case of bacteria. These time dependent changes in the microbial load inside the factories (Fig.2 and Fig.3) are in line with the established concept that the microbial population in the air of enclosed space tends to vary with time as a result of ventilation and deposition as well as changes in the amount of mechanical and human activities[15].

Temporal variations in the distribution of different types of bacteria in the air are shown in Fig.4. Throughout the day

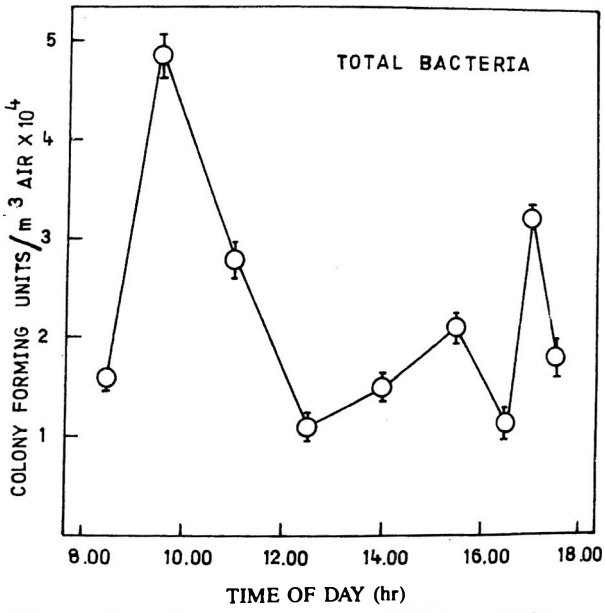

Fig.2. Bacterial load in the indoor air of a food factory (site B) monitored at various times during a normal working day. Values are the mean of 12 independent measurements.

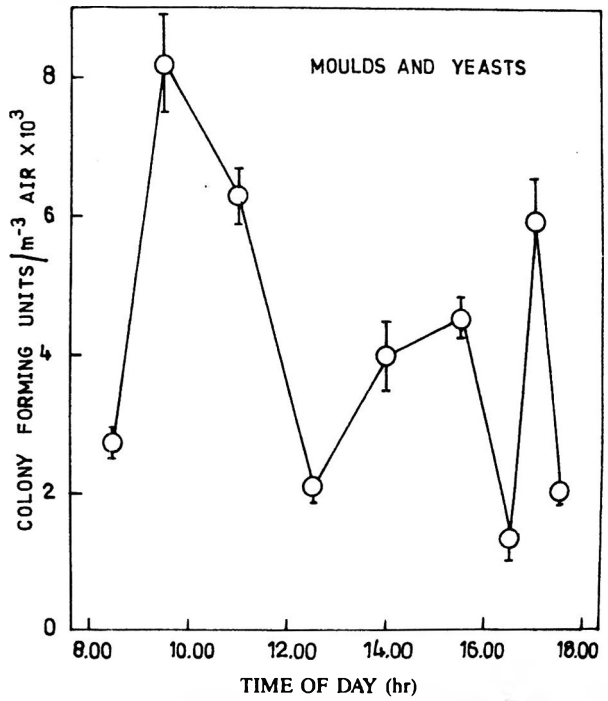

Fig.3. Yeast and mould colony forming units in the indoor air of a food factory (site B) monitored at various times during a normal working day. Values are the mean of 12 independent measurements.

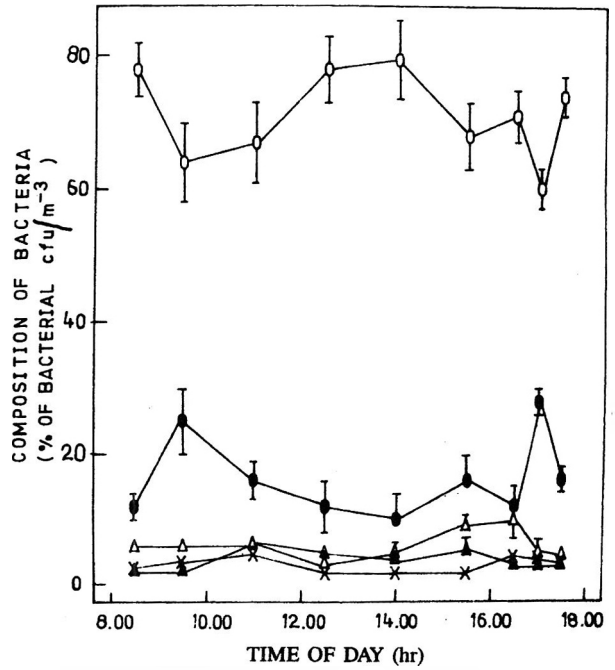

Fig.4. Time dependent changes in the composition of the bacterial population present in the indoor air of a food factory (site B). O, Gram-positive cocci; ●, Gram-positive rods (non-spore forming); △, Gram-positive rods (spore forming); ▲, Actinomycetes; X, Gram-negative rods.

Gram positive cocci were predominant, accounting for 60 per cent of total bacterial load followed by Gram positive rods of non-spore forming type. Both these types of bacteria exhibited an oscillatory behaviour where the distribution of Gram positive cocci was inversely reciprocated by the Gram positive rods. Other types such as Gram negative rods, actinomycetes, etc., constitute only a minor component of the bacterial population. At any given time, the Gram positive cocci and non-spore forming rods together accounted for about 84-90 per cent of the total bacteria in the air with a day's mean of 87.3 ± 2.9 per cent. While Gram negative rods were observed atleast at 2 per cent level most of the times, no Gram negative cocci were observed in the air at the sample sites.

Analysis of the species composition of moulds in air at various times of the day (Fig.5) showed a predominance of *Aspergillus sp.* including *A. niger* and *A. flavus* representing 43 per cent of the yeast and mould counts. *A. flavus* was observed throughout the study period with a mean occurrence of 13 per cent of the yeast and mould counts. Various unidentified fungal species with no particular pattern in individual occurrence constituted the second major category, while mucorales, particularly *Mucor* sp. and *Rhizopus* sp. were third in abundance. A consistently low count (< 10 per cent) was recorded for yeasts at all the times of sampling.

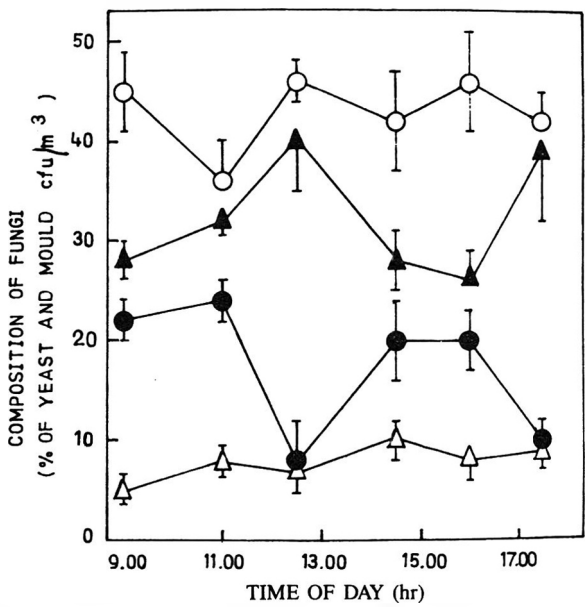

Fig.5. Time dependent changes in the composition of the yeast and mould colony forming units present in the indoor air of a food factory (site B). O, *Aspergillus* sp; ●, Mucorales; △, Yeasts; ▲, Unidentified moulds.

The oscillatory patterns observed in the case of bacterial types (Fig.4) and different species of moulds (Fig.5) can be explained atleast partly in terms of the settling properties of various suspended particles. Displacement of air due to movements such as turbulence, convection currents, etc., within the halls consequent to constant ventilation apart from the mechanical and human factors may also influence the temporal distribution. However, the inverse relationship observed in our study between the Gram positive cocci and Gram positive rods (Fig.4) during the day is not clear. But their abundance in the air (87 per cent) compares very well with a similar observation made on indoor air subjected to frequent changes due to intense human activity[14]. The fungal population mostly consisted of commonly known food spoilage organisms and is in line with corn processing houses[4,5] and atmosphere studies[16].

Air microbes at both the sites were also monitored to ascertain if the type of food item being processed had any bearing on their load and composition at any given time (Table 2). Since there was a characteristic time-dependent variation in the microbial population, air samples were collected during the second maximum phase in the afternoon (Fig.2 and Fig.3) to facilitate easy comparison between product dependent samples. Data shown in Table 2 indicate that the per cent contribution of bacterial and fungal population to the total air microflora remained more or less constant irrespective of the type of product manufactured. Also, the product independent means of the bacterial and fungal counts (82.4 ± 5.6 per cent and 17.6 ± 5.6 per cent, respectively) closely correlate with the corresponding mean counts shown in Fig.1. However, the cfu/m^3 for bacteria and yeast and moulds *per se* varied significantly between the products monitored. This fluctuation may be a reflection of the complex pattern of interaction between the intrinsic and extrinsic parameters that govern the prevalence of microorganisms in air. Detailed investigations need to be carried out in order to elucidate the exact nature of

TABLE 2. MICROBIAL CONTENT OF AIR* IN FOOD INDUSTRIES DURING THE PRODUCTION OF VARIOUS CONVENIENCE FOODS

Product	Total bacteria (cfu/m^3 air)	% of total air microflora	Moulds & yeasts (cfu/m^3 air)	% of total air microflora
Jamoon mix	6400 ± 1163	82.0	1400 ± 162	18.0
Veg. pulav (dehydrated)	7300 ± 639	82.0	1600 ± 402	18.0
Idli mix	4800 ± 400	80.6	1500 ± 162	20.8
Puliogere mix	12000 ± 1510	75.7	3850 ± 324	24.3
Spice mix	21000 ± 1050	82.2	4550 ± 306	17.8
Vada mix	41600 ± 2447	93.6	2850 ± 382	6.4

*Samples were drawn at 15.00 - 15.30 hr when the second maximal counts of the day were observed.

relationship between the products manufactured and the qualitative and quantitative composition of the air microflora in food industries.

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Studies of Some Assam Rice Varieties for Processing and Nutritional Quality

L. SAIKIA
Assam Agric. University, Jorhat - 785013 India
AND G.S. BAINS
Punjab Agric. University, Ludhiana, India.

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Studies on processing and nutritional quality of three varieties of Assam rice revealed a higher angle of repose with superior milling performance regarding yield of brown and head rice. 'Monoharsali' variety exhibited greater resistance to breakage among all the varieties tested. Assam varieties showed low protein content in brown (6.3 to 7.2%) and raw milled rice (6.0 to 6.7%). The differences in reducing sugars and amylose contents among different Assam varieties of rice were not so conspicuous. Assam varieties showed higher fat content both in brown and raw milled rice.

Rice (*Oryza sativa* L.) is the most important food crop in India, supplying, on an average, one third of the calories. The protein content of rice is low, however, the quality of the protein is superior. Different tests were proposed for assessing quality of white milled rice[1-6]. Studies on milling[7] and quality of milled rice[8-9] were extensively carried out. However, the information about the processing and nutritional characteristics of the rice varieties grown in Assam was lacking and hence the present study was undertaken.

Materials and Methods

Paddy samples of 'Monoharsali', 'Prosadbhog' and 'Jaha' were collected from Rice Research Station, Titabar, Assam Agri. University, Assam. The samples were adequately cleaned.

Physical parameters: Angle of repose was done by the method of Anderson.[10] Thousand kernel weight, Length/Width (L/W) ratio and bulk density were estimated by the method of Juliano *et al.*[11] Density was determined by the method of Bhattacharya *et al*[12]. and moisture by AACC[13].

Milling tests: The paddy crop was harvested at commercial maturity (full turning of colour to grey yellow in respect to 'Monoarsali' and 'Prasadbhog' and light black colour for 'Jaha'), and the grains dried to about 15 per cent moisture content. Laboratory Satake sheller with rubber rollers was used to dehusk representative paddy samples. Breakage was minimised by adjusting the rubber rolls optimally suited to size of the grain. The yield of whole, brown and broken rice were determined using Burrows Rice Sizing Machine. To determine the yield of head rice, weighed shelled rice (50g) was subjected to 5 per cent polishing in the Kett Polisher, Type TP-2 (Kett Electrooic Laboratory, Japan). Varietal interaction to extended milling was determined taking 50 g well mixed shelled samples and subjecting them to polishing for 10, 20, 30, 40, 50, 60, 70, 80, 100, 130, 160 and 200 sec, and the yields of brown, broken and head rice were expressed as per cent on the basis of cleaned paddy.

Biochemical parameters: Amylose, protein, reducing sugars and free fatty acids (FFA) were determined as per Juliano[14], micro Kjeldahl, potassium ferricyanide and AACC methods[13] respectively. Fat was estimated by Soxhlet extraction method, using petroleum ether (b.p. 60° to 80°C) for 16 hr.

Results and Discussion

Physical characteristics: The analysis was carried out in four replicate samples selected at random. The physical characteristics of paddy, brown and milled rice (5 per cent polish) are shown in Table 1. Considerable variation in the grain dimensions was noted. Significant variation in 1000 kernel weight, L/W ratio, porosity were found for paddy, brown and milled rice. Slender grained fine variety 'Jaha' had lowest 1000-kernel weight for paddy (14.57), brown rice (11.54) and milled rice (11.40). 'Monoharsali' gave the highest 1000-kernel weight as it was a coarse variety. No significant difference was found in case of density and bulk density of the paddy, brown and milled rice. The findings are equally in line with the results reported by Bhattacharyya *et al*[12]. and Sidhu *et al*[8]. Porosity varied slightly with the variety. Coarse variety 'Monoharsali' (51.17 per cent) and 'Prosadbhog' had the higher porosity (53.44 per cent), than the 'Jaha'. Angles of repose were found to be higher in the varieties tested.

Yield of intact brown and total rice: Milled at appropriate moisture content (15 per cent), the yield of brown rice including brokens, husk and moisture differentials are presented in Table 2. Significantly higher yields of intact rice were found for 'Jaha' (78.9 per cent) with 2.8 per cent of broken rice resulting in the total yield of 81.70 per cent brown

TABLE 1. PHYSICAL CHARACTERISTICS OF PADDY BROWN AND MILLED RICE

Variety	1000 kernel wt	L/W ratio (g/ml)	Density (g/ml)	Bulk density (%)	Porosity (%)	Angle of repose (<)
			Paddy			
Jaha	14.57	3.28	1.18	0.60	49.68	47.00
Monoharsali	28.30	3.24	1.22	0.60	51.17	45.00
Prosadbhog	18.66	3.80	1.24	0.58	53.44	47.50
S. Em.	± 0.0574	± 0.0047	±0.0058	±0.0058	± 0.5773	± 0.5774
C.D at 0.05	0.1301	0.0107	0.0130	0.0131	1.3059	1.3059
,, 0.01	0.1870	0.0153	0.0187	0.0188	1.8763	1.8763
			Brown rice			
Jaha	11.54	3.11	1.39	0.79	43.53	—
Monoharsali	22.33	2.76	1.39	0,82	41.49	—
Prosadbhog	14.54	2.99	1.39	0.80	42.96	—
S. Em	± 0.5773	± 0.0058	—	±0.0058	± 0.5773	—
C.D at 0.05	1.3059	0.0188	—	0.0131	1.3059	—
,, 0.01	1.8763	0.0188	—	0.0188	1.8763	—
			Milled rice (5% polishing)			
Jaha	11.40	2.63	1.41	0.80	42.26	—
Monoharsali	21.65	2.43	1.41	0.83	40.92	—
Prosadbhog	14.23	2.90	1.41	0.81	42.41	—
S. Em	± 0.5773	± 0.0337	—	±0.0058	± 0.5773	—
C.D at 0.05	1.3059	0.0761	—	0.0131	1.3059	—
,, 0.01	1.8764	0.1094	—	0.0188	1.8763	—

TABLE 2. YIELD OF BROWN RICE AND MOISTURE DIFFERENCE IN PADDY VARIETIES

Variety	Brown rice (%)			Husk (%)	Moisture (%)		
	White or intact grain	Broken	Total		Paddy	Brown rice	Husk
Jaha	78.9	2.8	81.7	18.3	14.5	14.9	10.5
Monoharsali	75.7	5.3	81.0	19.9	14.6	16.0	10.4
Prosadbhog	73.5	4.8	78.3	21.7	14.4	15.0	10.7
S.Em	± 0.0577	± 0.0577	± 0.0577	± 0.0577	± 0.0568	± 0.0570	± 0.0577
C.D at 0.05	0.1305	0.1305	0.1305	0.1305	0.1303	0.1306	0.1305
C.D at 0.01	0.1876	0.1876	0.1876	0.1876	0.1872	0.1878	0.1876

rice. Yield of total rice in 'Monoharsali' (81.0 per cent) was at par but with 5.3 per cent broken rice. Contrary to what has been reported by Matthews and Spadaro[26] long grained variety did not always imply higher breakage, because medium fine grain variety 'Jaha' had the lowest breakage.

Milling interactions: Milling interaction is greatly affected by variety and environment of growth[15] time of harvest[16,17], field moisture contents[18] storage condition[19] and drying temperature[20]. The results of analysis of the interactions of varieties and milling time on yield and susceptibility to breakage are illustrated in Fig 1,2 and 3, respectively.

Highest yield of total milled rice was recorded for 'Jaha' (Fig.1) which was maintained till 130 sec of milling but dropped to the lowest level (55.6 per cent) when milled for 200 sec, exhibiting soft texture of the endosperm in comparison to 'Monoharsali' which gave the highest yield of 68 per cent for 200 sec. A significant effect of variety on milling yield per cent breakage and extent of degree of polish in the initial stages of milling compared with extended milling has been observed by Sidhu *et al*[8]. Degree of polish suffered by the varieties due to extended milling, was approximately similar when milled for 130 sec, (Fig. 2) but behaved differently afterwards according to the hardness of endosperm. Minimum polishing was suffered by 'Monoharsali (16.3 per cent) when milled for 200 sec. Varietal differences in the yield of head rice for different milling times were found to be significant (Fig. 3). The highest yield of head rice was obtained for 'Jaha' (79.7 per cent) when shelled. The trend was maintained up to 130 sec of milling (46.8 per cent) but dropped to 21.1 per cent for 200 sec. 'Monoharsali', which gave lower head rice up to 130 sec yielded highest head rice (25.0 per cent) when milled for 200 sec.

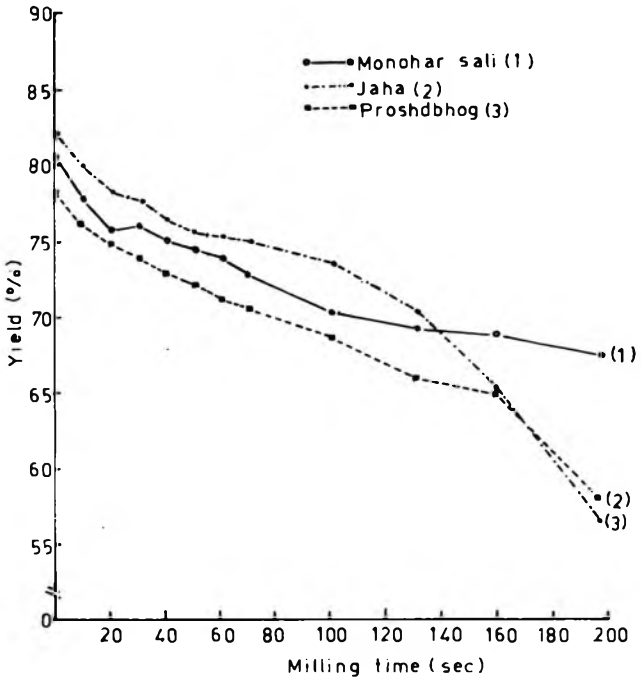

Fig.1. Effect of milling time on the yield of rice (%) of different varieties

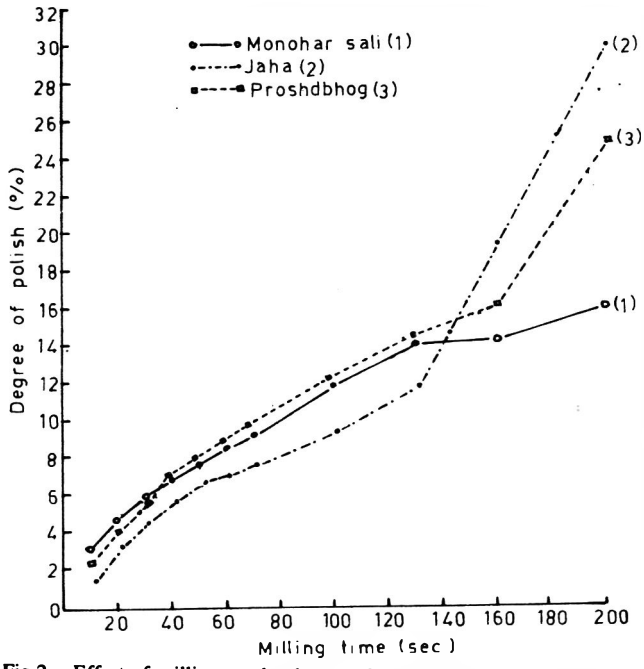

Fig.2. Effect of milling on the degree of polish of different varieties.

Nutritional quality: The results of composition of brown and milled (5 per cent polish) rice are presented in Table 3. Significant variations in protein, reducing sugars, amylose, fat and FFA contents for brown and milled rice were recorded. Brown rice found to contain higher protein, reducing sugars, fat and FFA but lower amylose. Protein content ranged from 6.3 to 7.2 and 6.0 to 6.7 per cent in brown and milled rice, respectively, which agreed with the reported result of Chakrabarthy *et al*[1]. and Cruz *et al*[21]. Breeding of varieties with high protein content needs identifying high protein

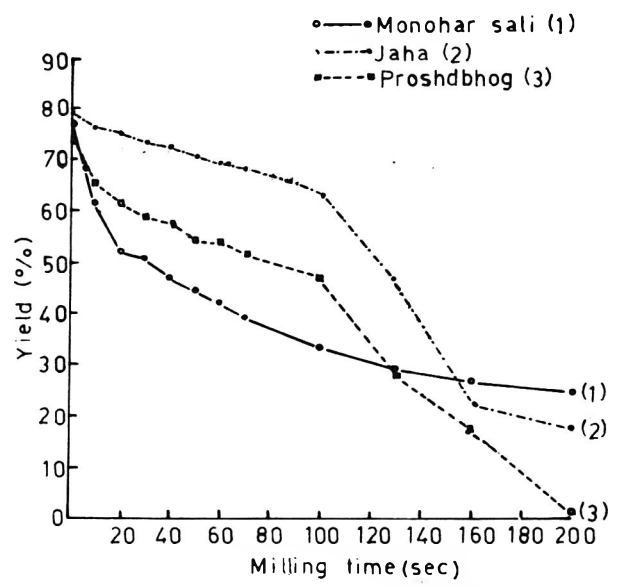

Fig.3. Effect of milling time on yield of head rice of different varieties.

sources from all possible materials or enforce their genetic factors for protein, along with those high yield and quality[22]. 'Jaha' had the highest reducing sugar (0.42 per cent) as maltose which dropped to 0.31 per cent on milling. Amylose content is the principle factor for the rice quality which causes great increase in volume, water-up-take ratio, texture and gloss of cooked rice[23]. 'Monoharsali' had the highest amylose (20.9 per cent) in milled rice. Similar data were obtained by Chakraborthy *et al*[7]. and Sidhu *et al*[8]. for brown and milled rice. The values for fat content ranged from 2.18 to 2.57 and 1.07 to 1.74 per cent in the brown and milled rice, respectively. The data were in agreement with the reported value of fat content for brown rice by Juliano *et al.*[11] and for milled rice by Milev and Vice[24]. However, the values were higher than the reported value of Chakraborthy *et al*[7]. FFA contents of brown rice were higher (31.1 to 37.5 per cent) than the milled rice (20.4 to 30.6 per cent). The outer bran fractions contained more FFA than the inner endosperm comprising milled rice. The values obtained were reasonably low testifying the sound condition of paddy samples. The values were slightly higher than the reported values of Yasumatsu and Moritake[25].

Bran characteristics: The results of replicated samples of protein, fat and FFA of the bran when milled to 5 per cent polish are presented in Table 4. Significant variations in the protein, fat and FFA content were observed. Protein and fat contents ranged from 11.2 to 12.1 and 18.0 to 19.5 per cent, respectively. Higher FFA (216.8 to 279.2 per cent) content in the bran may be due to the non-stabilization of the bran immediately after polishing. However, higher fat content in the bran signified its stability and hence suitability for oil extraction, commercially.

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TABLE 3. COMPOSITION OF DIFFERENT VARIETIES OF BROWN AND MILLED RICE

Variety	Protein* (%)	Reducing sugars (%)	Amylose (%)	Fat (%)	FFA (mg KOH/100g)
			Brown rice		
Jaha	7.2	0.42	18.3	2.57	31.3
Monoharsali	6.3	0.40	19.2	2.18	37.5
Prosadbhog	6.3	0.41	19.5	2.26	31.1
			Milled rice		
Jaha	6.7	0.31	19.7	1.74	26.9
Monoharsali	6.1	0.20	20.9	1.07	30.6
Prosadbhog	6.0	0.31	20.4	1.38	20.4
S.Em	± 0.0577	± 0.0058	± 0.0577	± 0.0058	± 0.0578
CD at 0.05	0.1305	0.0131	0.1305	0.0130	0.1305
,, 0.01	0.1876	0.0188	0.1876	0.0188	0.1878

*N × 5.96

TABLE 4. COMPOSITION OF BRAN OF VARIOUS VARIETIES (w.b)

Variety	Bran yield (%)	Protein* (%)	Fat (%)	FFA (mg KOH/100g)
Jaha	3.98	12.10	18.60	279.20
Monoharsali	4.88	11.80	19.50	234.30
Prasadbhog	3.87	11.20	18.00	216.80
S.Em	± 0.1756	± 0.0577	± 0.0578	± 0.0601
CD at 0.05	0.3970	0.1305	0.1305	0.1359
,, 0.01	0.5700	0.1876	0.1876	0.1953

* N×5.96

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Changes in the Functional Characteristics of Wheat During Soaking and Subsequent Germination

K. LEELAVATHI, R. VETRIMANI AND P. HARIDAS RAO
Central Food Technological Research Institute,
Mysore - 570 013, India

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Changes in the functional characteristics during soaking and germination of wheat for different periods (24, 48 and 72 hr) were studied. Soaking wheat overnight considerably decreased the hardness from 9.6 to 4.0 kg/grain, hectoliter weight from 74 to 70 kg/Hl and 1000 kernel weight from 74 to 50 g. Subsequent germination further lowered their values gradually depending on the germination period. Flour yield decreased from 68.3 to 62.8 per cent and colour grade value increased from 2.1 to 7.4 due to germination for 72 hr. Soaking improved the colour of flour, but germination gradually darkened it as seen from the increase in colour grade value from 2.1 to 7.4. Germination lowered the gluten, sedimentation value, damaged starch, non-reducing sugars, free lipids and bound lipids, while increased the free fatty acid contents. Soaking did not affect various farinograph characteristics, but germination gradually lowered the farinograph water absorption, stability and dough development time considerably. Among the different enzymes, lipase and protease increased while lipoxidase decreased during germination. Overall quality of bread improved on germinating wheat upto 24 hr. Germination of wheat for 72 hr increased the spread (5.0 to 5.4 cm) and raise (0.66 to 0.76 cm) and improved flavour and taste of biscuits. Soaking of wheat slightly deteriorated the quality of cake, while germination further affected the quality. However, cake made from wheat germinated for 24 hr was acceptable.

Germination of wheat during harvest due to unseasonal rains is a serious problem in several countries including India. Germination results in the loss of flour yield and deterioration in the flour quality[1-3]. However, the work reported so far, was mainly concerned with the deteriorating effect on bread quality due to increase in the level of α-amylase activity[4,5] and possible methods to improve the quality of such breads[6]. The information on the effect of germination for different periods on the functional characteristics of Indian wheats is scanty[7]: Narpinder *et al*[8]. in their studies observed that heat treatment of germinated Indian wheats brought about considerable improvement in the quality of bread.

The present study aims at finding the changes in the functional characteristics of Indian wheat at different degrees of germination and to determine the maximum extent of germination that can still yield acceptable quality bakery products. Results of the above studies are presented in this paper.

Materials and Methods

Commercially available medium hard wheat procured from the local market was used in the study. About 15 kg wheat was soaked in excess of water at 25°C for about 16 hr with one change of water. The soaked wheat obtained by draining the water was spread on a wet gunny bag and allowed to germinate at room temperature (25-27°C) and humidity (60-65 per cent). About 4 kg samples were drawn at intervals of 24, 48 and 72 hr of germination and dried in a cabinet drier maintained at 35°C to a moisture level of about 10 per cent. The roots were removed by rubbing them over a sieve and the samples were stored at 4°C. About 4 kg of soaked ungerminated wheat was also dried and stored similarly.

Hectoliter weight and thousand kernel weight of wheat samples were determined using standard methods. Kernel hardness was determined using grain hardness tester (Kiya Seisa-Kusho Ltd. Japan). The average of 20 values was calculated. Pearling index of wheat samples was determined by polishing 20 g wheat in a 'Concorna' barley pearler for 1 min, and the loss of bran was expressed in percentage.

Milling: Wheat samples of ungerminated, soaked and germinated for 24, 48 and 72 hr were tempered to 15.5 per cent moisture content overnight and milled into straight grade flours using Buhler Pneumatic Laboratory Mill (MLU-202).

Chemical and rheological characteristics of flours: Moisture, ash, gluten, diastatic activity, proteolytic activity, falling number, sedimentation value, damaged starch, amylograph, and farinograph characteristics were determined according to AACC approved methods[9]. Free fatty acids, total and reducing sugars were determined according to standard AOAC procedures[10]. Lipase and lipoxidase activities were determined by the method of Kantharaj Urs *et al.*[11] and Tappel[12], respectively.

Baking characteristics: Bread making quality of different flour samples was assessed by the 'Remix' baking test of

Irvine and McMullan[13], with the following modifications (i) omission of malt flour and (ii) use of 1 per cent fat. Loaf volume was determined by the rape seed displacement method[14]. The other characteristics such as colour and shape of the crust, and texture, colour, grain size, and uniformity of the crumb, and taste were assessed by the scoring method described by Pyler[15].

Biscuits were prepared and evaluated for various physical parameters according to the methods described earlier[16]. The other sensory parameter like colour, surface smoothness, crumb colour, crispness and taste of biscuits were evaluated by a panel of judges by giving scores to each parameter.

Cup cakes were made with flour (100 g), sugar (84 g), fat (84 g), salt (1.0 g), whole egg (84 g), baking powder (2.0g) and essence (1.0 ml). All in-one method of mixing was used for preparing the batter. The specific gravity of the batter was determined by noting the weight of glass cuvette filled with batter and water. Sixty five grams of the batter was poured into each cup and baked for 25 min at 190°C in an oven. The volume of the cakes was determined by the rape seed displacement method. The other quality characteristics such as crust colour, softness and grain size and shape of the crumb and taste were assessed by a panel of 6 judges. All the experiments were carried out in triplicate and the statistical evaluation of the data was carried out by Duncan's Multiple Range Test.

Results and Discussion

Hectolitre weight as well as thousand kernel weight which reflected the flour yield potential of wheat decreased during soaking as well as germination (Fig. 1). The changes in these parameters were considerable and sudden in soaked wheat while they were gradual during germination. The lowering of values of these characteristics could be attributed to the loss of solids during soaking and germination.

Germination made the grain brittle but softer as indicated by the kernel hardness value. The hardness decreased from 9.6 to 4.0 kg/grain during soaking and to 2.4 kg/grain during germination for 72 hr. The decrease in the hardness was also

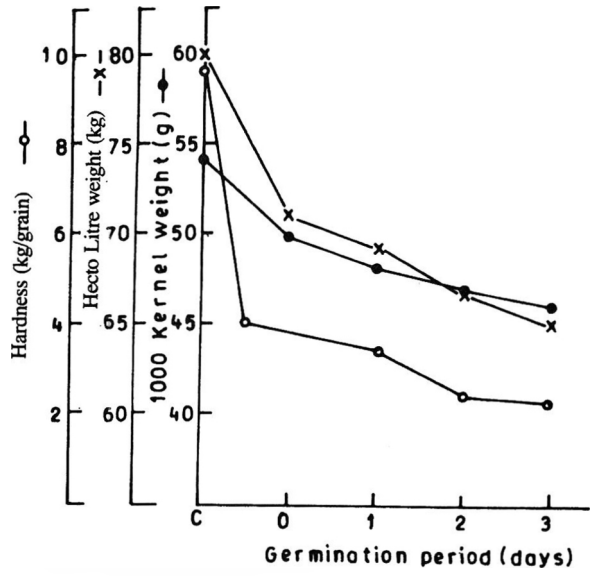

Fig.1. Effect of germination on the physical characteristics of wheat.

reflected by the increase in the pearling index. The lower values for physical characteristics in germinated wheat indicated its lower flour yield potential. Similar observations were also made by earlier workers[2,17].

Milling characteristics: Milling characteristics of wheat (Table 1) indicated increase in the yield of break flour and decrease in the yield of reduction flour due to soaking as well as germination. The changes in the yield of these flours were considerable due to soaking (about 7.0 per cent) as compared to germination.

Total flour yield decreased from 68.3 to 62.8 per cent when wheat germinated for 72 hr. Similar results were also reported by Lukow and Bushuk[2] and Huang and Bushuk[18], while Narpinder Singh *et al.* reported about 2 per cent increase in the yield of flour due to germination for 48 hr. The colour of flour as indicated by the colour grade value, decreased from 3.2 to 2.1 in flour milled from soaked wheat, while it gradually increased from 2.1 to 7.4 in flour obtained from wheat germinated for 72 hr. Similar observations were also made by other workers. The ash content increased from 0.45

TABLE 1. EFFECT OF GERMINATION OF WHEAT ON THE MILLING CHARACTERISTICS

Germination period (hr)	Break flour (%)	Reduction flour (%)	Total flour yield (%)	Colour grade value	Ash (%)
Untreated	17.3[a]	83.2[a]	68.3[a]	3.2[a]	0.45[a]
Soaked (0)	24.3[b]	75.8[b]	67.2[b]	2.1[b]	0.41[b]
24	25.7[c]	74.3[c]	66.1[c]	2.7[c]	0.40[b]
48	25.4[c]	74.6[c]	64.3[d]	4.0[d]	0.44[c]
72	27.2[d]	72.8[d]	62.8[e]	7.4[e]	0.50[d]
S.Em (df = 15)	±0.29	±0.29	±0.24	± 0.05	±0.003

Means in the same column followed by different superscripts differ significantly at 5% level.

to 0.50 per cent in flour milled from wheat germinated for 72 hr. This increase in the ash was reported to be due to loss of carbohydrates due to respiration and not because of actual increase in mineral matter[19].

Chemical characteristics: Changes in the chemical characteristics given in Table 2, indicate a gradual decrease in wet as well as dry gluten content in flour obtained from soaked wheat as well as from subsequently 24 hr germinated wheat. However, germination for more than 24 hr resulted in a steep decrease in these values. Similar observations were made by Hwang and Bushuk[18] who attributed these to the degradation of gluten proteins as a result of cleavage of peptide bonds.

In contrast, slight increase in the protein content was observed in wheat germinated for 72 hr. Lorenz[20] observed increase in the protein content, while Lukow and Bushuk[2] observed gradual decrease in the protein content during germination and attributed it to the translocation of amino acids due to the developing embryo.

Damaged starch content in flour decreased from 11.0 to 8.5 per cent during 24 hr germination and further increase in the germination period considerably increased the damaged starch content to 10.8 per cent. This is in agreement with the result of Dronzek *et al.*[21] and Narpinder Singh *et al.*[8] and was attributed to the degradation of starch.

Free lipids decreased from 1.18 to 0.76 per cent while bound lipids decreased only slightly from 0.62 to 0.54 per cent during germination for 72 hr period as reported. Similar observation was also made by Lukow *et al.*[22] and they attributed it to the breakdown of triglycerides. Soaking did not result in any change in the free fatty acid contents in flour. However, germination for 72 hr increased substantially the free fatty acid contents from 13.8 to 82.1 mg per cent. This is possibly because of increase in the lipase activity.

Reducing sugars as expected, increased during soaking as well as germination from 0.36 to 2.0 per cent while non-reducing sugars decreased from 2.15 to 1.6 per cent.

Various enzyme activities in soaked and germinated wheat given in Fig. 2 and 3 indicated a considerable increase in the diastatic activity from 341 mg/10 g flour, observed for ungerminated wheat to 967.5 mg/10 g flour in 72 hr germinated wheat. Maltose value which also indicates the

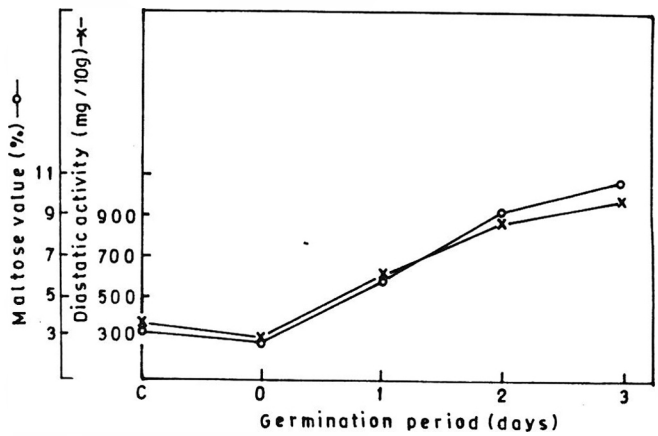

Fig.2. Effect of germination of wheat on the diastatic activity and maltose value.

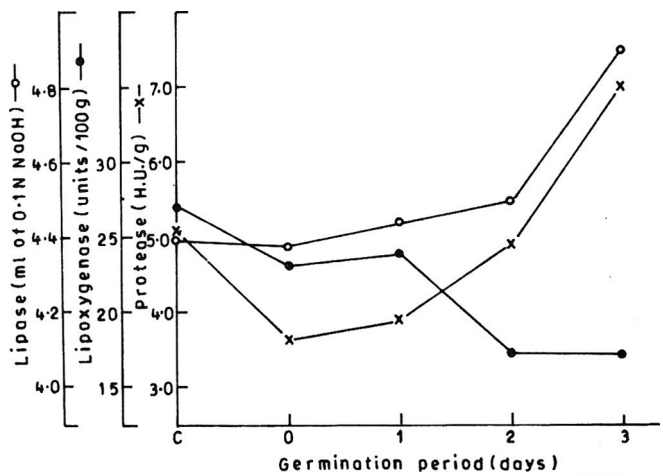

Fig.3. Effect of germination of wheat on various enzyme activities.

TABLE 2. EFFECT OF GERMINATION OF WHEAT ON THE CHEMICAL CHARACTERISTICS OF FLOUR

Germination period (hr)	Dry gluten (%)	Protein* (%)	Sedimentation value (ml)	Damaged starch (%)	Lipids (%)		Free fatty acids (mg/KOH/100 g)	Sugars (%)	
					Free	Bound		Reducing	Non-reducing
Untreated	8.3[a]	8.9[a]	26.0[a]	11.0[a]	1.18[a]	0.52[a]	13.8[a]	0.36[a]	2.15[a]
Soaked (0)	7.7[b]	8.7[b]	25.0[b]	9.8[b]	0.97[b]	0.58[b]	13.8[a]	0.46[ab]	1.79[b]
24	7.0[b]	8.0[c]	22.0[c]	8.5[c]	0.86[c]	0.60[b]	22.1[b]	0.50[b]	1.30[c]
48	5.3[c]	9.5[d]	20.0[d]	9.4[d]	0.80[c]	0.57[b]	59.9[c]	1.0[c]	1.50[d]
72	0.8[d]	9.8[e]	12.0[e]	10.8[e]	0.76[d]	0.54[c]	82.1[d]	2.0[d]	1.60[e]
S.Em (df = 15)	±0.18	±0.03	±0.26	±0.07	±0.02	±0.0798	±0.17	±0.03	±0.03

Means in the same column followed by different superscripts differ significantly at 5% level.
*N × 5.7

amylase activity showed a similar pattern. Falling number which is an index of alpha amylase activity, decreased slightly during soaking from 492 to 301. However, germination for 24 hr itself decreased the value to a minimum notable value of 60. Proteolytic activity decreased considerably from 5.02 to 3.50 HU/g during soaking and thereafter gradually increased to 7.01 HU/g with subsequent germination up to 72 hr period similar to that observed by Lukow and Bushuk[2]. On the other hand, lipoxidase activity gradually decreased from 25.4 to 3.3 units/100 g in flour from 72 hr germinated wheat.

Rheological characteristics: Farinograph characteristics of flours obtained from soaked and germinated wheat shown in Fig. 4 and Table 3 indicate a reduction in water absorption capacity from 62.3 to 55.0 per cent during germination for 72 hr. Soaking, however, did not affect the water absorption capacity. Similar observations were made by several workers[8,16,23]. Dough development time and dough stability were not affected by soaking, but considerably decreased as the germination period increased. These changes are in agreement with the report of Ibrahim and D'Appolonia[3] and were attributed to the reduction in the gluten content as well as to quality. Amylograph characteristics indicated that soaking of wheat increased the gelatinization temperature from 55.5 to 57.5°C, peak viscosity from 2790 to 3200 BU and temperature at peak viscosity from 75.5 to 86.5°C suggesting thereby modification of starch during soaking. Amylograph peak heights were zero for 24, 48 and 72 hr germinated samples.

TABLE 3. EFFECT OF GERMINATION OF WHEAT ON THE FARINOGRAPH CHARACTERISTICS OF FLOUR

Germination period (hr)	Water absorption (%)	Dough development time (min)	Dough stability (min)	Mixing tolerance index (B.U.)
Untreated	62.3[a]	3.55[a]	4.00[a]	93.0[a]
Soaked (0)	62.2[a]	3.75[a]	3.75[a]	126.5[a]
24	58.2[b]	1.25[b]	1.25[b]	260.0[b]
48	56.6[c]	1.05[b]	1.05[b]	340.5[c]
72	55.0[d]	0.55[c]	0.55[b]	380.0[d]
S.Em	±0.05	±0.13	±0.20	±10.75
Df	15	10	10	10

Means in the same column followed by different superscripts differ significantly at 5% level

Bread making quality: The quality characteristics of bread given in Table 4 indicate that the loaf volume increased due to soaking as well as germination of wheat upto 24 hr. Further, increase in the germination period reduced the loaf volume.

The grain size and shape improved when bread was made from that soaked for 24 hr germinated wheat. However, the grains became undesirably coarser when bread was made from wheat germinated for more than 24 hr period. The bread made from 24 hr germinated wheat flour having maltose value of 5.2 per cent had better soft texture and desired light brown crust colour.

Biscuit making quality: The quality of biscuits made from germinated wheat is given in Table 5. The spread of biscuits gradually increased with increase in the germination period, similar to the observations made by earlier workers. It increased to 54.4 from 50.4 mm when wheat was germinated to 72 hr. The raise in biscuits also gradually increased with increase in the period of germination.

The crispness as well as taste and flavour of biscuits improved with germination period as indicated by the higher scores. The biscuits made with germinated wheat had a pleasant malty flavour. The crust colour however, became undesirably darker with increase in the germination period.

The overall quality of biscuits was highest when biscuits were made from flour from 48 hr germinated wheat having maltose value of 7.2 per cent.

Cake making quality: The specific gravity of cake batter increased with increase in the germination period from 0.89 to 0.94 in 72 hr germination (Table 6). Volume as well as

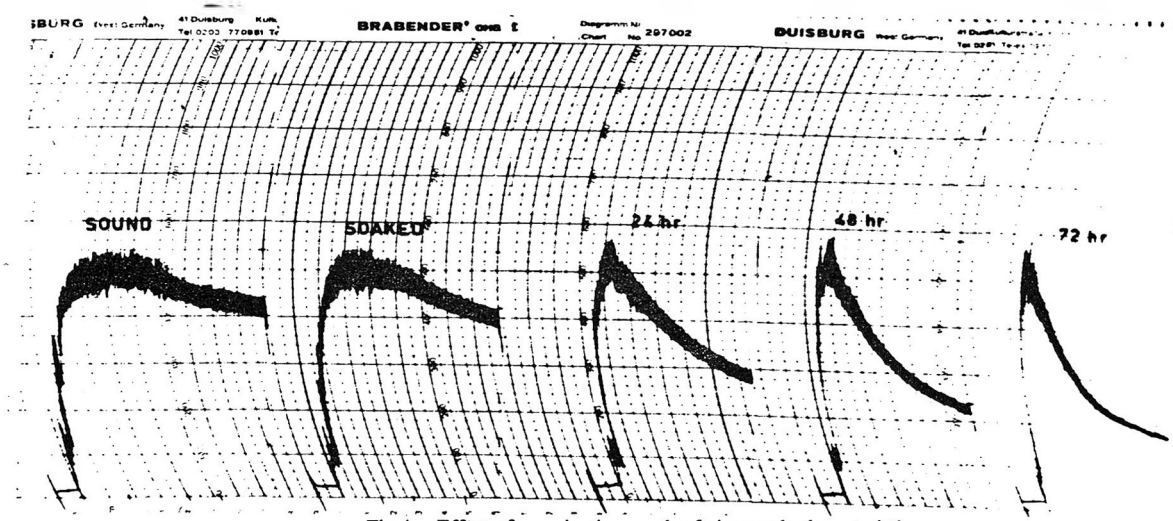

Fig.4. Effect of germination on the farinograph characteristics.

TABLE 4. EFFECT OF GERMINATION OF WHEAT ON THE BREAD QUALITY OF FLOUR

Germination period (hr)	Physical characteristics			Sensory characteristics						
				Crust		Crumb			Taste (20)*	Overall quality (80)*
	Wt (g)	Vol (m)	Specific vol (ml/g)	Colour (10)*	Shape (10)*	Colour (10)*	Grain size and uniformity (10)*	Texture (20)*		
Untreated	141.6	460[a]	3.07[a]	8.45[a]	7.75[a]	7.88[a]	7.02[a]	15.00[a]	16.38[ab]	63.58[a]
Soaked (0)	142.4	520[b]	3.23[b]	7.95[a]	8.25[a]	8.50[b]	7.92[b]	14.88[a]	17.38[b]	64.00[a]
24	138.7	520[b]	3.75[c]	7.85[a]	9.25[b]	7.50[a]	8.95[c]	17.20[b]	17.70[b]	69.65[b]
48	138.4	465[a]	3.36[d]	5.62[b]	6.38[c]	5.02[c]	5.65[d]	11.05[c]	12.85[c]	45.50[c]
72	137.5	440[c]	3.20[b]	4.00[c]	3.75[d]	3.28[d]	3.10[e]	7.88[d]	7.25[d]	28.65[d]
S.Em (df = 15)		±2.52	±0.019	±0.24	±0.19	±0.19	±0.22	±0.28	±0.36	±0.72

Means in the same column followed by different superscripts differ significantly at 5% level.
*Maximum score.

TABLE 5. EFFECT OF GERMINATION OF WHEAT ON THE BISCUIT QUALITY OF FLOUR

Germination period (hr)	Physical quality				Sensory characteristics					
	width (W) (cm)	Thickness (T) (cm)	W/T ratio	Spread factor (%)	Crust colour (10)*	Surface smoothness (10)*	Crumb colour (10)*	Crispness (25)*	Taste (25)*	Overall quality (80)*
Untreated	5.04[a]	0.66[a]	7.64[a]	100[a]	8.78[a]	7.98[a]	9.12[a]	20.70[a]	17.55[ab]	64.10[a]
Soaked (0)	5.19[b]	0.70[ac]	7.41[b]	95.72[b]	9.17[b]	8.98[b]	8.28[b]	20.55[a]	19.10[b]	66.72[bb]
24	5.23[b]	0.72[b]	7.26[bc]	94.62[ab]	7.56[c]	9.55[b]	8.28[b]	22.12[b]	21.99[c]	70.25[c]
48	5.38[c]	0.74[bc]	7.23[cd]	94.38[b]	6.54[d]	9.54[b]	7.25[c]	23.65[c]	22.00[c]	69.02[c]
72	5.44[c]	0.76[c]	7.15[d]	93.10[c]	5.08[e]	10.45[c]	6.05[d]	23.50[c]	21.54[c]	66.75[d]
S.Em	±0.02	±0.02	±0.04	±0.34	±0.17	±0.23	±0.22	±0.31	±0.42	±0.47
df	15	15	15	12	15	15	15	15	15	15

*Maximum score
Means in the same column followed by different letters differ significantly at 5% level.

TABLE 6. EFFECT OF GERMINATION OF WHEAT ON THE CAKE QUALITY OF FLOUR

Germination period (hr)	Physical characteristics			Sensory characteristics						
				Crust		Crumb			Taste (20)*	Total score
	Batter (sp gr)	Wt (g)	Specific vol (cc/g)	Colour (10)*	Shape (10)*	Colour (10)*	Grain (10)*	Texture (20)*		(80)*
Untreated	0.89[a]	56.6	1.76[a]	9.05[a]	8.88[a]	8.95[a]	8.78[ab]	16.20[ab]	19.08[a]	70.55[a]
Soaked (0)	0.91[ab]	56.8	1.72[a]	8.82[a]	9.40[a]	8.88[b]	8.92[a]	15.85[a]	18.72[a]	67.88[b]
24	0.91[bc]	56.7	1.73[a]	8.48[a]	7.28[b]	8.00[b]	8.18[b]	12.55[b]	15.55[b]	60.90[c]
48	0.93	57.1	1.67[a]	5.20[b]	4.70[c]	6.32[c]	6.52[c]	10.50[c]**	13.56[c]	56.45[d]
72	0.94	57.5	1.50[b]	3.55[c]	4.12[c]	4.80[d]	4.08[d]	8.22[c]**	10.05[d]	32.40[e]
S.Em (df = 15)	±0.007	±0.016	±0.18	±0.21	±0.21	±0.21	±0.21	±0.31	±0.41	±0.78

*Maximum score
**Sticky crumb
Means of the same column followed by different letters differ significantly at 5% level.

specific volume of cake gradually decreased with increase in the germination period due to poor air retention capacity of the batter. The crust colour changed from desired golden brown to dark brown particularly in cake made from wheat germinated for 48 hr on longer period. Cake made from wheat germinated for a period of 24 hr or more had a flat and caved-in crust. The crumb colour also became more yellowish and the grain tended to be coarse and non-uniform, and the crumb became sticky as the germination period increased. Only the cake made from wheat germinated upto a period of 24 hr was acceptable.

Germination of wheat for 24 hr resulting in the increase in maltose value to 5.2 per cent improved the bread quality. In case of biscuits, germination though increased the spread which was undesirable, the taste and flavour improved considerably. However germination had an adverse effect on the cake quality.

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Development of Ready-to-Eat Traditional Indian Sweet Dishes Based on Jaggery and Coconut*

T.S. SATYANARAYANA RAO, N.M. KAVERAPPA, T. HEMAPRAKASH REDDY AND K.S. JAYARAMAN
Defence Food Research Laboratory, Mysore-570 011, India.

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Traditional Indian sweet meat like Holige (sweet stuffed chapathi), Modaka (karji kai) and burfi spoil quickly due to high moisture, improper handling during preparation and packaging. These products with reduced moisture content were developed using dehydrated coconut powder and jaggery and stored for 6 months. These products, without any preservative when packed in paper-aluminium foil polyethylene laminate pouches were found to be acceptablee for ٤ period of 6 months at 19-26°C (ambient temperature) and 3 months at 37°C.

In spite of the fact that most of the traditional Indian sweet dishes have attained familiarity in all parts of the country, their manufacture is still best done in their place of origin. To trace the origin of the names of various Indian sweet dishes is a big problem[1]. Some preparations are known for centuries, whereas others are quite recent. Available information on the method of preparation and marketing of these traditional Indian sweet meats is scanty.

Holige, Modaka and Burfi, the popular Indian sweet meats, are made from coconut, jaggery or sugar, wheat flour, maida (refined wheat flour) semolina and vanaspati (hydrogenated fat) after adding flavouring substance like cardamom. The proportion of these ingredients used and the method of preparation may vary as these are not standardised.

Defence Food Research Laboratory has recently developed some dehydrated sweet mixes like sooji halwa made of wheat semolina, sugar and a pumpkin halwa made out of pumpkin, sugar, and milk powder for introduction into Service ration packed in flexible pouches. But these items require to be reconstituted by addition of water or facilities for warming up. This paper describes the methods for preparation and preservation of three types of ready-to-eat traditional Indian sweet meats, namely Holige (sweet stuffed chapathi), Modaka (karji kai) (stuffed sweet fried in oil) and Burfy based on jaggery, dehydrated coconut powder and their changes in chemical properties and sensory evaluation during storage when packed in flexible pouches stored for different periods and also the effect of preservatives in these products.

Materials and Methods

Fully matured coconuts, light golden coloured jaggery, vanaspathi (hydrogenated fat), cardamom, poppy seeds, whole Amul milk powder, sooji (semolina) and maida (refined wheat flour) were procured from the local market.

The outer fibre from the coconut was first removed by hand and then the coconuts were dipped in 2 per cent bleaching powder solution for 30 min to eliminate any foreign contamination. They were then washed in running tap water, broken into two halves and grated by using a coconut electrical grater. The grated coconut along with its original water was minced well. in a waring blender with addition of extra quantity of water to get uniform smooth paste. The paste was spread uniformly on Kilburn aluminium trays (90 × 45 × 2.5 cm) at the rate 1.5 kg per tray and dried in a Kilburn cabinet drier at a temperature of 60°C for 5-6 hr at an air velocity of 180 meters/hr. The dehydrated coconut powder was then collected and used for the preparation of sweet meats.

The required quantity of water was added to powdered jaggery and boiled for 10 min on a low flame. The thin syrup was then filtered through a 60 wire mesh sieve or on a muslin cloth to remove all extraneous materials present in the jaggery. Cardamom seeds after peeling the skin were powdered in electrical blender to get powder of 30 mesh. Poppy seeds after initial cleaning were roasted for 5 min on a iron pan (tawa).

Preparation of dough for Holige and Modaka: Equal proportions (24 per cent) of semolina and maida (24 per cent) were mixed after initial sieving, the required amounts (34 per cent) of water and vanaspathi (18 per cent) were added and kneaded well to get a uniform dough. The mix was kept for 3 hr in moist condition by covering it with wet muslin cloth.

Preparation of stuffing: Jaggery syrup was then further boiled to get Brix of 80° or to a temperature of 105° C, the dehydrated coconut powder was added and cooked further to get a final Brix of 85°. At this stage, cardamom powder and poppy seeds were added and it was allowed to cool. The stuff was powdered and used in the preparation of Holige and Modaka.

Preparation of Holige: Twenty five g of dough was rolled into disc first and then 20 g of sweet stuffing material was placed at the centre and folded. Using a wooden roller it was rolled into disc of 10-11 cm diameter. It was then baked over an uniformly heated iron pan at 120°C for 5 min. A little

*Adjudged as best paper in IFCON-88

vanaspathi was added and both sides baked. The product (four numbers) was then cooled and covered with polypropylene (300 gauge) and then packed in paper-aluminium foil (0.02 mm) polyethylene laminate pouches.

Preparation of Modaka: Twenty five grams of dough was rolled as above, sweet stuffing material (20 g) was placed at the centre of the disc and folded into semi circular shape. The edge was then closed and trimmed ornamentally. The modakas were then deep-fat-fried in oil hydro at a temperature of 200-205°C for 5 min and cooled. They were then packed as described above.

Preparation of Burfi: The required quantity of jaggery was made to syrup after preliminary cleaning, mixed with dehydrated coconut powder and cooked to get a Brix of 80°. The Amul milk powder was mixed well with vanaspathi and cardamom powder were added at the end. The burfi was then poured on aluminium moulds pressed and cut into 5 × 5 cm pieces and packed as above.

The free fatty acids (FFA), peroxide value (PV, milli eq. O_2/kg fat) were determined as per A O A C. method[2]. Non enzymatic browning, thiobarbituric acid (TBA) value (μg MA/kg), citric acid and pH were determined[3-5]. The colour, texture, appearance, taste and overall acceptability were evaluated using a taste panel (8 judges) and expressed as scores on a 9-point Hedonic scale.

One batch (120 numbers) of jaggery holige and one batch of modaka (150 numbers) were prepared by incorporating antioxidants butylated hydroxy anisole BHA (0.02 per cent), citric acid (0.004 per cent) and potassium sorbate (0.2 per cent) and packed as described above. Another batch of holige (120 numbers) and modaka (150 numbers) prepared and packed as above without preservatives but sterilized at 85°C for 60 min in hot air oven.

All the above samples were stored at ambient temperature (19-26°C) for 6 months and 3 months at 37°C. Samples stored at 4°C were served as control. These samples were analyzed periodically for chemical and sensory changes.

Large scale preparations consisting 200-250 pieces in each batch of jaggery holige and modakas were undertaken in laboratory to carry out user trials. Chemical and microbiological examinations were done to assess variations of parameters due to raw materials and this will help while drawing up specifications.

Results and Discussion

It is seen that the conditions and preparation of holige and modakas are almost same except modakas are deep-fat-fried at higher temperature.

Table 1 gives the per cent composition of raw materials used for preparation of jaggery holige and modaka. It is seen that water content of the stuff is only 10.5 per cent as dehydrated coconut powder has helped in keeping the final moisture content of the product at 10-11 per cent. Dough requires nearly 34 per cent water to have good elasticity which will facilitate its easy rolling. It was observed that the above formula gave the acceptable product as judged by taste panel.

TABLE 1 COMPOSITION OF DOUGH AND STUFFING MATERIAL USED IN THE PREPARATION OF JAGGERY HOLIGE AND MODAKA

Dough composition	percent
Semolina	24
Refined wheat flour	24
Water	34
Vanaspathi	18
Stuffing material composition	
Coconut powder, dehydrated	18.8
Jaggery	67.0
Water	10.5
Poppy seeds	2.2
Cardamom	1.5

Table 2 indicates the chemical and organoleptic changes of jaggery holige and modaka with and without preservatives and sterilization during storage at different temperatures. It was observed that moisture (12.6, 11.2 per cent), total fat (18.8, 19.9 per cent), sugar (35.2, 35.6 per cent) did not change significantly.

The FFA contents of the samples have increased significantly from 0.15 per cent to 3.1 to 3.3 per cent at 37°C and at ambient temperature both in treated and untreated samples by the end of the storage period. Similar observations were made in both the products. The peroxide values were slightly higher in holige in all the three samples at the end of 6 months at ambient temperature. The increase either in FFA or P V did not influence the sensory qualities in both treated or untreated samples. This clearly indicates that either preservatives or sterilization did not enhance the shelf life of the product. This may be due to the presence of tannins, reducing sugars[6] and SO_2 (180 p.p.m.) in jaggery.

Table 3 indicates the per cent composition of jaggery coconut burfi. Khoa which used to be the normal ingredient in burfi has been replaced by Amul whole milk powder and vanaspathi. Burfi with this composition was highly acceptable. The chemical and sensory qualities of jaggery burfi during storage at different temperatures are given in Table 4. It is seen that there are considerable increases in FFA, PV and TBA values during storage at 37°C and at 19-26°C. Similarly NEB values have doubled during the same period. However, these changes have no effect on sensory qualities like taste, colour, aroma, texture and overall acceptability. The energy value has indicated that 100g of coconut burfi will contribute 500 kcal.

The proximate composition of several batches of jaggery holige and modaka is presented in Table 5. It is observed that modaka had less moisture and high fat content when compared to holige because of deep-fat-frying of modaka. There are no significant changes in other parameters between

TABLE 2. CHEMICAL AND ORGANOLEPTIC CHANGES IN JAGGERY HOLIGE AND MODAKA WITH, WITHOUT PRESERVATIVES AND STERILIZATION DURING STORAGE AT DIFFERENT TEMPERATURES

Storage temp. °C	Storage period (months)	F.F.A. (% oleic acid)			P.V. (milli. eq. of O_2/kg fat)			Organoleptic score		
		I	II	III	I	II	III	I	II	III
					Holige					
Initial		0.15	0.16	0.16	3.75	4.65	12.2	8	8	8
37	2	2.3	2.4	2.3	11.8	10.7	12.2	7	8	7
	3	3.1	3.1	3.1	28.3	17.9	29.7	7	6	6
19-20	2	1.9	1.9	1.8	7.3	7.2	8.17	7	7	7
	4	2.5	2.8	2.7	19.7	19.3	19.8	7	7	7
	6	3.2	3.1	3.3	29.8	28.7	28.7	7	7	6
4	2	1.1	1.1	1.1	—	7.9	—	8	8	8
	4	1.3	1.1	1.3	—	—	—	8	7	7
	6	1.3	1.4	1.4	13.2	13.9	14.1	8	7	7
					Modaka					
Initial		0.15	0.10	0.16	3.5	3.6	4.1	8	8	8
37	2	1.3	1.3	1.4	10.8	9.7	9.8	7	7	7
	3	2.9	2.7	2.8	26.3	21.8	25.7	7	7	7
19-26	2	1.9	1.0	1.8	6.9	6.8	6.9	7	8	7
	4	2.7	2.5	2.9	18.7	17.8	19.1	7	7	7
	6	3.0	3.2	3.3	28.3	27.4	27.9	7	7	7
4	2	0.8	1.0	1.0	4.7	1.5	2.2	8	8	8
	4	0.9	1.0	1.0	—	4.7	5.3	8	8	8
	6	2.0	1.9	1.9	14.1	11.3	13.9	8	8	8

I No preservatives added
II Preservatives: BHA 0.02%, + citric acid 0.004% + potassium sorbate 0.2% added
III Sterilized

TABLE 3. COMPOSITION OF JAGGERY COCONUT BURFI

Ingredients	Per cent
Dehydrated coconut powder	31.50
Jaggery	52.50
Amul whole milk powder	3.15
Vanaspathi	3.80
Cardamom powder	0.75
Water	8.30

the two products. The coconut burfi is having high fat (30 per cent), protein (10 per cent) and total sugars (40 per cent).

Table 6 indicates the microbiological status of jaggery holige and modaka prepared on large scale. It is seen several batches of holige and modaka had less than 1000 colonies of bacteria with negligible counts of yeast and moulds. No pathogens were encountered. Thus, it is seen with proper care during preparation, handling and packaging, products with

TABLE 4. CHEMICAL AND SENSORY CHANGES IN COCONUT BURFI (JAGGERY BASED) DURING STORAGE AT DIFFERENT TEMPERATURES

Storage									
Temp. (°C)	Period (months)	Moisture (%)	PV*	FFA (% lauric acid)	TBA*	pH	Acidity (% citric acid)	NEB** (O.D at 420 nm)	Sensory score
—	Initial	8.5	1.15	0.11	21	6.39	0.34	0.12	8
37	1	8.7	1.36	0.33	32	6.26	0.34	0.165	8
	2	8.5	2.36	0.49	53	6.23	0.35	0.24	7
	3	8.5	6.74	0.48	52	6.40	0.33	0.29	7
19-26	2	8.5	1.44	0.37	41	6.37	0.34	0.16	7
	4	8.3	4.58	0.47	64	6.46	0.33	0.18	7
	6	8.6	7.96	0.53	74	6.25	0.34	0.24	7
4	2	8.4	1.39	0.20	21	6.36	0.35	0.13	8
	4	8.3	1.51	0.28	32	6.45	0.33	0.14	8
	6	8.5	1.55	0.28	32	6.32	0.32	0.15	7

*Milli eq of O_2/kg fat **NEB: Non enzymatic browning + g melonaldehyde/kg sample

TABLE 5. PROXIMATE COMPOSITION OF JAGGERY BASED HOLIGE* AND MODAKA

Parameter	Holige	Modaka
Moisture (%)	11.62 ± 0.40	10.36 ± 0.33
Total fat (%)	17.23 ± 0.50	21.77 ± 1.05
Total proteins (%) (N × 6.25)	5.61 ± 0.15	6.05 ± 0.12
Total ash (%)	1.29 ± 0.03	1.28 ± 0.02
Acid insoluble ash (%)	0.34 ± 0.05	0.33 ± 0.05
Crude fibre (%)	2.78 ± 0.07	2.98 ± 0.11
Total sugars (%)	34.85 ± 0.80	34.10 ± 0.88
Reducing sugars (%)	2.78 ± 0.11	2.91 ± 0.15

*Values are mean ± SD of 6 batches

low counts of bacteria, yeast and moulds can be obtained/manufactured.

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TABLE 6. MICROBIOLOGICAL ANALYSIS OF DIFFERENT BATCHES OF JAGGERY HOLIGE AND MODAKA PREPARED ON LARGE SCALE

Batch No.	S.P.C.	Organism	
		Yeast and moulds	Others
	Modaka		
1	$7.5 \times 10^1 - 47.5 \times 10^1$	Nil to 1×10^1	Neg
2	$25 \times 10^1 - 97 \times 10^1$	Neg	Nil to 1×10^1 (spore)
3	$49.5 \times 10^1 - 51.0 \times 10^1$	Nil to 1×10^1	Neg
4	51.5×10^1	Nil	Neg
5	$49.5 \times 10^1 - 51.0 \times 10^1$	1×10^1	Neg
6	41.0×10^1	Neg	Neg
7	210.0×10^1	Neg	Neg
	Holige		
1	$15.0 \times 10^1 - 78.0 \times 10^1$	Nil	Neg
2	$4.0 \times 10^1 - 8.0 \times 10^1$	Neg	Nil to 3×10^1 (spore)
3	$31.0 \times 10^1 - 39.5 \times 10^1$	Nil to 1×10^1	Neg
4	$167.0 \times 10^1 - 266.0 \times 10^1$	Nil to 1×10^1	Neg
5	$29.0 \times 10^1 - 50.0 \times 10^1$	Neg	1 to 2×10^1
6	$31.0 \times 10^1 - 39.5 \times 10^1$	1×10^1	Neg
7	207.0×10^1	Neg	1×10^1 (micro spore) 2.5×10^1 (thermo spore)

Coliforms, Salmonella and Shigella were nil in all batches

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Development of Shelf Stable Ready-to-Eat Indian Sweet Meats Based on Sugar and Coconut

T.S. SATYANARAYANA RAO, N.M. KAVERAPPA AND K.S. JAYARAMAN
Defence Food Research Laboratory, Mysore - 570 011, India.

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Sugar holige (sweet stuffed chapathi) and Modaka (karji kai) the known traditional Indian sweet meats have a very short shelf-life under normal conditions of preparation and storage. These two products were prepared using normal ingredients like sugar, coconut, copra, semolina, refined wheat flour, vanaspathi and cardamom and were packed in polypropylene and then paper foil aluminium polyethylene laminate pouches and stored at various temperatures. These two items were found to be acceptable for a period of two months at 37°C and 4 months at ambient temperatures, without use of any preservatives.

Sugar holige (sweet stuffed chapathi) and Modaka (karji kai), the popular Indian sweet meats are prepared from sugar, coconut, refined wheat flour, semolina, vanaspathi and cardamom as a flavour component in different proportions in different parts of the country. The proportion of these ingredients used and method of preparation vary depending upon the sweet maker or a house wife. These Indian sweet meats are having limited storage life and hence considered as perishable items. Keeping quality of many sweets is not more than a few days and in some cases not more than 3-4 weeks[1].

Packaging of these products to ensure longer shelf life has become important to supply to our Armed forces at far off places and because of the increase in exports of sweet meats from India to some Middle East countries. The main steps involved in the preparation are stuff making, dough making, baking or frying and packing under hygienic conditions. Therefore, a study was undertaken to study the effects of antioxidants, preservatives and packaging materials on the shelf life of the product during storage at different temperatures and the results are presented in this paper.

Materials and Methods

Dehydrated coconut powder was prepared as per method described earlier[2]. Sugar, cardamom, poppy seeds, hydrogenated fat (vanaspathi), copra, semolina and maida (refined wheat flour) were procured from local market.

Preparation of dough for Holige and Modaka: Preparations of dough for sugar holige and modaka were done as per the procedure shown in Fig 1.

Preparation of stuffing: Powdered sugar was mixed with dehydrated coconut powder/dry copra in the ratio 2:1 and roasted on a stainless steel pan (tawa) for 5 min, then roasted poppy seeds and cardamom powder were added and mixed well before cooling to room temperature.

Preparation of sugar holige: Twenty gram dough was rolled into small disc using a wooden roller and 15 g of stuff was placed at the centre and folded to get spherical shape which was then rolled into a disc 10-11 cm dia meter. It was then baked over uniformly heated iron pan (tawa) at 120°C for 5 min. A little vanaspathi was added on Holige while baking, similarly the other side of it was baked and cooled to room temperature. The product (four in number) was packed in polypropylene (300 gauge) and then it was packed in paper-aluminium foil polyethylene laminate pouches (0.02 mm).

Preparation of sugar Modaka: Dough (20 g) was rolled as above, stuffing (15 g) was placed at the centre of disc and folded into semi circular shape. The edge was trimmed ornamentally. The modakas were then deep fat-fried in vanaspathi at a temperature 200-205°C for 5 min and cooled. Four such modakas were packed as described above.

One batch containing (120 number) sugar holige and one batch of modaka (150 number) were prepared by incorporating antioxidant BHA (0.02 per cent) and citric acid (0.004 per cent) and potassium sorbate (0.2 per cent) and packed as described above (II) and another batch of same number of holige and modaka was prepared and sterilized in air oven at 85°C for 1 hr (III). A batch without these chemicals and sterilization was used as control (I). All these samples were stored at 19-26°C (ambient temperature) and 4°C (control) for 6 months and at 37°C for 3 months. Samples were analyzed periodically for chemical and sensory changes during storage.

The moisture, free fatty acids (FFA), total sugars, peroxide value (PV) were determined as per AOAC method[3]. The colour texture, appearance, taste and overall acceptability were evaluated using a taste panel consisting of 8 judges and expressed as scores on 9-point Hedonic scale.

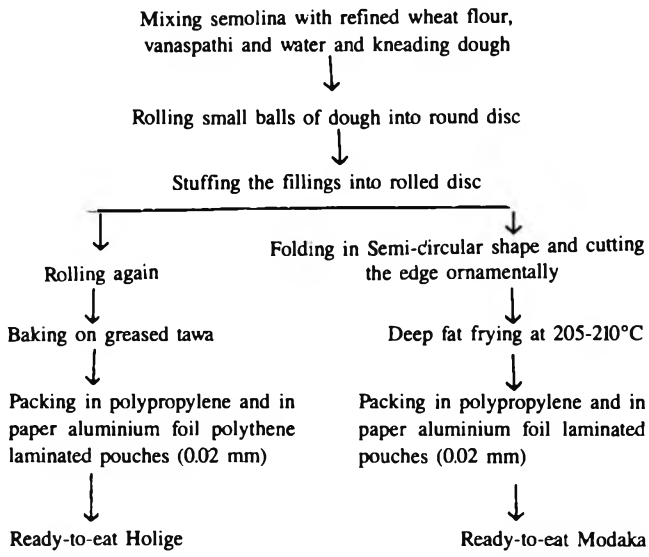

Fig.1. Flow sheet for the preparation of sugar holige and modaka

The recipes used for sugar holige and modaka are as follows - for dough sooji and maida 24 per cent each, water 34 per cent, vanaspathi 18 per cent. For stuffing dehydrated coconut or grated copra 32 per cent, sugar 64 per cent, poppy seeds 2.2 per cent and cardamom 1.4 per cent.

Results and Discussion

It is seen from the flow sheet that preliminary steps are common for preparation of both holige and modaka except that modakas are fried at 200-205°C for 5 min.

Table 1 indicates the chemical and sensory changes of sugar holige with and without preservatives and sterilization stored at various temperatures. There were no significant losses in moisture, fat and total sugars during storage both in preservative treated (sample II) and sterilized (sample III) upto a period of 3 months at 37°C and 6 months at 19-26°C (ambient) and control 4°C (I). The FFA contents at 37°C stored sample at the end of 2 months increased from 0.1 to 2.9 per cent. When the FFA contents further increased to 3.0 and 3.8 per cent, the treated, sterilized and untreated samples showed off-flavour by showing rancid smell of coconut oil as indicated in sensory score 3. Similar observation was made at the ambient temperature stored sample at the end of 6 months. The product became unacceptable when the peroxide value increased to 26-27 meqO_2/kg fat at 37°C at the end of 2 months, but when the peroxide values of the stored samples were between 19.0 and 20 per cent at ambient temperature at the end of 4 months both treated and untreated products were found to be acceptable. Whereas control samples remained acceptable throughout the storage period.

TABLE 1. PHYSICO-CHEMICAL AND SENSORY CHANGES OF SUGAR HOLIGE WITH AND WITHOUT PRESERVATIVES AND STERILIZATION DURING STORAGE AT DIFFERENT TEMPERATURES

Storage		Moisture (%)			Total fat (%)			Total sugars (%)			F.F.A. (% oleic acid)			P.V. (meq. of O_2/kg fat)			Sensory score		
Temp (°C)	Period (months)	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Initial		6.8	7.9	7.1	17.8	20.1	19.8	31.0	33.2	33.2	0.1	0.12	0.12	3.4	3.3	3.4	8	7	8
37	2	6.6	7.8	7.1	19.3	20.1	19.2	31.9	33.2	32.2	2.7	2.3	2.91	13.1	13.7	13.6	7	7	7
	3	6.6	7.5	7.2	19.3	20.2	20.0	33.1	33.2	—	3.4	3.0	3.8	27.9	26.3	26.3	6	3	3
19-26	2	6.7	7.8	7.1	19.5	20.6	19.8	32.1	34.0	32.6	1.9	1.7	1.91	8.1	8.1	8.3	7	7	7
	4	6.8	7.7	7.3	19.1	20.4	20.2	33.0	35.0	33.0	2.0	2.0	2.71	20.7	19.3	19.3	6	6	6
	6	6.8	7.6	7.1	19.4	20.7	19.7	34.6	36.1	—	3.5	3.2	3.1	—	—	—	7	3	3
4	2	6.8	7.8	7.2	19.4	20.3	19.8	31.0	35.0	32.1	0.13	0.14	0.14	—	—	3.8	7	7	7
	4	6.8	7.8	7.3	19.1	20.6	19.9	31.0	35.3	32.1	0.14	0.14	0.15	—	—	9.3	7	7	7
	6	6.7	7.8	7.2	20.1	20.5	19.8	32.0	34.6	—	0.15	0.17	0.15	—	—	—	7	6	6

I = No preservatives, II = with preservatives (BHA 0.02% citric acid 0.04% , and potassium sorbate 0.2%) III = with preservatives (BHA 0.04%, citric acid 0.08%, and potassium sorbate 0.2%) and with sterilization

TABLE 2. PHYSICO-CHEMICAL AND SENSORY CHANGES OF SUGAR HOLIGE WITH COPRA, WITH AND WITHOUT PRESERVATIVES AND STERILIZATION, DURING STORAGE AT DIFFERENT TEMPERATURES

Storage		Moisture (%)			Total fat (%)			Total sugars (%)			F.F.A. (% oleic acid)			P.V. (meq. of O_2/kg fat)			Sensory score		
Temp (°C)	Period (months)	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Initial		7.3	7.6	8.3	20.8	21.0	19.3	33.3	35.1	36.2	0.11	0.11	0.13	3.21	3.4	3.5	8	8	8
37	2	7.2	7.5	8.3	20.2	20.5	19.2	31.4	32.6	37.0	2.5	2.8	2.7	14.2	14.0	14.8	7	7	7
	3	7.4	7.3	8.3	20.7	20.9	20.0	33.8	—	36.2	3.7	3.3	3.5	30.6	30.1	29.2	3	3	3
19-26	2	7.3	7.5	8.4	20.3	20.4	19.2	33.8	32.7	35.9	1.8	1.8	1.7	9.1	9.8	9.8	7	7	7
	4	7.3	7.4	8.4	21.0	20.6	20.2	34.2	31.4	—	3.8	3.1	3.0	28.7	30.6	30.1	3	3	3
4	2	7.3	7.5	8.4	21.3	20.4	19.8	34.1	32.6	36.6	1.4	1.2	1.2	—	—	—	8	7	7
	4	7.3	7.5	8.2	21.0	20.0	20.1	33.7	33.2	36.7	1.3	1.8	1.7	—	—	—	7	7	6

I = No preservatives; II = with preservatives (BHA 0.02%, citric acid 0.004% and potassium sorbate 0.2%) III = as in II with sterilization.

TABLE 3. PHYSICO CHEMICAL AND SENSORY CHANGES OF SUGAR MODAKA WITH, WITHOUT PRESERVATIVES DURING STORAGE AT DIFFERENT TEMPERATURES

Storage		Moisture (%)			Total fat (%)			Total sugars (%)			F.F.A. (% oleic acid)			P.V. (meq. of O_2/kg fat)			Sensory score		
Temp (°C)	Period (months)	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Initial		7.2	8.1	7.9	21.3	21.0	21.1	33.3	33.2	34.0	0.11	0.13	0.13	3.2	3.2	3.3	7	8	7
37	2	7.2	8.1	7.9	21.3	20.9	20.2	33.4	33.1	34.7	2.9	2.8	2.9	17.8	17.2	17.8	7	7	7
	3	7.2	8.1	7.9	21.3	20.8	20.3	34.6	33.8	34.1	3.9	4.3	3.8	35.6	33.8	36.1	3	3	3
19-26	2	7.2	8.2	7.7	21.5	20.9	20.4	34.2	34.0	34.0	2.6	2.7	2.6	16.7	18.3	16.7	7	7	7
	4	7.3	8.2	8.0	21.8	21.9	20.4	33.9	33.9	35.1	3.0	3.2	3.2	22.3	21.1	23.3	7	7	7
	6	7.3	8.2	8.0	20.4	20.9	20.0	33.6	33.7	34.2	4.7	4.8	4.3	33.2	31.7	29.9	3	3	3
4	2	7.2	8.2	7.8	21.8	21.1	20.3	34.2	33.5	34.5	1.9	2.0	1.9	4.1	4.3	4.3	7	7	7
	4	7.2	8.1	7.9	21.8	20.7	20.2	34.0	34.1	34.7	2.2	2.2	2.4	10.2	12.0	—	7	7	7
	6	7.2	8.2	7.9	21.5	20.7	21.1	33.2	33.8	34.6	2.3	3.0	3.8	—	—	—	7	6	6

I = No preservatives; II with preservatives (BHA 0.02%, citric acid 0.004% + potassium sorbate 0.2%) III as in II with sterilization.

The chemical and sensory changes of sugar holige prepared from copra instead of dehydrated coconut with and without preservatives and sterilization are given in Table 2. It was observed that there were no significant changes in moisture, fat and total sugars during storage in all the three samples. Similar observation was made regarding the changes in FFA, P.V. and sensory score in sugar holige prepared from copra also. Changes observed in products containing graded coconut powder and copra were also similar.

In sugar modaka prepared with and without preservatives and sterilization, the contents of total moisture, fat and sugar did not show any significant changes (Table 3). When FFA increased from 0.11 to 3.2 per cent at 37°C and at 19-26°C (ambient temperature) the products were found to be acceptable at the end of 2 months and 4 months, respectively. Further increase in FFA beyond 4.3 per cent, the products turned brown, with off-taste and rancid smell, which is indicated clearly in sensory evaluation score of 3. When P.V. increased from 3.2 to 18.3, the samples stored at 37°C and at 19-26°C (ambient temperature) were acceptable. Further increase in P.V. in samples at 37°C, showed a distinct off-flavour and became unacceptable. When P.V. at 19-26°C increased to 23.3 the samples did not develop any significant off-flavour or colour and thus the samples showed a score of 6 out of 9 and the overall acceptability of the product was good. When P.V. crossed 30 the products became unacceptable. Thus, it is seen that the sugar holige and modaka made out of dehydrated coconut powder or copra have shelf life of 2 months at 37°C and 4 months at 19-26°C as against 3 months and 6 months respectively in jaggery holige and modaka as reported earlier[2].

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Effect of Protease on Flavour Development and Biochemical Changes in Buffalo Milk Cheddar Cheese

S.K. KANAWJIA AND S. SINGH
Dairy Technology Division, National Dairy Research Institute,
Karnal-132 001, India.

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Attempts were made to enhance the ripening process of buffalo milk cheddar cheese by addition of protease enzyme. Protease, 0.005, 0.010 and 0.025% (W/W of curd) were added in milled curd along with salt. The flavour development and biochemical changes in cheeses with added protease were faster than in those without protease. A level of 0.010% protease addition resulted in getting best cheese.

The flavour formation of cheddar cheese made from buffalo milk is slower than that of cow's milk[1-3] and the main reason could be slower proteolysis. Proteolysis plays an important role in the conversion of calcium-para-caseinate curd to mature cheddar cheese. It also influences flavour since free amino acids have typical flavour characteristics[4] and contribute to back ground flavour[5]. The rationale of protease addition is to increase casein breakdown which would provide more substrate for microbial peptidases, thus accelerating the production of flavour precursors and flavour compounds[6-9]. In this study, attempts were made to enhance the flavour development and biochemical changes in buffalo milk cheddar cheese by addition of protease enzyme.

Materials and Methods

Manufacture of cheddar cheese: Buffalo milk was procured from the experimental Dairy of this Institute. Cheddar cheese was manufactured from 90 l of milk by presalting method[10]. The milk was standardized to casein to fat ratio of 0.70, pasteurized at 63°C for 30 min and cooled to 28°C. To this milk, 1 per cent sodium chloride and 2 per cent LF-40 starter culture were added. Modilase (coagulating enzyme) was used for setting the curd within 45-50 min. The curd was cut and cooked to 37°C in 50 min and cheddaring was done for about 3 hr to attain acidity of 0.5 per cent lactic acid. The cheese curd was milled, salted and pressed. The cheese blocks were ripened partially at 15°C for 3 weeks and then transferred to 8 $\pm$1°C for complete ripening.

Modilase: Modilase produced from *Mucor miehei* in liquid form, was procured from Wisconsin, USA and used at the rate of 14.07 ml/100 l of milk.

Protease (Type II) produced from *Aspergillus Oryzae* was procured from M/S Sigma Chemical Company, USA. The protease was incorporated in the milled curd along with salt. The rates of additions were 0.005, 0.010 and 0.025 per cent of the milled curd.

The sensory characteristics of cheddar cheese in terms of flavour and body and texture were evaluated by a panel of 5 judges using cheddar cheese score card[11] at 2 months interval upto 10 months of curing.

Cheddar cheese was analysed for its biochemical changes, at 4 months interval upto 8 months of curing. The fat in milk was determined by Gerber method and in cheese by Mojonnier method[12]. The PH of the cheese was measured by using Digital PH meter (Elico Pvt. Ltd., Hyderabad). The soluble protein content in cheese was analysed by the method described by Kosikowski[13]. The total free fatty acids were determined by the method recommended by Rama Murthy and Narayanan[14].

Results and Discussion

The effect of protease on flavour and body and texture qualities of cheddar cheese is shown in Table 1. The flavour of control cheese was flat upto 4 months of ripening, whereas protease added cheese was normal right from the beginning. Thus, protease had stimulatory effect on flavour of cheese. The 0.005 per cent protease had marginal beneficial effect on flavour formation in cheese. The higher doses and increased ripening period further enhanced the flavour development. The maximum acceptable flavour was attained in cheese containing 0.010 per cent protease at 6 months of ripening. The highest concentration of enzyme resulted in bitter flavour development. This may be due to accumulation of large amount of bitter peptides. It is reported that enzyme treated cheese developed higher levels of soluble proteins and free volatile fatty acids and displayed better flavour and acceptability than control cheese[15]. Similar observations were also reported by several workers for cow's milk cheese[7-9,16,17].

Similarly, the body and texture development was also faster in protease added cheeses. The cheese made with 0.010 per cent protease resulted in the most acceptable body. The

TABLE 1. EFFECT OF PROTEASE ON FLAVOUR AND BODY TEXTURE OF CHEEDAR CHEESE DURING RIPENING

Treatment	Concn. (%)	Flavour during indicated ripening period (months)				Body and texture during indicated ripening period (months)			
		2	4	6	8	2	4	6	8
Control	—	34.0 F	34.5 F	36.0	37.5	25.0 H,Cu	25.0 H,Cu.	26.0	26.5
Protease	0.005	35.5	36.6	38.0	38.5	25.5	26.5	27.0	27.2
Protease	0.010	36.0	38.5	40.2	40.0	26.0	27.5	28.2	28.2
Protease	0.025	36.5	37.0 Sl.B	34.0 B	32.0	27.0	27.5	26.0 W,B	24.0 W,B

*Average of 3 trials;
Flavour: F-Flat, Sl.B-Slight bitter, B-Bitter;,
Body and Texture: Cu-Curdy, H-Hard, W-Weak, B-Brittle

TABLE 2. EFFECT OF PROTEASE ON CHANGES IN PH, SOLUBLE PROTEINS AND FREE FATTY ACIDS IN CHEDDAR CHEESE DURING RIPENING

Treatment	Concn. (%)	PH during indicated ripening period (months)			Soluble proteins during indicated ripening period (months)			Free fatty acids during indicated ripening period (months)		
		0	4	8	0	4	8	0	4	8
Control		5.20	5.28	5.41	1.16	3.26	4.16	2.42	10.10	17.20
Protease	0.005	5.21	5.33	5.46	1.40	4.96	6.92	2.92	12.50	18.40
Protease	0.01	5.23	5.37	5.50	1.80	6.05	7.54	3.30	13.10	19.20
Protease	0.025	5.27	5.42	5.62	2.20	7.62	9.10	3.68	13.86	20.10

* Average of 3 trials.

highest level of enzyme addition resulted in softer, brittle, less springy and less cohesive cheese. These defects were more pronounced towards the end of ripening period. This may be due to excessive proteolysis. Our findings are in agreement with those reported by Fedrick *et al*[9]. and Ridha *et al*[16].

The effect of protease on change in pH, soluble protein and free fatty acids in cheddar cheese during ripening is presented in Table 2. Initially, the pH ranged from 5.20 to 5.27 being the maximum for cheese containing 0.025 per cent protease and the minimum for control. These pH values increase with the progressive ripening time. During ripening the protease added cheese showed higher values as compared to control. This may be due to release of more basic amino groups by higher proteolysis that took place in experimental cheese.

The proteolysis in control cheese was the slowest as compared to that of experimental. Proteolysis enhanced by the addition of protease in cheese curd. The casein hydrolysis was positively related with increasing enzyme concentration and storage time. The values of soluble proteins in protease containing cheese of 2 to 3 months old were equivalent to that of 8 months old control cheese. The greater acceleration of proteolysis was achieved by protease by many workers[9,15,17,18].

The lipolysis in experimental cheeses also was accelerated by the addition of protease. Free fatty acids (FFA) in cheese increased with the increase ripening time. The lipolysis was positively related with concentration of enzyme and period of storage. The maximum free fatty acids were noticed in cheese containing maximum protease. Whereas, the values of FFA in control cheese remained at lower side throughout the ripening. The stimulatory effect of protease on lipolysis may be due to split of lipo-protein system by which the lipolytic activity got accelerated.

The flavour formation in buffalo milk cheddar cheese could be enhanced by addition of protease enzyme and hence considerable amount of time and energy can be saved.

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Effect of Cooking on Lipid Composition of Buffalo Bone Marrow

N. SHARMA, R.C. KESHRI AND G. GANDEMER*
Division of Livestock Products Technology, Indian Veterinary Research Institute,
Izatnagar - 243 122, U.P., India.

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Samples of femur marrow of buffalo were analysed for total, neutral and phospholipids (TL, NL and PL). Effect of cooking of bones on lipids and their fatty acid composition were determined. Major lipid was triglyceride (TG) in femur marrow. In PL fraction, phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) were predominant. Bone marrow contained 42-46% saturated fatty acids of which the major components were palmitic and stearic acids. Monounsaturated fatty acids (50-56%) comprised mainly of oleic and palmitoelic acids. The polyunsaturated fatty acid (PUFA) contents were limited to 1% in TL and 3% in PL. Linoleic and linolenic acids were present in PUFA. Cooking had influence on lipid content and pressure cooking released greater amount of NL from marrow into the soup. Pressure cooking caused a decrease in saturated fatty acids.

Buffalo meat is usually separated from bones for export or preparation of meat products. Further bones are utilised for making bone soup. Besides proteins and minerals, bone provides variable quantities of marrow which is a rich source of lipids[1]. The importance of lipids and fatty acids as dietary constituents is well known. Few studies have been made on lipid and fatty acids of buffalo muscles[2-4]. Information on lipid and fatty acid composition of bone marrow from buffalo is, however, not available.

Cooking affects composition of lipid and common methods of cookery are known to accelerate the meat lipids[5]. In preparation of a certain meat product viz. *Nihari* buffalo meat with bone is cooked for 5-6 hr on slow fire. For preparation of bone soup also, bones are cooked for long time. Thus, it is necessary to know the effect of cooking on marrow lipids. This study was, therefore, undertaken to determine the effect of cooking on lipid and fatty acid composition of bone marrow from buffalo.

Materials and Methods

Femur bones of freshly slaughtered buffalo were obtained from local meat market and subjected to two types of cooking (i) simmering under water in open kettle for 6 hr and (ii) pressure cooking (15 lb PSI) for 90 min. Marrows from fresh and cooked bones were removed and mixed separately. Ten gram marrow samples from each group were taken for extraction of lipids by method of Folch *et al.*[6] Extracted samples were taken for estimation of neutral (NL) and phospholipids (PL) and free fatty acids (FFA). Thin layer chromatography (TLC) of NL and PL was carried out on silica gel plate (250 thickness) for separation of NL and PL[8]. For separation of NL, triple solvent systems containing hexane: diethyl ether: glacial acetic acid were tried in proportions of 60:40:1, 90:10:1 and 30:70:1 respectively[7]. For separation of PL, chloroform: methanol: 7M ammonium hydroxide in the ratio of 115:45:75 (v/v/v) were employed. Spots of NL and PL were identified by comparing their Rf values with authentic standards co-chromatographed with each run. Each spot was scrapped and glyceride-glycerol from mono-di- and triglycerides was estimated[9]. The procedure of Bartlett[10] as modified by Marinetti[11] was used for the estimation of PL phosphorus. Certain PL having very close Rf values viz. phosphatidyl serine (PS) and lysophosphatidylcholine (LPC), lysophosphatidyl ethanolamine (LPE) and sphingomyeline (SPH) and phosphatidic acid (PA) and polyglycerol phosphatide (PA+PGP) were not always distinct in buffalo marrow lipid. Therefore, to keep the uniformity in expression of results, these PL fractions were eluted together. Free fatty acids (FFA) were estimated colorimetrically[12]. For estimation of cholesterol, method of Hanell and Dam[13] was followed.

For fatty acid analysis, methyl esters of the TL, NL and PL were prepared as described by Hornstein *et al*[14]. Gas liquid chromatography of the methyl esters was performed on a Girdel 300C gas chromatogram paired with an integrator Enica 10 (Delsi Instruments). The capillary column (50 m long and with a 0.25 mm internal diameter) containing stationary phase-BDS was maintained in the oven at 190°C and the flow rate of hydrogen was 1 bar. The injector and detector temperatures were 230°C. The individual fatty acid peaks were identified by comparing equivalent chain length (ECL) with those of standard fatty acid mixtures (Sigma standard 189-5, Suelco Inc., PUFA 24-7015) and the ECL published by Massart-Leen *et al.*[15] The results were expressed as per cent of the weight of methyl ester injected.

*INRA, LAOA, Nantes, France.

Results and Discussion

The lipid composition of buffalo bone marrow and bone soup fat are presented in Table 1. Pressure cooking of bones resulted into more release of the total lipids from marrow as compared to the simmering. Accordingly, soup fat obtained out of pressure cooked bone had higher amount of total lipids than those of simmered bone. The predominant lipid present in marrow was NL. In the NL fraction, the majority of the lipids (97 per cent) were triglycerides (TG). Cholesterol content was 1.6 per cent (Table 2). Mellow *et al.*[16] reported almost similar value for cholesterol in bovine marrow of femur bone. Increase in monoglycerides (MG), diglycerides (DG) and FFA and simultaneous decrease of TG due to cooking could be due to hydrolysis of TG to MG and DG[17].

Individual fractions of PL are shown in Table 3. Almost 60 per cent of all fractions was PC. Next predominant fraction was PE. Distribution of these PL fractions of buffalo femur marrow approximated to that found in cow marrow[18]. Contrary to the observation in cow marrow, the buffalo femur marrow contained appreciable amounts of LPC, LPE and PS. There was reduction in values of PE due to cooking but same was not true for PC which seemed to be more stable to heat[19].

The composition of fatty acids of total, neutral and polar lipids of fresh and cooked buffalo marrow are given in Table 4. The identifiable fatty acids which represent a minimum of 0.1 per cent of the total fatty acids are reported in the Table.

In bone marrow, the principal fatty acids found were myristic (10:0), palmitic (16:0), stearic (18:0), palmitoleic (16:1, N-9, 7) and oleic (18:1, N-9, 7). There were about 4 per cent branched chain fatty acids. Linoleic acid was almost 4 times more than linolenic acid.

To our knowledge, there is no report on fatty acids of buffalo bone marrow for direct comparison. However, patterns of fatty acids obtained in the present study are almost similar to those of marrow fatty acids of cow[18] with major difference that oleic acid (18:1) was comparatively more in buffalo marrow lipid as compared to that of beef marrow.

In NL fraction of marrow C: 16, C: 18, C16: 1 and C18: 1 accounted for approximately 87 per cent of the total fatty acids. Oleic acid (C18: 1) was the major fatty acid in NL fraction, which is in agreement with the findings of other workers[16,18].

With respect to the PL fraction (Table 4), the percentage of total monosaturated fatty acids was less than those of the TL and NL fractions. Only two fatty acids i.e. linoleic and linolenic were in PUFA.

On the basis of results obtained, it can be said that marrow as a food is a rich source of energy with some essential fatty acids.

TABLE 1. TOTAL LIPIDS (TL), NEUTRAL LIPIDS (NL) AND PHOSPHOLIPIDS (PL) (G PER 100 G SAMPLE) OF BUFFALO BONE MARROW

	TL	NL	PL	NL*	PL*
Raw bone marrow	54.71 ± 0.21	50.24 ± 0.23	4.46 ± 0.05	91.85 ± 0.10	8.15 ± 0.10
Simmered bone marrow	48.46 ± 0.51	44.95 ± 0.48	3.50 ± 0.04	92.77 ± 0.04	7.23 ± 0.04
Pressure cooked bone marrow	38.14 ± 0.08	35.55 ± 0.23	2.60 ± 0.24	93.16 ± 0.64	6.84 ± 0.64
Simmered bone soup fat	47.11 ± 0.04	45.10 ± 0.03	2.01 ± 0.02	95.71 ± 0.03	4.28 ± 0.03
Pressure cooked bone soup fat	62.22 ± 0.06	59.81 ± 0.03	2.41 ± 0.03	96.12 ± 0.06	3.88 ± 0.06

*g/100 g of total lipids. (Mean ± SE)

TABLE 2. EFFECT OF COOKING ON NEUTRAL LIPID FRACTIONS (%) OF BUFFALO BONE MARROW

Sample	TG	MG	DG	TC	FFA
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Raw bone marrow	97.04 ± 0.16	—	0.65 ± 0.02	1.61 ± 0.14	0.51 ± —
Simmered bone marrow	95.55 ± 0.13	0.20 ± —	0.73 ± 0.01	1.57 ± 0.02	1.49 ± 0.01
Pressure cooked bone marrow	94.23 ± 0.08	0.51 ± 0.03	1.50 ± 0.04	1.81 ± 0.04	1.78 ± 0.03

TG Triglyceride, MG Monoglyceride, DG Diglyceride, TC Total cholesterol, FFA Free fatty acids.

TABLE 3. EFFECT OF COOKING ON PHOSPHOLIPID FRACTIONS (%) OF BUFFALO BONE MARROW

Fractions	RBM	SBM	PCBM
	Mean ± SE	Mean ± SE	Mean ± SE
Org + PI	4.01 ± 0.03	3.89 ± 0.01	3.65 ± 0.06
PS + LPC	7.22 ± 0.06	9.35 ± 0.07	10.91 ± 0.05
LPE + SPH	6.42 ± 0.06	6.81 ± 0.07	6.75 ± 0.05
PE	15.11 ± 0.03	8.54 ± 0.07	9.20 ± 0.03
PC	58.10 ± 0.06	61.46 ± 0.73	60.34 ± 0.05
PA + PGP	8.20 ± 0.08	8.98 ± 0.05	8.53 ± 0.04

RBM Raw bone marrow; SBM Simmered bone marrow; PCBM Pressure cooked bone marrow.

TABLE 4. FATTY ACID COMPOSITION OF TOTAL LIPIDS (TL), NEUTRAL LIPIDS (NL) AND PHOSPHOLIPIDS (PL) OF BUFFALO BONE MARROW

Fatty acid (%)	Raw bone marrow			Simmered bone marrow			Pressure cooked bone marrow		
	TL	NL	PL	TL	NL	PL	TL	NL	PL
14:0	2.50	1.65	2.50	2.70	2.30	2.35	1.25	0.75	1.35
15:0	1.35	1.65	1.25	0.95	0.90	1.00	0.35	0	0.55
16:0	24.15	23.45	25.45	26.05	24.45	25.75	13.95	12.95	16.90
17:0	1.35	1.56	1.75	1.65	1.60	1.70	0.60	1.00	0.80
18:0	9.30	9.45	12.35	9.75	9.5	11.85	3.55	4.25	5.90
Saturated (satu)	38.70	38.10	43.30	41.10	38.85	42.70	19.70	19.10	25.40
Branched satu.	3.80	3.20	3.55	4.00	3.50	3.40	4.25	2.40	3.10
Total satu.	42.45	44.32	46.85	45.10	42.60	46.10	23.90	21.55	28.50
16: 1 N-9	0.85	0.80	0	0.60	0.55	0	0.40	0.65	0
16: 1 N-7	7.90	6.55	5.60	6.25	6.10	6.05	13.55	11.00	11.05
17: 1	1.65	1.55	1.45	1.80	1.80	1.56	2.65	2.30	2.20
18: 1 N-9	44.20	45.55	40.05	40.65	44.90	41.55	54.60	56.05	51.85
18: 1 N-7	1.25	1.60	1.95	3.60	1.85	1.95	2.60	5.90	2.55
20:1	0.40	0.55	1.15	0.50	0.65	1.10	0.90	1.20	1.30
Monounsatu.	56.25	56.60	50.20	53.35	55.80	52.15	74.40	77.20	68.90
18:2 N-6	0.95	1.55	2.70	1.20	1.20	1.45	1.15	1.10	2.30
18:3 N-3	0.30	0.40	0.05	0.35	0.35	0.25	0.30	0.20	0.35
Polyunsatu.	1.25	1.95	3.20	1.56	1.60	1.70	1.45	1.30	2.65

Distribution of fatty acids were altered due to cooking and method of cooking had influence on fatty acids. The pressure cooking caused major loss of saturated fatty acids. As pressure cooking resulted in higher loss of total lipids in the form of soup fat, the lower values were recorded in pressure cooked as compared to those of simmered marrow. The concentration of monosaturated fatty acids increased with pressure cooking. It is hard to offer a suitable explanation for such changes but results were consistent for mono-unsaturated fatty acids in TL, NL and PL fractions. No appreciable difference due to cooking was observed in PUFA[20].

Alteration in marrow lipids during cooking appears to be, in part, a function of temperature and pressure. However, more work should be performed towards the transfer of heat in a multiphasic system such as bone, cooking condition and their relationship to fatty acids.

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Storage Studies on Coorg Mandrin Juice Concentrates Packed in Various Containers

R.S. RAMTEKE AND W.E. EIPESON
Central Food Technological Research Institute,
Mysore - 570 013, India.

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Coorg mandrin juice concentrate (68°Brix) was prepared using forced circulation evaporator. One batch of concentrate was prepared after aroma recovery. The fresh juice concentrate, aroma stripped juice concentrate and stripped juice concentrate to which recovered aroma was added were packed in plain OTS cans (A 2½), high density polyethylene bottles (1 kg) and paper/aluminium foil laminate pouches (750 g). They were stored at 25-28°C (RT), 0°C, -8°C and -18°C and analysed for various quality parameters like browning index, carotenoids, ascorbic acid, cloud and sensory quality. It was found that concentrate could be stored without appreciable changes for six months at 0°C. For longer storage, temperature below 0°C are preferable. Orange juice concentrate with and without aroma addition did not show any difference.

The literature concerning changes which occur during the storage of citrus juice concentrate showed that non-enzymatic browning, ascorbic acid degradation, changes in lipid constituents and the terpenes, are the main reactions which govern the shelf life of citrus concentrates[1-3]. Browning in citrus products has been associated with the degradation of ascorbic acid[4]. It is shown that, browning in citrus juices appears when 10-15 per cent ascorbic acid has been degraded[5]. Water insoluble solids increased during storage of orange concentrate[6]. At 15.5°C, decrease in water soluble pectin and loss of cloudiness have been reported by Rouse *et al.*[7].

For optimum flavour, aqueous essences are added to the concentrates to impart fresh aroma, Dougherty *et al.*[8] indicated that in orange juice there was an optimum level for essence and above this level a decrease in flavour quality resulted. The present study was undertaken to arrive at the shelf life of mandrin juice concentrate packed in cans, HDPE bottles and laminate pouches during storage at RT, 0°C, -8°C and -18°C.

Materials and Methods

Raw material: Coorg Mandrin oranges of main crop with fully developed colour were purchased from the local market.

Juice extraction: Oranges (2000 kg) were washed in tap water and peel was removed by hand. The orange segments were fed into screw type juice extractor and the juice was collected. The juice was centrifuged in a basket centrifuge to reduce the pulp content to 1 per cent. The juice was pasteurized in the pasteurization section of aroma recovery unit.

Aroma recovery: Aroma concentrate from orange juice was recovered in a pilot plant model aroma recovery unit (Holstein and Kappert, W, Germany) using the principle of evaporation and fractional distillation. The per cent water evaporation required to separate 90 per cent of the orange aroma was determined in the laboratory by distilling off different percentage of water from 500 ml orange juice and determining the chemical oxygen demand (C.O.D.) of the distillate[9]. It was found that 25 per cent water evaporation was sufficient to recover 90 per cent of the volatiles. In the aroma recovery unit, orange aroma could be concentrated to 80 fold strength. The aroma concentrate obtained had about 80 per cent of the volatiles initially present in the juice.

Concentration of juice: Pasteurized orange juice as well as aroma stripped juice were concentrated to 68° Brix in a forced circulation evaporator operating at 45°C.

Packaging and storage of juice concentrate: The aroma stripped juice concentrate was divided into two equal lots and to one lot, half of the recovered aroma concentrate was added (100 per cent level). The fresh juice concentrate, balanced stripped juice concentrate and aroma stripped juice concentrate to which recovered aroma was added were packed in plain A 2½ size OTS cans, HDPE containers (1 kg capacity) and laminate pouches (750 g capacity). The containers were stored at room temperature (RT) (25-28°C), 0°C, -8°C and -18°C. Fresh orange juice was also canned in plain A 2½ size cans and stored at 0°C which served as a control in sensory evaluation.

Preparation of beverage: The beverages from concentrates were prepared to give the final product having 15 per cent juice, 15 per cent total soluble solids content and 0.25 per cent acidity.

Sensory evaluation: The important criteria of sensory quality of orange was analyzed. A group of 20 descriptive-communicative panel was initially trained for (i) identification

of orange aroma at threshold in ascending concentration sequence and (ii) testing of orange juice prepared as ready-to-serve (RTS) by ranking method for attributes of colour, aroma and overall quality. These methods were chosen to identify aroma difference more critically and also follow with observations at use level. The test samples were tested along with canned fresh juice from the same lot as control sample. The data obtained were analyzed for statistical significance using Bureau of Indian Standard Specifications[10].

Chemical analysis: Ascorbic acid was determined by indophenolxylene extraction method as described by Robinson and Stotz[11]. Alcohol soluble colour (ASC) i.e. browning index was determined as detailed by Chan and Cavaletto[12]. Carotenoids were determined according to the standard A.O.A.C. method[13]. Cloud was determined according to McCollach and Randall[14].

Results and Discussion

The material balance data for orange processing are presented in Fig 1. The analysis of orange juice and concentrate is reported in Table 1. Winter (main crop) crop oranges have better sugar/acid balance and lower bitterness as compared to rainy season oranges[15]. Therefore, for processing into concentrate the former was preferred. The concentrates stored at -18°C in all the three types of containers did not show any increase in browning (Fig 2A). At -8°C and 0°C, there was only gradual increase in browning upto 6 months storage; the extent of browning in all the three containers was same. While browning of the concentrates stored at 0°C became perceptible only after 6 months of storage, those at RT showed perceptible browning after 3 months of storage. Among the containers, cans showed least browning followed by laminate pouches and HDPE jars. Marcy *et al.*,[16] found that storage temperature and time significantly affected the absorbance at 420 nm of non-enzyme treated orange concentrate (66° Brix). The role of stannous ions and the reducing atmosphere inside the plain cans are the major factors which retard the browning reactions. The laminate pouch with aluminium foil has a lower oxygen transmission rate than the HDPE container which has shown the highest browning rate. At -18°C, there was no loss of ascorbic acid (Fig 2 B). However, at higher temperatures, the losses were significant. Ascorbic acid loss at 0°C and RT (25-28° C) after six months of storage were 30 and 80 per cent respectively. Among the containers, cans and laminated pouches were better compared to HDPE jars in retaining ascorbic acid during storage. Similar findings have been reported by Bisset and Berry[1] for frozen concentrated orange juice. Changes in carotenoid content during storage at different temperatures in different packages are represented in Fig 2C. There was a negligible change in carotenoids content during storage of 6 months at -18°C and -8°C in all the three types of containers. At 0°C, there was about 20 per cent decrease in carotenoid content in cans and pouches and about 30 per cent in HDPE jars. At RT, there was about 40 per cent decrease in cans while there was about 46 per cent decrease in pouches and HDPE jars. Although the all trans configuration of carotenoids predominates in most fresh plant extracts, approximately one-third of these molecules undergoes spontaneous isomerization to the cis form[17]. The rate of isomerization is enhanced by exposure of the extract to light and/or heat.

Fig 2 D shows the extent of cloud loss in orange juice concentrate in different containers during storage. The cloud loss was dependent on storage temperature. Among the containers, there was no difference in the extent of cloud loss. Cloud loss is believed to be the result of pectic enzyme activity on natural pectin in the juice. Rouse *et al.*[7] reported that at 40°C, satisfactory cloud was retained in eight orange concentrates (42°Brix) having various levels of pectin esterase activity and pectin. When pectin esterase was same, orange concentrate containing high content of pectin had a longer storage life than that with low content of pectin.

The threshold of orange aroma in fresh juice was 1:900 as recorded by the panel while in the product after concentration, the strength was only 20 per cent of the original (1:180). Addition of aroma improved it to 50 per cent (1:450).

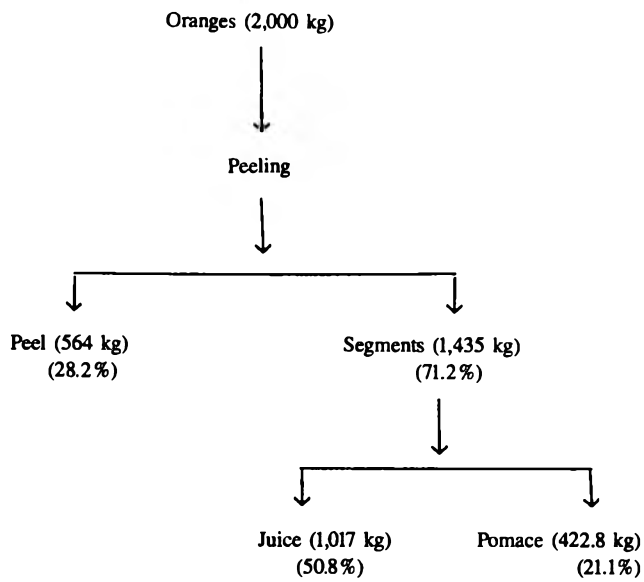

Fig.1. Flow diagram for mandrin orange processing.

TABLE 1. ANALYSIS OF ORANGE JUICE AND JUICE CONCENTRATE

	Juice	Concentrate
Soluble solids (°Brix)	10	68
Acidity (% anhydrous citric acid)	0.40	2.7
pH	4.0	3.8
Total carotenoids (μg/100 g)	74	500
Ascorbic acid (mg/100 g)	35	205
Pectin (%)	0.52	3.51
Browning index (O.D at 420 nm)	0.082	1.15

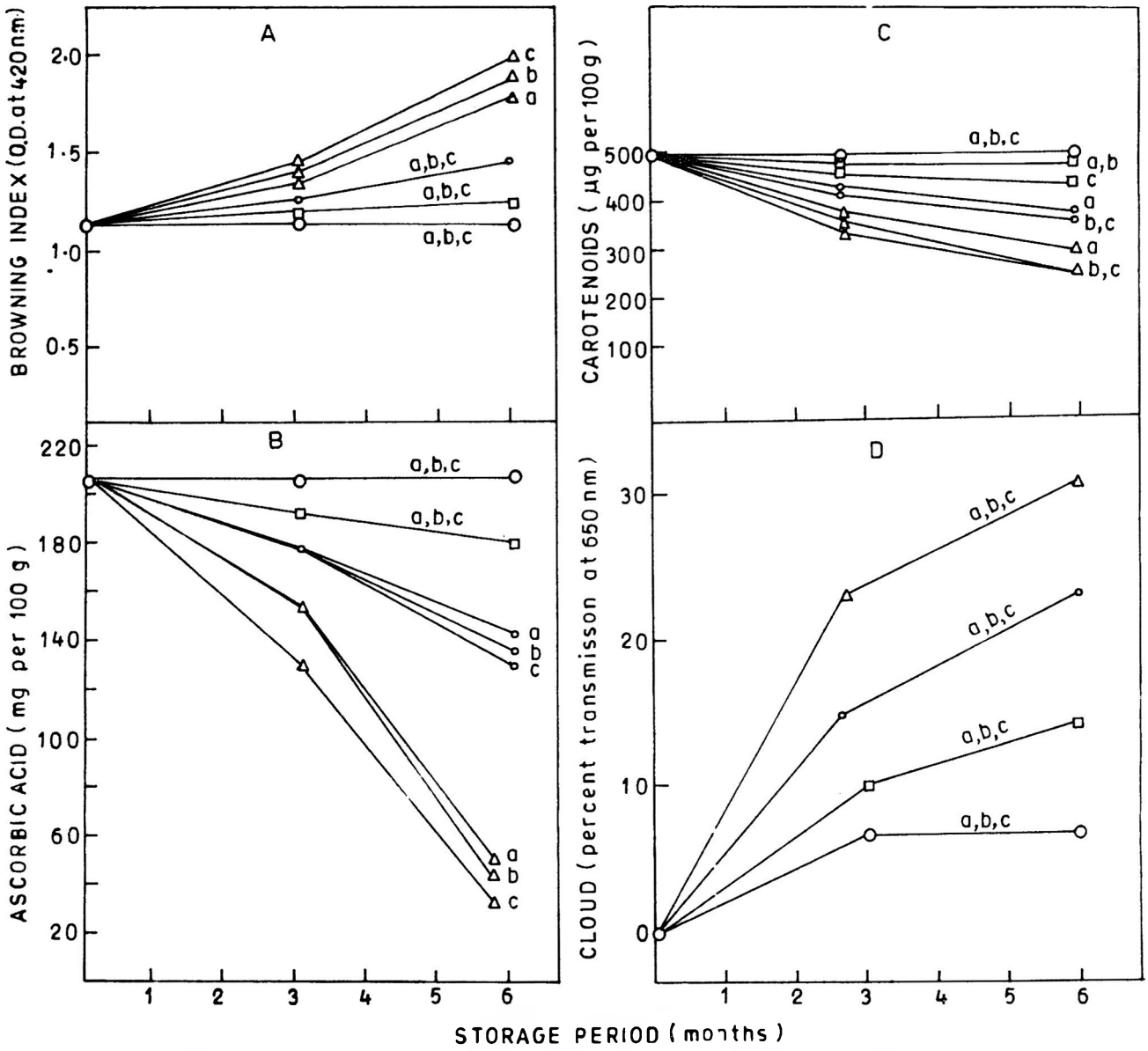

Fig.2. Effect of temperature and package on the changes in orange juice concentrate during storage

O———O = −18°;
□———□ = −8°C
o———o = 0°C
△———△ = 25-28°C
a = Cans (OTS); b = Laminated pouch; c = HDPE bottles

However, the quality was modified and found to be more 'green' 'fruity' and lacked typical orange aroma. The difference between the aroma profiles of fresh orange juice and recovered aroma reconstituted juice from concentrate could be due to the loss of oil fraction of orange aroma. In the aroma recovery unit, though most of the water soluble aroma volatiles could be recovered into the aroma concentrate, a larger portion of the oil fraction is lost in the column's bottom. Storage of these concentrates into three containers and as four temperatures revealed that upto three months, no significant difference was seen among stored samples.

Analysis of RTS beverage by ranking test showed that at 3 months, the HDPE stored sample had picked up off-note and was darker in colour than the samples stored in laminate and in can at all the temperature conditions. The concentrate samples were also confirmed to be different and less intense than fresh sample as found by threshold test. The study therefore, concludes that if care is taken in initial concentration of orange juice and necessary aroma concentrate and peel oil is added, the producct can be stored well in either metallized polyester or can at all the four temperatures for a period of three months while HDPE

packing is not suitable to retain sensory properties.

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Conformational Changes in Lysozyme Due to Food Dyes

J.M. TEIJON, J.A. ONRUBIA AND M.D. BLANCO
Departmento de Bioquimica Y Biologia Molecular. Faculty de Medicina,
Unisidad Complutense - 28040 Madrid, Espana

ISSA KATIME*
Departmento de Quimica Fisica Grupo de Nuevos Materiales,
Facultad de ciencias, Universidad del Pais Vasco, Apartado 644, Bilbao, Espana

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Lysozyme interaction with food dyes causes conformational changes in the former. The dye concentration at which conformational transition appears is lower in the case of FAO/WHO-accepted food dyes such as tartracin and indigotin, than non-accepted ones such as eosin gelblich, although the absolute intrinsic protein viscosity in the presence of the latter dye is twice that of the former ones. However, these conformational changes do not alter the lytic activity of the enzyme, as evidenced by bacterial susceptibility tests, either through diffusion in agar or through dilution in culture broth, at these dye concentrations.

The interaction that takes place between molecules and its physico-chemical environment may affect its structure[1]. When a non-covalent ligand bond to macro-molecules takes place, a fixing of such ligands to certain areas of the macromolecular architecture ensues stoichiometric complex, as well as a modification of the macromolecular environment (preferential sorption)[2], leading to a possible local or general reorganization of the macromolecular coil, finally resulting in the appearance or disappearance of different functional activities from those present up to that point.

When biomolecules are in solution, they show characteristically distinct conformational parameters such as superficial accesible area, and molecular shape and volume. These factors are responsible for thermodynamic and hydrodynamic properties that characterize solutions containing such macromolecules[3]. These properties are altered when an agent induces conformational changes in a macromolecule. Differential refractometry and viscometry are capable of assessing some of the aforementioned properties, and in an indirect way inform the conformational parameters previously mentioned.

In order to determine whether the conformational changes in lysozyme, may affect its biological function, a bacterial susceptibility test is performed using a microorrganism susceptible to such an enzyme, employing agar diffusion or culture broth dilution methods.

Materials and Methods

Two types of dyes were used in this study, tartracin[4-7] and indigotin[4,5,8-11] permitted in food industry and eosin gelblich[4,5,13,14] not permitted. Tartracin belongs to category A of the FAO/WHO organization[12]. It is a monoazoic dye with a molecular weight of 534.7 g/mol. The latter, indigotin, belongs to category B of the FAO/WHO orrganization[12]. It is an indigoid dye with a molecular weight of 466.36 g/mol. Eosin gelblich is included in the toxicological category C-II of the FAO/WHO organization[12]. This dye is a xanthic derivative with a molecular weight of 691.88 g/mol, and is used in different cosmetic products (lip sticks, makeup, etc), and as dye in the textile and paper industries. The protein chosen was lysozyme (EC. 3.2.1.17) from egg whites, supplied by Serva at 25,000 U/mg and a molecular weight of 140,000 g/mol.

The binary mixture dye-water was prepared at a percentage weight concentration, with mixtures ranging between 0 and 0.05 per cent. The lysozyme concentration for each mixture was 2.5 per cent by volume. The intrinsic viscosity of the lysozyme in the different solutions was determined in a modified Ubbelhode suspended level viscometer immersed in a thermostatic bath at 298°K, with a temperature control of ± 0.05°K, by double extrapolation using the Huggins[15] and Kraemer[16] equations.

The differential refractive index increments of the solutions, dn/dc, were measured at 298°K and 633 nm in a Brice Phoenix differential refractometer model BP 2000, equipped with a He-Ne laser. The refractive indices of the pure solvents and of the mixtures were measured at 298°K in a Warszawa precision refractometer.

The partial specific volume of lysozyme, $\bar{V}$, has been calculated from refractometric measurements using the equation:

$$dn/dc \; \bar{v} \; (n - n_o)$$

*Correspondence address: Avda Basagoiti, 8-1°C 48990 Guecho, Algorta Vizcaya, Espana.

where dn/dc is the differential refractive index increments of the solutions, n and n_0 the refractive index of the lysozyme in water and water, respectively.

Two different procedures were used in the bacterial susceptibility test; agar diffusion, and dilution in culture roth. The agar diffusion technique used[17], was that modified by Bauer[18], with Mueller-Hinton II (BBL) agar as culture medium. Each lot underwent a quality control test[19], using control strains[20]. The different agar plates were inoculated with *Micrococcus luteus* ATCC-9341, grown in blood-agar medium. Six filter paper discs impregnated with the corresponding solutions were placed on the agar plate in the following arrangement. Four discs contained lysozyme dissolved in water at concentrations ranging between 1 and 10 per cent; one contained the ternary mixture lysozyme/water/dye with 2.5 per cent lysozyme and at the dye's critical concentration; and one control disc only containing dye, used as control.

The culture broth dilution test was used for determining lysozyme's minimum inhibitory concentration (MIC), compared with the lysozyme dye systems which induced conformational changes, as determined by other methods. The starting solution contained 100 mg of enzyme in 1 ml sterile Mueller-Hinton culture broth, the usual protocol for the case was followed. In the case of eosin gelblich, a minimum bactericidal concentration (MBC) had to be done, since the MIC reading was not possible to do.

Results and Discussion

The changes in the protein's intrinsic viscosity, [n], as a function of the binary mixture water-dye composition, U_2, for each of the dyes under study can be observed in Fig. 1. Eosin gelblich induced a conformational change in lysozyme at dye composition of 0.045 per cent; while tartracin and indigotin gave rise to conformational transition at a concentration of 0.01 per cent. Therefore, tartracin and indigotin, induce conformational changes at lower concentration than eosin gelblich. However, the value in absolute terms of the protein's intrinsic viscosity is almost twice as high in the case of eosin gelblich as can be seen in Fig 2. This finding suggests that this dye shows greater affinity for the biomolecule.

The changes in the refractive index increments as a function of dye concentration can be seen in Fig. 2. Tartracin induces conformational changes at a lower concentration of 0.01 per cent, indigotin came next at concentration 0.015 per cent, and finally eosin gelblich at concentration of 0.025 per cent, the degree of change induced by each dye, being 5.1, 6.8 and 8.5 per cent respectively. Nevertheless, that degree of change experimented by the partial specific volume (Table 1), indicated that the lysozyme-dye interaction was more effective in the following order:

eosin gelblich > indigotin > tartracin

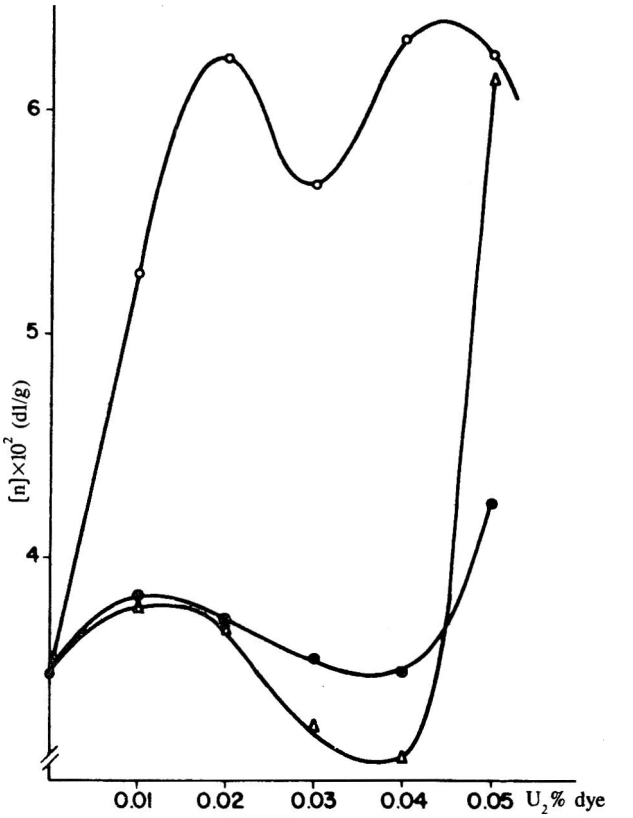

Fig.1. Dependence of intrinsic viscosity on the volume fraction of dye for lysozyme in the water/dye system. (o) Eosina, (△) tartracin and (●) indigotin.

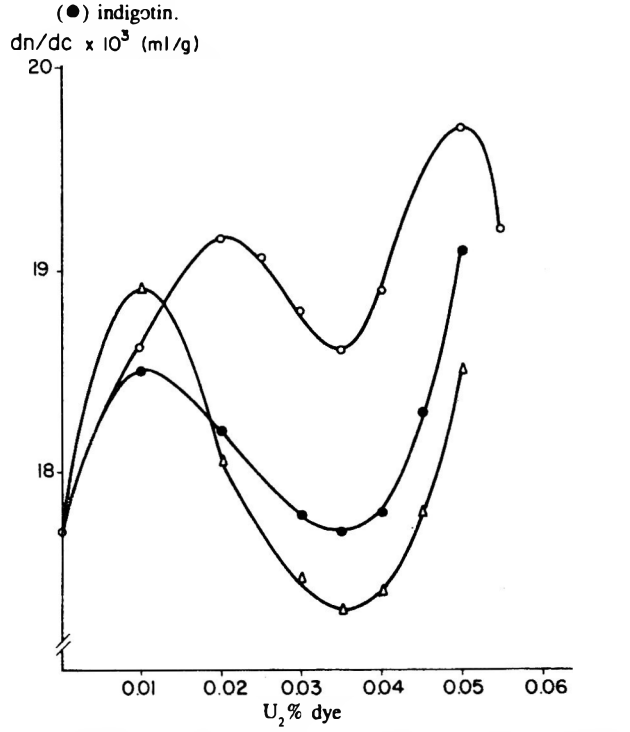

Fig.2. Dependence of dn/dc on the volume fraction of dye for lysozyme in the water/dye system. (o) eosin, (△) tartracin and (●) indigotin.

Attempts were made to determine whether the conformational changes iinduced by the dyes on lysozyme also affects its biological function using *Micrococcus luteus*

TABLE 1. PARTIAL SPECIFIC VOLUME VALUES (ML/G) OF LYSOZYME AS A FUNCTION OF DYE CONCENTRATION

U2 (%)	Tartracin	Indigotin	Eosin gelblich
0.00	0.733	0.733	0.733
0.01	0.945	0.925	0.930
0.02	0.900	0.910	0.955
0.025	—	—	0.960
0.03	0.875	0.890	0.940
0.035	0.865	0.885	0.930
0.040	0.870	0.890	0.945
0.045	0.890	0.915	—
0.05	0.925	0.955	0.985

ATCC-9341 as the susceptible microorganism. Linear regression and correlation analyses were done between the inhibition halos and their respective lysozyme concentration by the agar diffusion method. A Pearson linear correlation coefficient of r = 0.9211 was found. This value was contrasted with the hypothesis p = 0 (population correlation coefficient), through the statistician $t_{n-2} = r\sqrt{n-2}/\sqrt{1-r^2}$, with the result that such hypothesis was rejected, indicating the existence of a strong random dependence, very close to a functional one, between both variables studied. The regression line equation way $y = 9.9502.10^{-5}_{x} + 7.6574$, with an estimation error of $S_{cy} = 0.8231$, showing the accuracy of the fit. The presence of significant statistical differences among the inhibition halos of each lysozyme concentration used, was verified by means of one-way ANOVA, with a resulting value of $F_x = 85.885^{***}$ ($p < 0.001$). The hypothesis of normality and homo-cedasticity were confirmed previously through Barlett test, with a resulting value of $X^2 = 3.3463$, which was not significant, against the critical value for four degrees of freedom corresponding to the five groups studied ($X_4 = 9.94$ for $\alpha = 0.01$).

In order to test whether the dye modified the lytic activity of lysozyme, a contrast between the means of both groups was done at the same concentration, when the dye was added to the disc at a concentration which in the refractometric and viscometric studies produced conformational changes in the protein (0.04 per cent for tartracin and indigotin and 0.03 per cent for eosin gelblich). Using Student's test, a value of t = 0.5425 was found ($t^*_{10} = 2.228$), which was not significant.

With regard to the culture broth dilution procedure, the MIC value was the lowest lysozyme concentration at which disc did not show any visible growth. In our case, this parameter was 0.78 mg/ml, either in the presence or absence of dye. In the case of eosin gelblich, the reading was not possible due to the presence of aggregates, thus a MBC determination was done, which was the lysozyme concentration that induced the death of 99.9 per cent of the bacteria incubated on petri discs for 48 hr. The MBC value was 1.56 mg/ml for lysozyme in water and 3.125 mg/ml in the presence of eosin gelblich.

The results of the microbiological tests showed that there was significant bacterial susceptibility to lysozyme in the medium, and that the introduction of dyes to such medium did not affect to a significant degree the enzyme's lytic activity. Therefore, the conformational changes observed by physico-chemical methods, must be due to dye adsorption to residue which do not form part of the enzyme's active site, thus not involving its enzymatic activity.

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EFFECT OF PROCESSING CONDITIONS ON EXTRUSION COOKING OF SOY-RICE BLEND WITH A DRY EXTRUSION COOKER

R.T. PATIL, D.S. SINGH* AND R.E. TRIBELHORN**
Central Institute of Agricultural Engineering,
Nabi Bagh, Berasia Road, Bhopal-462 018, India.

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A dry extrusion cooker Insta Pro 2000 was used in the study for extrusion of soy rice blend in proportion of 30:70. It was observed that extrusion at lower level of moisture (12% wb) is most suitable. The highly puffed product having bulk density of 266.23 kg/m^3, WAI of 4.09 was obtained at this level of feed moisture. The moisture content of the final product was also lower (6.0%) avoiding need for further drying.

Extrusion cooking is a thermally efficient process and offers many advantages in processing of soy-based products[1]. Soybean contains antinutritional factors like trypsin inhibitor urease enzyme, which are to be eliminated without degradation of the protein[2]. For this purpose, a high temperature short time process of extrusion is very effective. The low cost autogenous extruders have been found effective for producing nutritious products based on soybean for human food especially for less developed countries. The processing based on low cost extrusion cookers (LEC) is mainly a dry process, hence need for a dryer and boiler is eliminated thereby lowering the cost of the plant.

One such dry extruder model Insta Pro 2000 (Fig 1) was evaluated for extrusion of soy and rice blend and the results are given in this note.

The extrusion cooker shown in Fig 1 consisted of cast iron barrel and screw segments. Screw segments sliped over a central keyed shaft with ring like restrictions called steam locks placed between screw segments. Barrel segments are clamped together to form a continuous barrel. Internal wear rings in barrel segments are replacable. The die was a single hole with adjustable clearance. A variable speed injection auger feeds the extruder. The production rate for this machine varies between 450 and 750 kg/hr at 540 r.p.m. Extrusion temperature for cereal based product ranges between 150 and 170°C. Pre-grinding of ingredients is required for efficient extrusion operation and product uniformity. Addition of 3-5 per cent water to dry feed (12-18 per cent feed moisture) eases the extrusion. Maximum feed moisture limit in this unit is reported to be 20 per cent. Adjustments of die clearance, feed rate and moisture addition control extrusion temperature. All the energy required for cooking/processing is due to mechanical dissipation of frictional energy provided by the primmover of 75 hp.

The study was conducted to produce soy rice blended extruded product. The processing variables (independant) were moisture content (12, 14 and 16 per cent wet basis) and die opening (6.35, 8.89 and 11.43 mm). The percentage of soybean in soybean rice mixture was kept at 30 per cent. The observations were recorded for temperature of extrudate, mass flow rate, bulk density, water absorption index, torque and moisture content of the final product by the standard procedures[3]. The soybean and rice brokens were milled in the proportionate mill to 40 mesh size and then fed to extruder. The water in known quantity was added by rotameter to get required moisture level.

The data recorded on the Insta Pro 2000 extruder are given in Table 1. The transit section temperature ranged from 79.4 to 93.33°C for all the treatments and the melt temperature varied from 168 to 177°C. The temperature is well within the range required for elimination of antinutritional factors[4]. The mass flow rate is almost constant at 5.20 kg/min (range 4.96-5.45 kg/min) as screw speed for all the treatments is same at 600 r.p.m. The bulk density data indicated that it increased with increase in moisture content in all treatments. However, it is not found to vary much with clearance. The lower bulk density indicated higher expansion and better cooking which is further reflected in the observations on water absorption indices (WAI). The WAI is higher at lower moisture contents. Another advantage seen for lower moisture feeding is the lower moisture content of extrudate i.e. final product. The moisture content is in the range of 5.8 to 6.1 per cent (wb) so that product could be used for further grinding and storage without further drying. The torque is also measured while in operation. It indicated that torque requirement variation is mainly due to moisture content because at higher moisture, the flowability of material is relatively higher compared to lower moisture feeds. However, the total hp or energy requirements do not vary significantly even at lower moisture.

From the above study, it is concluded that soy rice blend (30:70) may be extruded at 12 per cent moisture content at the clearances of 6.35 mm to 11.43 mm which gives the product having average values of bulk density as 266.23 kg/m^3 water absorption index as 4.09, and moisture content of 6.00 per cent wb.

*JNKVV, Jabalpur

**Colorado State University, Ft. Collins, USA.

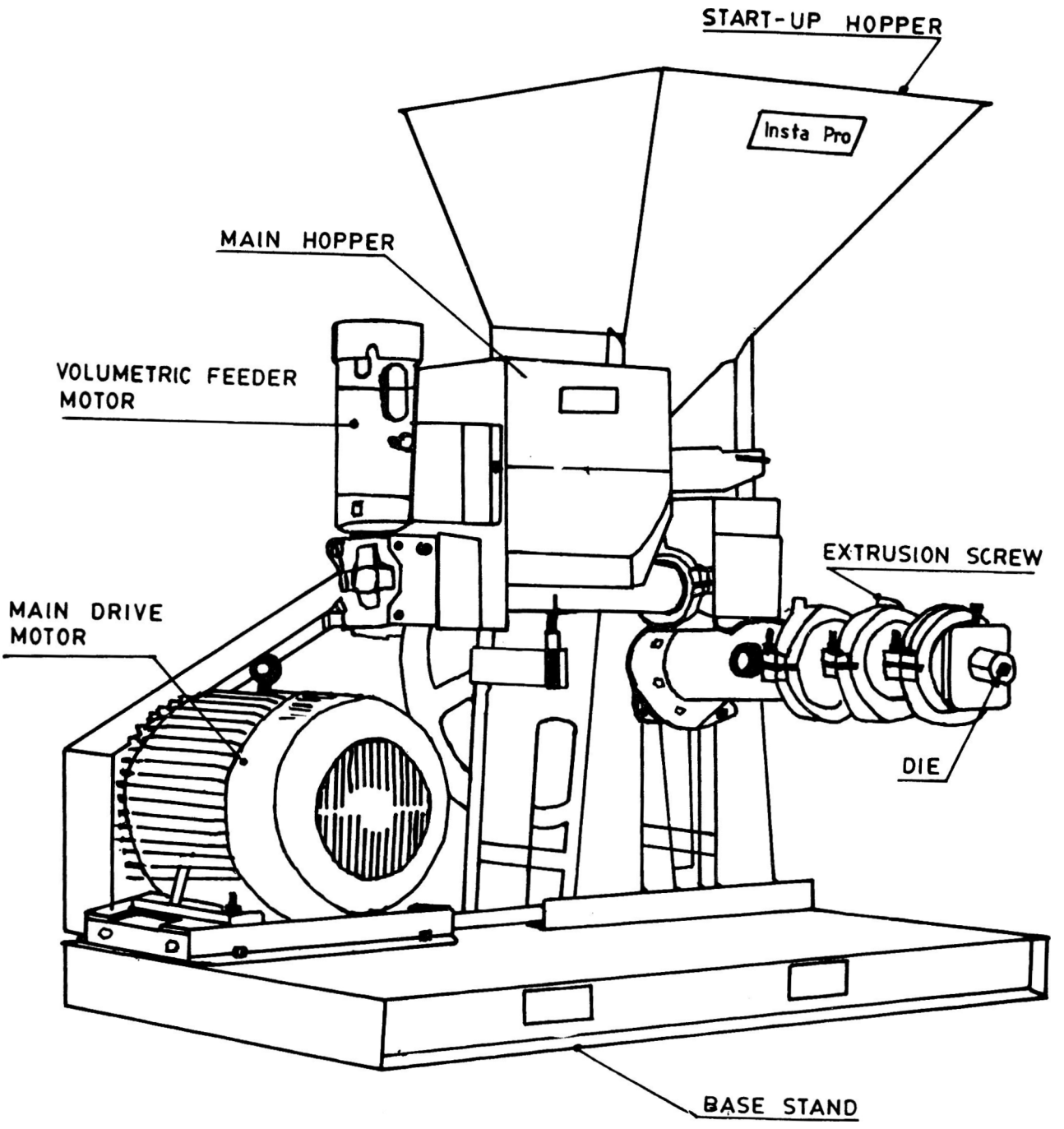

Fig.1. INSTA PRO 2000 EXTRUDER

TABLE 1. OBSERVATIONS RECORDED ON INSTA PRO 2000 DRY EXTRUDER

Moisture content of feed (%)	Clearance (mm)	Temperature °C T_1	Temperature °C T_2	Mass flow rate (kg/min)	Bulk density (kg/m^3)	Moisture content of final product (% wb.)	Water absorption index
12	6.35	79.44	168.33	4.96	242.80	5.80	4.20
12	8.89	93.33	176.66	5.14	266.90	6.10	3.98
12	11.43	93.33	173.88	4.96	289.10	6.10	4.10
Mean				5.02	266.23	6.00	4.09
14	6.35	93.33	171.11	5. 9	404.20	8.40	3.20
14	8.89	93.33	176.66	5.02	410 70	8.70	3.20
14	11.43	93.33	176.66	5.18	390.40	8.50	3.30
Mean				5.13	401.77	8.53	3.23
16	6.35	93.33	176.66	5.45	448.00	10.20	3.10
16	8.89	79.44	176.66	5.19	434.40	9.80	3.05
16	11.43	93.33	176.66	5.40	439.00	10.20	3.30
Mean				5.35	440.47	10.06	3.15

Each data is an average of three replications.

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COMPARATIVE STUDY ON THE ESTIMATION OF ETHANOL IN FERMENTED SAMPLES BY DIFFERENT METHODS

O. SREEKUMAR AND S.C. BASAPPA
Department of Microbiology and Sanitation,
Central Food Technological Research Institute, Mysore - 570 013, India.

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Five known methods such as specific gravity method, Kozelka and Hine method, Caputi's method, Gas chromatographic and enzymatic methods have been used for the estimation of ethanol in different fermented samples, keeping the specific gravity method as a reference. Of these methods, gas chromatographic method was simpler, more rapid, more sensitive and less expensive than the other methods. The data obtained were statistically analysed and found to be in favour of the gas chromatographic method.

In most of the potable fermentation industries, ethanol is estimated generally by the specific gravity method[1]. Since this method is time consuming, not very sensitive and requires a large quantity of sample (about 100 ml) for analysis, the other known physical, chemical and enzymatic methods which required less quantity of sample were employed in the studies.

The main objective was to find out the most suitable method for analysing larger number of samples in terms of rapidity, simplicity, sensitivity and cost. Some of the well known methods were compared in analysing certain fermented samples and the data obtained were statistically analysed and presented in this paper.

Both yeast *(Saccharomyces cerevisiae, S. diastaticus, Schizosaccharomyces pombe)* and bacterial *(Zymomonas mobilis)* cultures were drawn from the stock maintained at the Discipline of Microbiology and Sanitation, C.F.T.R.I., Mysore. These were maintained on Wickerham's[2] agar medium and glucose-yeast-extract medium[3] respectively by periodical subculturing and preserving at 5°C. These were grown separately in 2 per cent glucose-yeast-extract medium (100 ml in 250 ml conical flask) after sterilizing at 121°C for 15 min) at 30°C on rotary shaker (200 r.p.m) for a period of 18 hr to serve as the inoculum. Sterile 12 per cent glucose-yeast-extract medium (150ml in 250 ml conical flask) was inoculated with all the cultures at 10 per cent inoculum level separately, except in cases of *S. cerevisiae* and *Schizosaccharomyces pombe*, wherein 20 per cent glucose-yeast-extract medium was used. The inoculated flasks were incubated at 30°C (sometimes at 37°C) under stationary conditions for periods ranging from 40 to 72 hr. The fermented samples were centrifuged for 10 min at 10,000×g and the ethanol content in the clear liquid was estimated by the following 5 different methods.

One hundred ml of the sample along with 40 ml of distilled water was distilled to collect 100 ml of distillate on ice bath and ethanol was determined at 20°C by the specific gravity method[1].

The Kozelka and Hine method[4] is originally used for the estimation of ethanol in blood and urine samples, wherein 1-2 ml of the sample is deproteinized by sodium tungstate and distilled in all-glass apparatus. The distillate (25-30 ml) is reacted with acidic 0.1 N potassium dichromate solution and ethanol is estimated by iodometric titration.

A 1.0 ml sample was distilled into an acidic potassium dichromate solution in a 50 ml volumetric flask and heated for 20 at 60°C. Per cent ethanol by volume was then determined spectrophotometrically (Spectronic 21) at 600 nm as per Caputi's method[5].

In gas chromatographic (GC) method, n-propanol internal standard is added to sample and ethanol is determined in gas chromatograph (Packard 437) using flame ionization detector[6]. The ethanol concentration (% v/v) is determined by the following formula.

$$\frac{\text{Peak height ethanol}}{\text{Peak height propanol}} \div F$$

where F is the slope, from the standard curve.

The principle involved in enzymatic method is that ethanol in presence of NAD and alcohol dehydrogenase gives rise to acetaldehyde and NADH. The formation of NADH as measured by the increase in extinction at 334, 340 and 365 nm is proportional to the amount of alcohol[7].

All the estimated values were expressed as percent w/v. The data were statistically analysed by the use of Duncan's New Multiple Range test[8], when more than two methods were compared and Student's 't' test, when two methods were compared.

Five different fermented samples were analysed for ethanol using four different methods and the data are presented in Table 1. It shows that the values obtained by specific gravity method were more closer to those of the G.C. method than the other methods. The values obtained by the Kozelka and Hine method were significantly lower than those of the other methods. This is probably due to losses of ethanol during the particular type of distillation employed in the method. Even the statistical mean values by the Duncan's New Multiple Range Test obtained by Kozelka and Hine method are significantly lower than those of specific gravity method as well as other methods; whereas the values of GC method and Caputi's method were not significantly different from those of the specific gravity method (Table 1).

In the second set of experiments, the ethanol values of 8 fermented samples obtained by G.C. method were compared

TABLE 1. COMPARISON OF DIFFERENT METHODS FOR THE ESTIMATION OF ETHANOL (% W/V) IN VARIOUS FERMENTEED SAMPLES

Fermented sample	Sp. gr. method	Kozelka and Hine method	Caputi's method (distilled)	G.C. method
		Mean ± S.D.		
Zymomonas mobilis ZM 4 at 30°C	5.58 ± 0.07	4.15 ± 0.04	5.72 ± 0.08	5.44 ± 0.10
Z. mobilis ZM 4 at 37°C	4.98 ± 0.05	4.08 ± 0.10	5.30 ± 0.09	4.84 ± 0.08
Z. mobilis ZM 401 at 30°C	4.48 ± 0.08	3.62 ± 0.08	4.20 ± 0.18	4.30 ± 0.09
Z. mobilis ZM 401 at 37°C	5.66 ± 0.07	3.99 ± 0.18	5.89 ± 0.10	5.06 ± 0.09
Saccharomyces cerevisiae 101	5.13 ± 0.11	4.29 ± 0.07	5.30 ± 0.08	5.04 ± 0.11
Mean at 37°C	5.17_a	4.03_b	5.28_a	4.94_a

Mean (of 5 replicates) of this row followed by different letters differ significantly at $p < 0.05$.

TABLE 2. ESTIMATION OF ETHANOL (% W/V) IN VARIOUS FERMENTED SAMPLES BY G.C. AND ENZYMATIC METHODS

Fermented sample	Enzymatic method	G.C. method
1. *Z. mobilis* ZM 4	3.27 ± 0.10	2.90 ± 0.25
2. *Z. mobilis* ZM 1	2.80 ± 0.14	3.06 ± 0.12
3. *Saccharomyces diastaticus* 1046	2.98 ± 0.08	4.23 ± 0.18
4. *S. diastaticus* 3390	3.60 ± 0.08	4.27 ± 0.11
5. *S. cerevisiae* 101	6.96 ± 0.11	7.74 ± 0.07
6. *S. cerevisiae* S 29	7.19 ± 0.07	7.50 ± 0.07
7. *S. cerevisiae* S 28	6.97 ± 0.12	7.18 ± 0.07
8. *Schizo saccharomyces pombe*	6.91 ± 0.13	6.12 ± 0.07

Mean ± SD of five replicates., Difference between two treatment mean = 0.36 NS, NS = not significant.

with those or enzymatic method and found that the difference between the two treatment mean (0.36) was not statistically significant, as analysed by the Student's 't' test (two tailed method) (Table 2). In case of 6 out of 8 samples tried (sign method) the ethanol values were higher by G.C. method than the enzymatic method. Even here, the G.C. method could be used as a more suitable method than the others for the estimation of ethanol in fermented samples.

Among the 4 methods used, G.C. method has been found to be the best method for the estimation of ethanol. It was statistically comparable with the more common specific gravity method as well as the more specific one like the enzymatic method and the chemical method such as the Caputi's method. In addition, the G.C. method is rapid, simple and accurate. It could be used as a method of choice for analysing larger number of samples in a shorter time. The added advantages are that the samples could be used directly without distillation and the sensitivity is highest (0.2 μ g).

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SOME STUDIES ON RAPESEED MILLING

A.K. TIKKOO, U.S. AGRAWAL, AND D.K. GUPTA
G.B. Pant University of Agriculture and Technology
Pantnagar - 263145, U.P., India.

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Rapeseed *(Brassica campestris)* was milled, using four commercial oil expelling systems, according to recommended and conventional practices. The recommended practice gave higher oil recoveries (average value 430.8 ml/kg d.b.) as compared with those of conventional practices (average value 401.6 ml/kg).

Mechanical oil expellers are important devices in oilseed processing chain. These are extensively used at small scale or rural level and also as pre-milling devices for solvent extraction industry. In India, cold pressing is still the predominant practice in expeller milling of oilseed. On the basis of laboratory studies, Tikkoo, *et al.*[1] recommended that optimum moisture content for cold pressing of rapeseed in expellers should be 9-10 per cent (d.b.). This study was undertaken to analyse increase in oil recovery using the recommendation of Tikkoo *et al.*[1] at commercial scale over conventional practice. Four expeller enterprises, located in the Tarai region of U.P. were selected for the study. Each miller was supplied with 60 kg of rapeseed of same variety in 6 lots of 10 kg each. The samples were pressed using conventional practice as well as practice suggested by Tikkoo *et al.*[1] Three replications were made for both practices.

The data on oil recovery are expressed in terms of specific oil recovery i.e. ml of oil recovered per kg of rapeseed (dry basis) and average of three replications was taken. The specific oil recovery ranged from 395 to 454 ml/kg for recommended practice and 367 to 460 for conventional practice. The overall average specific oil recovery was 430.8 and 401.6 ml/kg for recommended and conventional practices, respectively (Table 1). Statistical analysis of data indicated that the effect of replication was insignificant whereas the effect of pressing practice (P), expelling system (E) and interaction (P × E) were found significant at 1.0, 0.1 and 2.5 per cent levels respectively with a corresponding critical differences of 27.4, 53.8 and 32.6. On average, recovery was 7.3 per cent higher for recommended practice (P_1) over conventional practice (P_2).

TABLE 1. SPECIFIC OIL RECOVERY DATA

Expeller No.	Specific oil recovery, ml/kg, (d.b.)	
	Recommended practice	Conventional practice
1	454.3	459.7
2	394.7	385.0
3	454.3	366.7
4	420.0	395.0
Average	430.8	401.6

Oil expelling systems E_1 and E_2 gave maximum and minimum recoveries respectively, whereas E_3 and E_4 gave intermediate recoveries. Combinations, P_2E_1, P_1E_1 and P_1E_3 gave maximum recoveries. Thus, the trend of interaction also indicates the superiority of the recommended practice. So far as superiority of one expelling system over the other is concerned, expellers E_1, E_2 and E_3 were 6 bolt expeller with 50 kg/hr rated capacity while E_4 was a 9 bolt expellers with capacity of 150 kg/hr. Even for the same type of expeller, condition of its components and skill of the operator play an important role and these must have been the cause of variation of oil recovery with the expelling system. However, from the grouping of interactions, it is clear that by use of the recommended practice, improved oil recoveries can be obtained even by the otherwise inferior expelling systems (e.g. $E_3 > E_2 : P_1E_2 > P_1E_3$ and $E_3 > E_4 : P_1 E_4 > P_2E_3$).

On the basis of results, it is be concluded that the adoption of recommended practice (Tikkoo *et al.*[1]) for expeller milling of rapeseed could result in higher oil recovery of about 7.3 per cent oil over conventional practice.

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EVIDENCE FOR THE PRESENCE OF A LIPOPROTEIN LIPASE IN ULTRA HEAT TREATED BOVINE MILK AND ITS IONIC BINDING TO HEPARIN

DEEPA PANDE AND M.P. MATHUR
Dairy Chemistry Division
National Dairy Research Institute
Karnal - 132 001, India.

Received 22 May 1989; revised 22 September 1989.

Raw bovine milk was ultra heat treated (UHT) at 140°C for 14 sec and packed in laminated pouches. The residual lipase activity was determined using different assay systems. The complete inefficacy of 'enzyme-substrate-buffer' system indicated that the enzyme requires certain co-factors for its optimum activity. Using five different assay systems, it was observed that blood serum is an indispensable component of the system, heparin is a stimulant and NaCl is an inhibitor thus establishing that the enzyme present in UHT milk is a lipoprotein lipase. The enzyme was purified using DEAE-cellulose chromatography followed by affinity chromatography on heparin sepharose-4B gel. A single broad peak with a fold purification of 279.48 was obtained on DEAE cellulose column whereas two very sharp peaks showing fold purifications of 2333.31 and 825.37 respectively were obtained on heparin sepharose 4B column.

The presence of a lipoprotein lipase in bovine milk was first reported by Korn[1]. The normal function of such enzyme is to liberate fatty acids from lipoproteins and chylomicrons of blood, which are then resorbed by secretory cells of mammary gland. However, in milk, this enzyme is non-specific and can act upon variety of substrates including acyl-glycerols but a low molecular weight activator protein (apo-lipo protein C II) is required for its function[2]. At the biochemical level, lipolysis correlates to binding of lipase to the fat globules and several workers have speculated that the underlying cause is transfer of activator protein from blood lipoproteins into the milk.[2-5].

The role of native lipase in UHT milk has not been established so far, as they cannot resist UHT sterilization treatment[6]. Nevertheless, Law *et al.*[7] and Muir *et al.*[8] have isolated and identified lipolytic bacteria from raw milk and suggested that the most active species belonged to genus *Pseudomonas*. Mottar[9] found a significant correlation between psychrotrophic organism in raw milk and residual enzyme activity in UHT milk. These residual enzymes can cause degradation of fat and the enzymatically catalyzed hydrolysis of triglycerides can give rise to rancidity that impairs the quality of product during long term storage and the so called 'long life milk' does not meet the desired standards of shelf life.

With a view to have basic information on the nature of lipase remaining after UHT treatment, the primary step is to isolate and study its biochemical characters. The present study established the exact nature of this enzyme surviving UHT ranges and the enzyme was purified to high degree using affinity chromatography. Such preparation can further be used to determine various kinetic and biochemical parameters of the enzyme.

Raw bovine milk was obtained from institutional herd maintained at National Dairy Research Institute, Karnal and was processed at ultra high temperature at a commercial UHT plant at Delhi employing 140°C for 14 secs. Enzyme was assayed as per the procedure of Olivecrona and Egelrud[11] with some modifications. Assay included 0.5 ml tris HCl; 1.0 ml BSA solution (2.0 per cent in Kreb's ringer phosphate buffer (pH 7.1); 0.5 ml heparin (20 IU/ml); 1.0 ml blood serum (dialyzed against 0.16 M NaCl); 2.0 ml substrate (10 per cent arabic gum to which 40 per cent tributyrin has been emulgated) and 2.0 ml enzyme fraction. Assay O included only buffer, substrate and enzyme. Assay 2 and 3 contained no blood serum and heparin respectively whereas assay 4 contained 1.0 ml of 1.5 M NaCl. The mixtures were incubated at 37°C for 3 hr and free fatty acids were extracted using diethyl ether and petroleum ether (1:1) and titrated with phenolphthalein as indicator; sample without any incubation was treated as blank.

The increase in free fatty acids over 3 hr incubation was taken as a measure of lipase activity and one unit was taken as = 1 μ mol free fatty acid produced under assay conditions.

Crude LPL was obtained from bovine UHT milk by centrifuging it at 8000 r.p.m. for 30 min to obtain skim UHT milk from which rennet casein was isolated. The whey removed was measured and equal amount of 0.02 M phosphatee buffer pH 7.0 containing 1.16 M NaCl was added to it. The solution was stirred overnight at 10°C (P_0). The supernatant obtained by centrifuging P_0 solution at 8000 r.p.m. for 30 min was subjected to 50 per cent ammonium sulphate precipitation at pH 7.0 and centrifuged at 10,000 r.p.m. for 30 min to separate the enzyme. The precipitate thus obtained was dialysed and lyophilized and was treated as crude enzyme (P_2). It was further purified using DEAE-cellulose chromatography[10] and heparin sepharose 4B chromatography[11]

Table 1 shows the enzyme activity in bovine milk and samples taken at various stages of purification using five assay systems. The maximum activity was measured using complete assay system indicating that the enzyme is a serum stimulated lipoprotein lipase requiring a serum activator. This serum co-factor is presumably a lipoprotein[12] that binds with lipid substrate during incubation[13] thus providing a natural

TABLE 1. ENZYME ACTIVITY USING DIFFERENT ASSAY SYSTEMS

Fraction	Assay 0	1	2	3	4
Skim milk	—	6.5	—	4.0	0.5
D_5*	—	8.0	—	6.0	0.5
D_{14}	—	27.0	—	24.0	2.0
D^{22}	—	30.0	0.5	24.0	2.0
D_{30}	—	9.0	0.5	6.0	1.0
H_5**	—	2.0	—	1.5	—
H_{16}	—	24.5	—	20.5	2.0
H_{20}	—	13.0	0.5	9.0	1.5
H_{32}	—	1.5	0.5	1.2	—

*DEAE-cellulose purified fractions; **Heparin sepharose-4B purified fractions.

substrate for the enzyme[2]. The inefficacy of the enzyme to act directly upon the substrate (Assay 0) shows that the presence of activator and stimulants are necessary for the enzyme to act optimally. Assay 3 reveals the role of heparin as a stimulant as the removal of heparin resulted in slight decrease in activity. This indicates a functional relationship between this enzyme and heparin. Robinson[14] reported that the enzyme can be released from its extracellular sites by heparin or any of the several other polyanions both *in vivo* and *in vitro*. Such interaction of heparin with LPL and subsequent increase in activity can be ascribed to an increased binding of enzyme to its substrate and/or an allosteric effect of heparin on enzyme. Assay 4 shows significant inhibition of enzyme with 1.5 M NaCl especially when enzyme is present in low concentrations. Thus, the role of blood serum, BSA and heparin in increasing enzyme activity and its inhibition by 1.5 M NaCl establish that the enzyme is a lipoprotein lipase.

Fig 1 shows the purification of LPL using heparin sepharose 4B chromatography. Essentially, all the protein applied was eluted at low ionic strength of buffer in contrast to enzyme that was eluted at 0.60 and 0.66 M NaCl concentrations, respectively. Both the peaks showed about 2333 and 825 fold purifications respectively. In comparison to our results, Olivecrona and Egelrud showed a 2000 fold purified peak eluted at an ionic strength of 0.83 M but no second peak was obtained.

In the present study, the survival of any native LPL can not be reasoned after such high heat treatment. Sugiura *et al.*[15] reported bacterial lipase from *Pseudomonas* that acts upon lipoprotein triglycerides and used it for the assay of serum triglycerides. It has already been evidenced by various workers[16-18], that the presence of heat resistant lipase from *Pseudomonas* is widespread. Nevertheless, the lipolytic spoilage of UHT milk is rarely reported. Having established the nature of lipase surviving UHT treatment, it is clear that

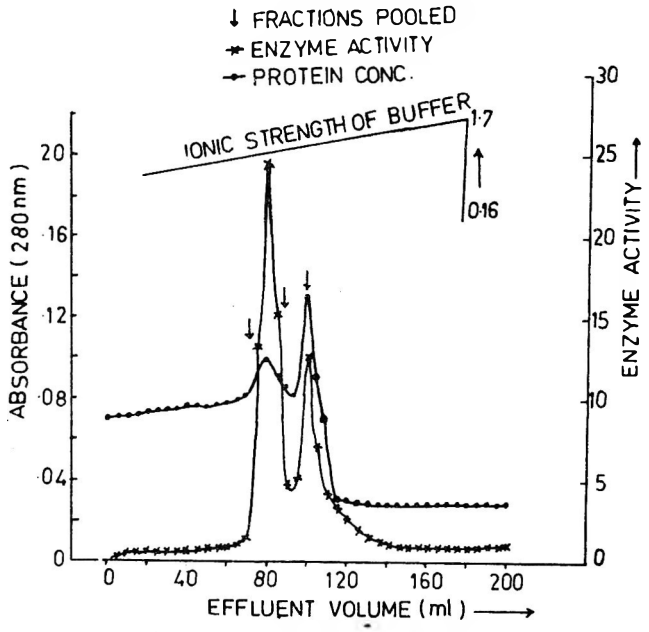

Fig.1. Heparin-Sepharose 4B chromatography of P_3 fraction.

it requires a system where activator is indispensable. Therefore, under normal processing and storage conditions, the enzyme will show very insignificant activity. This implies that lipolysis is not a grave problem in stored UHT milk as compared to gelation. Mottar[9] also established that proteolysis affects the texture and taste of stored UHT milk more negatively than lipolysis. Therefore, unless very poor hygienic conditions are prevalent and/or there is long term storage before processing the residual lipase does not play any significant role in relation to rancidity problems in UHT milk.

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NON-COAGULABILITY OF COW'S MILK WITH CHYMOSIN

LESLIE M. NSOFOR
School of Agriculture and Food Sciences,
Imo State University, P.M.B. 2000, Okigwe, Imo State, Nigeria.

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The production trend of non-coagulating milk by individual cows was monitored in a Holstein herd. A new method (coagulation efficiency) was developed to categorize the suitability of milk samples collected periodically from these cows for cheese making. The frequency of occurrence of non-coagulating milk with chymosin ranged from 0 to 54% at different periods of the year. Sixty-eight % of the cows sampled produced non-coagulating milk at least once during the test period. Milk samples from thirteen cows, that is 38% of those that produced non-coagulating milk had coagulation efficiencies below 40%, and they exhibited the greatest losses of cheese solids in whey. Milk having less than 40% coagulation efficiency should not be used for cheese making. A negative relationship existed between coagulation efficiency and cheese solids loss in whey, and milk samples which had greater coagulation efficiencies exhibited lower losses.

The incidence of uncoagulability of milk from individual cows in late lactation after chymosin addition has been reported[1]. Though the impact of this on the cheese making industry has not been widely studied, the reduction in curd strength of pooled milk, and consequent losses in cheese yield are known[2,3]. In countries like Australia and New Zealand, where milk production is highly seasonal, milk uncoagulability is commonly observed toward the end of the milk production season[4,5]. However, this does not appear to be a problem in countries of the northern hemisphere, where seasonal milk production is not widely practised.

It has been proposed that tighter control measures could be exercised on curd strength of cheese milk if individual cow's milk samples are tested periodically for coagulation properties[3], and cows which produced non-coagulating milk for long periods in a lactation be isolated, and their milk used for non-cheese purposes. A method for this periodic evaluation of coagulation properties of milk was developed, and a study to investigate the occurrence of non-coagulating milk with chymosin in individual 'Holsteins' in a herd which produces milk non-seasonally was undertaken.

Fifty cows were randomly selected in June 1981 in the Utah State University herd of one hundred and fifty 'Holsteins' which produced milk non-seasonally. Evening milk samples were obtained from each available animal on the last day of each of ten months as previously described[1]. A range of 29 to 50 milk samples was available at each sampling. Ten milk samples were simultaneously tested after addition of diluted chymosin of concentration 0.016 rennin units (RU) per ml milk in a Formagraph[1,6]. The instrument was left to run for 30 min after chymosin addition, and coagulation time (CT) determined for the samples that clotted. Coagulation efficiency (EC) was defined as:- $CE = a/b \times 1/c$ where, a = the number of samples of milk which coagulated within 30 min after addition of the enzyme, b = the total number of samples collected in the season, c = the mean of the coagulation times.

For cheddar cheese making, the schedule normally followed in cheese factories is to allow 2.5 times the CT before cutting. The ideal time is 30 min. Hence the average CT should be within 10 min. Hence for ideal CE, a = b (all the samples collected coagulate) and c = 10. Ideal CE = 1/10. If this is designated as 100 per cent by multiplying the above fraction by 1000, we have a measure for CE. Milk samples with poor coagulation property (PCM), average coagulation property (ACM) and good coagulation property (GCM) could thus be characterised based on the CE of the milks, expressed as percentage.

Milk samples were analyzed for fat, protein, casein, and total solids utilizing a calibrated Milko Scan instrument (Dickey - John Corp, Auburn, IL., USA)[1]. Somatic cells were counted with a Fossomatic cell counter (Dickey - John Corp.). Cheese making was simulated by using a modified Pearce Activity Test[7] to estimate loss of cheese solids (casein plus fat) from each milk sample in whey[1]. A calibrated Milko Scan instrument was used to determine whey solids as described[1]. Milk samples were obtained from cows in the sample group which gave milk continuously for 3 months from January through March 1982.

The occurrence of non-coagulating samples was first observed in July 1981 when 94 per cent coagulation was recorded (Table 1). More samples could not coagulate as the year progressed. The greatest number of unclotted samples was observed in November corresponding to only 46 per

TABLE 1. NON-COAGULABILITY OF INDIVIDUAL HOLSTEIN MILK WITH CHYMOSIN

Months (1981-'82)	Samples tested (No.)	Unclotted samples (No.)	% coagulation
Jun	50	0	100
Jul	47	3	94
Aug	43	5	88
Sep	40	7	83
Oct	39	10	74
Nov	39	21	46
Dec	35	14	60
Jan	35	13	63
Feb	29	6	79
Mar	30	7	77

cent. This indicates that at that time pooled milk from this herd had very weak curd after chymosin coagulation. It confirms that milk uncoagulability also occurs in a non-seasonal milk production system. Thus, it is expected that average coagulation properties would, at least, be maintained throughout the year. Higher percentage uncoagulability that was observed in fall and winter compared to summer strongly suggests that environmental temperature may have an effect on the rate of milk coagulation. A previous report[1] had suggested that an interaction occurred between season of the year and period of lactation of individual animals and that this affected coagulation. Average periods of lactation for the animals were 196, 219 and 230 days in summer, fall and winter respectively. Thirty-four cows (68 per cent of the sample size) produced non-coagulating milk, at least once, during the test period. Thirteen of these 34 cows produced samples with CE less than 40 per cent, hereby categorized as PCM, with a range of 19 to 37 per cent and average of 32 per cent. Three representative cows in this group are shown in Table 2. The mean CT shown excluded non-clotting samples. Cows which produced ACM and GCM samples had CEs equal to or greater than 40 and 70 per cent, respectively. Representative cows in the latter groups also are shown in Table 2. Milk samples from cows with mastitis, that is, those samples with somatic cell counts higher than 500,000 cells per ml milk were excluded from the coagulation tests. Mean CE for all the samples for the entire test period was 49 per cent with a range of 19 to 86 per cent.

An earlier report[1] established that a significant percentage of cows in late lactation produced non-coagulating milk. In the present report 14 cows (28 per cent) produced non-coagulating milk at other periods than just before their dry periods, for example cows with identification numbers 68 and 5038. Since the incidence of uncoagulability of milk from individual cows appears to be a random occurrence, even though the trend is very prominent in late lactation[1], it becomes imperative, that the concept of CE be adopted to categorize lactating cows for the overall coagulation properties of their milk. Thus cows that produce milk in a lactation period with CE less than 40 per cent should be noted. It is recommended that such milk be used for non-cheese purposes. Including this milk in bulk supplies would reduce curd strength of cheesemilk[2,3]. Curd strength and CT are highly correlated —.86[1]. Eventhough it has been reported that coagulation properties of PCM could be improved by adjusting the levels of factors that affect coagulation[2,8], cheese resulting from such milk had very high moisture content with bitter flavour[3].

Significant quantities of milk solids (casein plus fat) which are usually trapped in cheese curd, were lost in whey. The range of cheese solids loss was 13 to 24 per cent with a mean of 18 per cent. Forty six per cent of sample size was available for the experiment. Six per cent of the cows that produced milk which represented each coagulating group PCM, ACM and GCM and the corresponding losses of cheese solids in whey are shown in Table 3. The PCM shown exhibited the largest mean loss of 23 per cent and GCM the least, 15 per cent. Mean cheese solids loss for ACM was 18 per cent. Corresponding mean CEs for these representative samples were 29, 73 and 56 per cent respectively. Data in Table 3 indicate that a negative relationship exists between CE and cheese solids loss in whey. Thus if CE is high, less cheese solids are expected to be lost in whey. On the basis of this relationship, it is suggested that a modification of Van Slyke

TABLE 2. CHYMOSIN COAGULATION TIME OF INDIVIDUAL HOLSTEIN MILK SAMPLES OBTAINED PERIODICALLY*

Cow identity	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Mean	CE** (%)
Poor chymosin-coagulating milk (PCM)												
68	13	15	29	15	D	15	NC	NC	NC	NC	17	33
4986	9	24	–	21	NC	NC	NC	NC	NC	NC&	18	19
5038	11	NC	14	19	27	NC	26	NC	D	D	19	32
Average chymosin-coagulating milk (ACM)												
78	12	23	17	18	18	18	15	D	D	12	17	60
4958	8	29	D	D	30	13	17	7	21	15	18	57
4982	7	15	13	14	22	24	19	M	25	D	18	57
Good chymosin-coagulating milk (GCM)												
74	10	10	14	12	13	15	16	16		17	14	74
5012	13	14	12	10	11	23	D	13	16	16	14	71
5034	7	10	12	10	14	11	13	16	D	D	12	86

*Coagulation time was rounded up to the nearest minute; **CE = coagulation efficiency; D = dry period; NC = no values were assigned to samples which could not coagulate in 30 min; — = sample was not available; & = cow was dry after March; M = mastitis.

TABLE 3. CHEESE SOLIDS LOSS IN WHEY OF CHYMOSIN COAGULATED INDIVIDUAL HOLSTEIN MILK

Cow identity	Milk casein + fat (%)	Whey casein + fat (%)	Cheese solids loss (%)	Coagulation efficiency (%)
		Poor chymosin-coagulating milk (PCM)		
68	5.5	1.2	20.8	32
4986	6.9	1.6	23.9	19
4488	6.2	1.4	22.8	37
		Average chymosin-coagulating milk (ACM)		
4958	6.0	1.2	20.1	57
4656	5.1	1.0	20.2	50
4920	6.8	0.9	13.6	61
		Good chymosin-coagulating milk (GCM)		
74	7.6	1.1	14.3	74
5012	5.2	0.8	15.3	72
4948	8.4	1.3	15.3	72

and Price formula for cheese yield containing parameters for coagulation and curd strength, be considered for development.

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USE OF PRESERVATIVES FOR IMPROVING SHELF LIFE OF CURD (DAHI)

M.K. SANYAL, P. L. YADAV AND P. C. DUBEY
Division of Livestock Products Technology,
Indian Veterinary Research Institute,
Izatnagar - 243 122, India.

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A technique for the production of curd (dahi) with improved shelf life has been developed. Results of sensory evaluation revealed that dahi stored at 30±2°C and 4±2°C were acceptable upto 20 days and 45 days respectively while control samples of dahi kept good only for 2 days and 7 days respectively. Titratable acidity and pH of dahi samples did not change significantly during storage. Storage period had significant influence on physical appearance, body and texture, aroma and overall acceptability of dahi samples stored at 30±2°C but did not show any significant effect on the sensory attributes of dahi stored at 4±2°C.

The main constraint hampering large scale industrial production of dahi is its poor keeping quality both at room and refrigerated temperature. Use of preservatives in dahi, though not approved at present under PFA Act[1], could play an important role in extending shelf life which may pave the way for suitable modification of PFA Act for dahi subsequently. Use of food additives can be technologically justified when it improves the keeping quality and reduces the food losses. Attempts made earlier by several workers to improve the shelf life of dahi met with limited success[2-4]. Available literature on the production of long keeping dahi is also highly inadequate. An investigation was, therefore, undertaken to enhance the shelf life of dahi stored at 30±2°C and 4±2°C by using various additives and employing suitable processing techniques.

Fresh milk obtained from the herd of crossbred cattle, maintained at Indian Veterinary Research Institute (IVRI), Izatnagar was used in all the trials for the preparation of experimental and control samples of dahi. Freeze-dried organisms of *Streptococcus lactis*, procured from National Dairy Research Institute, Karnal was inoculated in sterile skimmed milk to obtain mother culture from which bulk culture was prepared. Freshly prepared starter culture was used each time for the production of dahi. Mother culture was maintained by weekly subculturing in sterilized skimmed milk.

Milk after straining through a fine muslin cloth was heated at 90°C for 10 min, cooled to 35°C and mixed with 0.5 per cent of agar agar as stabilizer by adding 1:10 melted solution. The milk-stabilizer mix was immediately inoculated with 3 per cent of *S. lactis* culture and incubated at 30°C for 16 hr. and the firmly set dahi was heated at 75°C for 10 min in a water bath with constant slow agitation, cooled to 30°C and mixed with 0.1 per cent sodium benzoate, 0.2 per cent potassium sorbate and 6.5 per cent cane sugar (pasteurized and filtered). Control samples of dahi were made by heating milk at 90°C for 10 min, cooling to 35°C, inoculating with 3% of *S. lactis* culture and incubating at 30°C for 16 hr. Both experimental and control samples of dahi were then filled into sterilized glass bottles and capped with aluminium caps. Samples of dahi were stored at 30±2°C in a B.O.D. incubator and at 4±2°C in refrigerator for further studies.

A panel of five judges evaluated the physical appearance, body and texture, aroma, taste and overall acceptability of the product on the basis of 9-point Hedonic scale. Fat percentage in milk was determined by Gerber's method[5]. Titratable acidity[6], casein, total protein, lactose, chloride and ash contents in milk were estimated by ISI methods[7]. The pH of milk and dahi was measured by using a digital pH meter.

The scores of sensory evaluation for different attributes of control and experimental samples of dahi at different intervals during storage at 30±2°C and 4±2°C are presented in Table 1. On sensory evaluation, experimental samples of dahi were acceptable upto 20 days and 45 days while the control samples kept well only for 2 days and 7 days during storage at 30±2°C and 4±2°C respectively. Samples of dahi preserved beyond these periods were rejected by the judges. The scores

TABLE 1. SENSORY ATTRIBUTES SCORES* FOR CONTROL AND EXPERIMENTAL DAHI SAMPLES STORED AT 30±2°C AND 4±2°C

Sensory attributes	Dahi stored at 30±2°C				Dahi stored at 4±2°C			
	Control	Experimental			Control	Experimental		
	2 days	1 day	15 days	20 days	7 days	25 days	35 days	45 days
Physical appearance	7.67	8.47	8.07	7.33	7.78	7.93	7.60	7.67
Body and texture	7.67	8.47	7.47	7.07	7.89	7.60	7.33	7.33
Aroma	7.00	8.33	7.80	7.13	7.89	7.47	6.93	7.13
Taste	6.89	8.33	7.80	7.27	7.67	7.80	7.27	7.20
Overall acceptability	7.22	8.47	7.60	7.07	7.44	7.87	7.27	7.20

*Average values of three replicates

decreased during storage and the decrease was more noticeable in the samples stored at 30±2°C than those preserved at 4±2°C. The scores for aroma and taste of dahi samples stored at 30±2°C and 4±2°C after 20 days and 45 days respectively were almost similar. Titratable acidity and pH of the stored samples of dahi did not change significantly.

Storage period had significant influence on all the sensory attributes excepting taste of dahi stored at 30±2°C but did not have any significant effect on the sensory attributes of dahi stored at 4±2°C upto 45 days.

Addition of agar agar, sodium benzoate and potassium sorbate are generally recognised as safe as per the accepted good commercial practice[8]. Both sodium benzoate and potassium sorbate are extensively used in preserving acid foods. Sodium benzoate is generally more effective against yeasts and bacteria[9] while potassium sorbate is an effective yeasts and moulds inhibitor but is less comprehensive in its action against bacteria as compared to fungi[10,11]. Heating of dahi at 75°C for 10 min killed all pathogenic organisms and almost all yeasts and moulds. Agar agar tied up free moisture and prevented heat coagulation of dahi. The menacing problems of growth of yeasts and moulds in the system were non-existent due to the heat treatment and presence of antimycotic agents.

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STUDIES ON THE PHYSICAL AND CHEMICAL PROPERTIES OF ICE CREAMS FORMULATED WITH DIFFERENT SUBSTITUTION LEVELS OF ARROWROOT POWDER FOR MILK SOLIDS - NOT - FAT

J.P. VENKATESWARLU, P.M. SASTRI, M.R. RAO AND M. RANGANADHAM

Department of Dairy Science, College of Veterinary Science, Rajendranagar, Hyderabad-500 030, India.

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Replacement of milk solids - not - fat (SNF) with arrowroot powder at 20, 40 and 60% in soft serve ice cream indicated that replacement at multiple levels of 29% increased the pH, relative viscosity and melt down duration values and decreased the titratable acidity, specific gravity, protein content and over-run percentage values. It was observed that arrowroot powder could satisfactorily replace milk SNF upto 40% without impairing organoleptic qualities with a reduction of 12.04% cost of production.

Scope exists for the reduction of cost of ice cream preparation through substitution of solids-not-fat of milk with the low cost arrowroot powder. In this investigation, the of replacement of milk SNF with arrowroot powder on the physico-chemical characteristics of ice cream mix and organoleptic attributes of ice cream was investigated.

Arbuckle[1] described a special ice cream containing sweet potato starch. Ash and Colmey[2] reported that starch can be safely used in frozen food product as a substitute for skim milk powder.

Four types of ice cream mixes of 2 kg capacity each in 10 replicates were formulated with proportionate replacement of milk SNF with arrowroot powder at levels of 20, 40 and 60 per cent giving due moisture allowances for arrowroot powder (T_1, T_2 and T_3) by keeping one type of mix as control (C) which did not contain any arrowroot powder solids. All the four mixes were prepared to contain an identical composition of 10 per cent fat, 11 per cent SNF, 15 per cent sugar, 0.3 per cent stabilizer in control mix and in 20, 40 and 60 per cent substituted mixes contained 0.25, 0.20 and 0.15 per cent stabilizer respectively. All the mixes were pasteurized at 72°C for 20 min followed by cooling and ageing for 3 hr at 5-10°C and were frozen in a soft ice cream freezer (Vulcan Laval Limited, Pune) after addition of permitted flavours and colours.

Fat contents of creams and skim milk were estimated as per procedure in ISI specifications[3]. The SNF content in skim milk was also estimated as per ISI specification[4] and SNF content of cream was calculated using the formula suggested by Sommer[5]. The moisture content was estimated as per AOAC procedure[6]. The pH of the mixes after ageing was estimated by a digital pH meter (Elico Private Limited Hyderabad) and the titratable acidity was estimated according to ISI specification[7]. Protein content was estimated as per AOAC procedure[6]. Using Baume Hydrometer, specific gravity was calculated by using the formula of Jannes and Patton[8]. Relative viscosity volumes were calculated with the formula recommended by Plummer[9] using Ostwald Viscometer. The percentage overrun of each type of ice cream was calculated by using the formula of Sommer[5]. The melt-down time of ice cream was recorded by noting down the time taken for complete melting of 100 g of ice cream placed on a glass piece resting on a funnel. Sensory quality of ice cream was assessed by a panel of 5 selected judges using the score card recommended by Harper and Hall[10]. Detailed cost economics were worked out taking the prevailing retail costs of ingredients into consideration.

The mean pH, titratable acidity, specific gravity and relative viscosity values are presented in Table 1. It could be observed that the pH of different ice cream mixes increased with a corresponding decrease in titratable acidity as the levels of replacement with arrowroot powder increased. This is due to the effect of the alkaline nature of arrowroot powder which is having a pH range of 7.2 to 7.4.

The specific gravity of ice cream mix decreased with increase in arrowroot powder level which is due to lower specific gravity of arrowroot powder (1.086) than the composite specific gravity of the constituents of milk solids-not-fat.

The relative viscosity increased significantly with increasing levels of arrowroot powder in the ice cream mix. The hydrophyllic colloids have the property of holding large quantity of water and setting into semi-rigid gel. Such gel may impart high viscosity.

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF DIFFERENT TYPES OF AGED ICE CREAM MIXES

Ice cream mix type	pH	Titratable acidity	Specific gravity	Relative viscosity
C	6.42^a	0.201^a	1.107^a	76.49^a
T_1	6.48^b	0.187^b	1.103^b	127.50^b
T_2	6.64^c	0.167^c	1.097^c	168.76^c
T_3	6.76^d	0.151^d	1.093^d	210.94^d
Least sig. diff.	0.03	0.006	0.002	9.98

The mean values with same superscript in each column do not differ significantly ($P<0.01$)

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The mean percentage overrun, melt down duration values and sensory quality assessment scores are presented in Table 2. It could be observed from the Table that the melt down duration values increased and the overrun percentage values decreased as the added arrowroot powder level increased. In general as the viscosity increases, the resistance to melting and smoothness of the body increases but the rate of whipping decreases. Over stabilization of the substituted ice cream mixes with different levels of arrowroot powder is one of the causes for slow melt down and smooth texture and decreased overrun per centage of the ice creams. The results of the organoleptic quality evaluation indicated that the ice creams prepared by replacing 20 and 40 per cent of SNF with arrow-root powder were equally acceptable as control.

The cost economics is presented in Table 3. The cost economics worked out in this investigation taking the prevailing retail market prices of the ingredients for different types of ice creams indicated a cost reduction of 7.10 and 12.04 per cent for 20 and 40 per cent replacement level of arrowroot powder for milk SNF respectively over control.

TABLE 2. OVERRUN, MELT DOWN DURATION VALUES, AND SENSORY QUALITY ASSESSMENT SCORES OF DIFFERENT TYPES OF ICE CREAMS

Ice cream type	Over run (%)	Meltdown time (min)	Sensory scores
C	35.81[a]	38.2[a]	93.4
T_1	32.60[b]	46.1[b]	92.4
T_2	26.50[c]	51.5[c]	91.8
T_3	15.95[d]	56.7[d]	86.2
Least sig. diff.	0.813	1.78	

The mean values with same superscript in each column do not differ significantly ($P < 0.01$).

TABLE 3. COST OF PRODUCTION OF DIFFERENT TYPES OF ICE CREAMS BASED ON THE PREVAILING RETAIL COST OF THE RAW MATERIALS.

Ingredients	Requirements/kg			Cost of ingredients	Cost/kg (Rs)		
	C	T_1	T_2	(Rs/kg)	C	T_1	T_2
Cream (50% fat)	200 g	200 g	200 g	20.00	4.00	4.00	4.00
Arrowroot powder	—	24 g	48.50	8.00	—	0.19	0.38
Skin milk powder	40 g	16 g	1.50 g	30.00	1.20	0.50	0.05
Skim milk	620 g	620 g	550 g	1.50	0.93	0.93	0.83
Sugar	150 g	150 g	150 g	6.00	0.90	0.90	0.90
Stabilizer	3 g	2.50 g	2 g	25.00	0.08	0.06	0.05
Essence	3 ml	3 ml	3 ml	12.00	0.36	0.36	0.36
Total					7.47	6.94	6.57
% cost reduction						7.10	12.04

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QUALITY CHARACTERISTIC OF FREEZE-DRIED EDIBLE OYSTER *CRASSOSTREA MADRASENSIS* (PRESTON)

G. INDRA JASMINE, C.B.T. RAJAGOPALSAMY*, G. SUGUMAR AND P. JEYACHANDRAN

Fisheries College, Tamil Nadu Veterinary and Animal Sciences University Tuticorin - 628 008, India.

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The flesh of edible oyster *Crassostrea madrasensis* (Preston) was freeze-dried. The biochemical, microbiological and sensory evaluation of the fresh as well as the freeze-dried sample were determined. There were no changes in the TMA, TVN, PV and FFA as a result of freeze-drying. The proximate composition was not altered.

Freeze-drying is recognised as one of the best methods of producing dried material of high quality. Edible oyster *Crassostrea madrasensis* was procured in fresh condition. The meat was washed well in running water and in 5 p.p.m. chlorinated water for 10 min and arranged in stainless steel trays in a laboratory model accelerated freeze-drier (Hindhivac LF 9S Model) of 10 kg capacity. The oysters were initially frozen at —40°C for 2½ hr. The chamber was evacuvated and a vacuum of 1.5 Torr was maintained.

The temperature was gradually raised to —20°C and the product was allowed to sublime. The final drying was done at 40°C. The entire drying took about 16 hr. The freeze-dried sample was taken and kept sealed in air tight pouches and quality assessed.

The proximate composition of the raw and freeze-dried oysters was determined. The moisture, fat and ash were determined by AOAC procedures[1]. Protein was analysed by Micro-kjeldhal method[2]. The carbohydrate content was estimated using anthrone reagent[3].

The following quality characteristics of raw and freeze-dried samples namely, trimethyl amine (TMA), total volatile base (TVB)[4], non-protein nitrogen (NPN)[5], free fatty acid (FFA)[6], peroxide value (PV)[7] and Ca^{2+} ATPase total activity in actomyosin[8] were determined. Microbial parameters such as total plate count were enumerated by pour plate technique using nutrient agar medium[9]. Total coliform and spore formers were enumerated quantitatively[9].

The results of the proximate composition of raw and freeze-dried oysters are given in Table 1. The percentage retention of various biochemical constituents after freeze-drying were more or less hundred per cent.

The freeze-dried oyster was reconstituted by soaking in water for 10 min, moisture content of the reconstituted sample was determined and the percentage reconstitution calculated. The moisture content of the reconstituted freeze-dried oyster was found to be 78.7 per cent thus giving 98 per cent rehydratability. TMA, TVN, FFA and PV were found to be nil in raw and freeze-dried samples. The decrease in NPN was from 0.77 to 0.08 g N per cent in freeze-drying. The total Ca^{2+} ATPase activity of actomyosin was found to be reduced from 64.9 to 60.9 μmol pi/min/10g (rehydrated weight basis) in freeze-drying. The TPC in the raw oyster was 3.0×10^4/g which decreased to 0.43×10^3/g as a result of freeze-drying. The coliforms decreased from 110 coliform/g in raw oysters to 12 coliforms/g in freeze-dried oysters (reconstituted weight basis). The spore formers were found to be very low (4.25×10^3/g) in the freeze-dried sample.

The acceptability of the freeze-dried sample was assessed by sensory evaluation. The reconstituted sample was cooked in 3 per cent brine for 10 min, and presented to a taste panel consisting of six members along with uncooked and control samples. Scorings were given for appearance, texture, taste and flavour of the product. The scores of sensory evaluation showed that the freeze-dried samples are acceptable in quality as the raw sample.

The above study indicates that acceptable freeze-dried product can be produced from edible oyster which could be

TABLE 1. PROXIMATE COMPOSITION OF RAW AND FREEZE-DRIED EDIBLE OYSTER *CRASSOSTREA MADRASENSIS*

Oyster type	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbo-hydrate (%)
Fresh	80.3	12.4	2.8	1.1	2.7
Freeze-dried	1.9	61.5	13.8	5.4	13.5
Fresh DWB*	—	62.7	14.2	5.6	13.3
Freed-dried DWB	—	62.7	14.0	5.5	13.0
Retention (%)	—	100.0	100.0	98.6	98.2

DWB* Dry wt basis: Average value from triplicate samples

**Present address:* Agricultural Research Station, Bhavanisagar.

reconstituted to regain fully all the quality attributes of the fresh samples.

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ORANGE CONCENTRATE BASED CARBONATED BEVERAGE

D.S. KHURDIYA
Division of Fruits & Horticultural Technology,
Indian Agricultural Research Institute
New Delhi-110 012, India.

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A formulation for orange concentrate based carbonated beverage has been standardized. The carbonated beverage made from syrup base containing 7.5% orange concentrate was equivalent to about 10% of single strength orange juice which was considered optimum. The carbonated beverage was made by post mix method and a beverage with a Brix to acid ratio of 45 and carbonated at 80 psi gas pressure was found to be the best.

Most of the carbonated beverages are based on synthetic colouring and flavouring agents which are potentially allergenic[1]. Inclusion of a fruit juice in soft drinks not only imparts its characteristic colour, taste and aroma but also obviates use of synthetic additives and has some nutritional value[2]. In carbonation of clarified pineapple juice, an overall preference for a 0.75 per cent titrable acidity and low carbonation product was observed[3]. A suitable method to prepare lime juice for carbonated drink has been reported[4]. Effect of carbonation and sweetness in carbonated apple juice on sensory attributes was studied by response surface methodology[5]. A procedure for production and bottling of carbonated beverages containing 5-25 per cent grape juice from surplus table grapes was described[6]. This investigation was conducted to formulate an orange concentrate based carbonated beverage.

The orange concentrate obtained from Agro Foods, Punjab was stored at 3°C until required. A syrup base containing orange concentrate (5, 7.5 and 10 per cent), cane sugar (45.8, 44.3 and 42.8 per cent) and citric acid (0.84, 0.65 and 0.47 per cent) was prepared. In the second experiment, the orange concentrate was 7.5 per cent, sugar and acid were varied. In the third experiment, the orange concentrate and Brix to acid ratio was kept constant and the beverage carbonated at variable gas pressure of 60, 80 and 100 psi. In the first and second experiments, the carbonation was done at 100 psi gas pressure. The carbonated orange beverage was made by post-mix method. A known valume (equal to 50 g) of the orange concentrate syrup base was placed in clean, pre-sterilized glass bottles (200 ml capacity), filled with chilled carbonated water, crown corked immediately and stored at 5°C until required. Chilled water was carbonated using a country made carbonating machine fitted with a 10 l stainless steel tank.

Total soluble solids (°Brix) was determined with a hand refractometer and the readings were corrected to 20°C. Acidity and pH were determined by the standard A.O.A.C. methods[7]. Ascorbic acid was determined by the titrimetric method[8]. Sensory evaluation was carried out by a panel of seven judges using a 9-point Hedonic scale[9]. The data were statistically analysed using analysis of variance. The density of the syrup base was estimated as the proportion of a mass to volume. The syrup and beverage were centrifuged for 10 min at 360 × g[10]. The percentage transmission of the supernatant was measured at 660 nm in a 1 cm cuvettes in a spectrophotometer (spectronic 1201, Milton Roy & Co.) and this value was considered a measure for the cloudiness. Increasing percentage transmission indicated loss of clouds or suspended matter.

The orange concentrate possessed °Brix 68, acidity 7.29 per cent and pH 2.6 was employed in the preparation of syrup base and then carbonated beverage. The variable percentage of orange concentrate in the syrup base has been found to influence the cloud and the sensory quality of carbonated beverage (Table 1). No change was noticed in respect of Brix, acidity, pH and Brix to acid ratio of the beverage, since these were kept constant while preparing the syrup base. The cloud was found to increase with increase in the orange concentrate in the syrup base. Thus, 10 per cent orange concentrate in the syrup base gave highest amount of cloud in the carbonated orange base. Probably, the cloud has influenced the colour score of the beverage. The sensory colour score increased with increase in the amount of orange concentrate in the syrup base, while the flavour score was maximum at 7.5 per cent concentrate level. Decrease in flavour score beyond 7.5 per cent level might be due to increased bitterness. The carbonated beverage made from syrup base containing 7.5 per cent orange concentrate was equivalent to about 10 per cent of single strength orange juice. The differences in the values of colour and flavour scores were found to be statistically significant. Thus, 7.5 per cent orange concentrate in the syrup base for the preparation of carbonated beverage was considered optimum in respect of colour and flavour.

Different concentrations of sugar (°Brix) and acidity were tried to see their influence on the quality of orange syrup base as well as carbonated beverage (Table 2). The results reveal that density and clouds were affected by the treatment. The density of the syrup base increased with increase in °Brix and was in the range of 1.16 to 1.31. The percentage transmission of the supernatant syrup increased when total soluble solids were increased by about 10 °Brix, due to floatation of greater amount of fruit particles in heavier syrup during centrifugation. Ascorbic acid was in the range of 3.77 to 4.71 mg per 100 ml of syrup base. The Brix to acid ratio was in the range of 24.0 to 56.8. The pH of the syrup base

TABLE 1. EFFECT OF ORANGE CONCENTRATE ON THE QUALITY OF CARBONATED BEVERAGE

Orai e conc.in base (%)	°Brix	Acidity (%)	pH	Cloud (% T* at 660 nm)	Brix to acid ratio	Sensory score		
						Colour	Flavour	Overall
5.0	10.7	0.27	3.21	14.92	39.6	5.53	6.23	5.88
7.5	10.4	0.28	3.20	12.00	37.1	6.85	6.70	6.77
10.0	10.4	0.28	3.20	9.41	37.1	7.32	5.50	6.41
S. Em.						±0.247	±0.252	
C D (P = 0.05)						0.49	0.50	

T*—Transmission

Analysed in duplicate

TABLE 2. EFFECT OF SUGAR AND ACIDITY ON THE QUALITY OF ORANGE CONCENTRATE SYRUP BASE AND CARBONATED BEVERAGES

	T.S.S. (°Brix)	Acidity (% W/V)	pH	Ascorbic acid (mg/100 ml)	Cloud (% T* at 660 nm)	Density (g/ml)	Brix to acid ratio	Sensory score		
								Colour	Flavour	Overall
Syrup base										
I	42.0	0.86	2.2	3.77	3.7	1.16	48.8	—	—	—
II	43.0	1.32	2.2	4.24	3.1	1.18	32.6	—	—	—
III	43.5	1.81	2.2	4.71	3.8	1.19	24.0	—	—	—
IV	50.0	0.88	2.2	4.70	6.3	1.28	56.8	—	—	—
V	53.5	1.34	2.2	4.24	6.2	1.31	39.9	—	—	—
VI	53.0	1.80	2.2	4.24	5.6	1.30	29.4	—	—	—
Beverage										
I	9.0	0.20	3.45	—	18.85	—	45.0	6.0	6.9	6.45
II	9.0	0.22	3.50	—	17.40	—	40.9	6.0	6.5	6.25
III	9.5	0.37	3.55	—	15.90	—	25.7	6.0	6.2	6.10
IV	11.8	0.24	3.60	—	17.75	—	49.2	6.0	6.1	6.05
V	12.3	0.25	3.52	—	15.00	—	49.2	6.0	6.7	6.35
VI	12.0	0.40	3.55	—	16.90	—	30.0	6.0	6.3	6.15
								(n.s.)	(n.s.)	

n.s. — not significant

T = transmission.

remained unchanged even though the acidity varied and it might be due to the buffering action of the constituents.

Carbonation of orange beverage was found to reduce the difference in the cloud values (Table 2). The pH values varied in the narrow range of 3.45 to 3.60. The Brix to acidity ratio was in the range of 25.7 to 49.2. Based on sensory quality, all the samples were found acceptable. The colour score was same while the flavour scores varied slightly and statistically not significant. However, the orange carbonated beverage with 9 °Brix and 0.2 per cent acidity (45 Brix to acid ratio) got the highest flavour score (6.9).

The effect of CO_2 gas pressure on the quality of beverage was also evaluated. The carbonated orange beverage possessed °Brix 10, acidity 0.21 per cent and pH 3.05; these parameters were not influenced by variable gas pressure. The percentage transmission was 26.4, 27.7 and 29.6 in the supernatant of centrifuged beverage which was carbonated at 60, 80 and 100 psi gas pressure, respectively. Thus, cloud has decreased to some extent with increase in gas pressure. This might be due to more floatation of suspended fruit particles during centrifugation after release of gas pressure. The sensory colour score remained same (6.7). The flavour scores of the beverage were 5.5, 6.2 and 6.3 when carbonated at 60, 80 and 100 psi gas pressure. The differences in the flavour scores at 60 and 80 psi levels were statistically significant and beyond 80 psi level no significant difference was observed. Thus, carbonation level of 80 psi was considered optimum so far as flavour was concerned.

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DETOXIFICATION OF AFLATOXIN B_1 IN MAIZE BY DIFFERENT COOKING METHODS

FASIHA REHANA AND S.C. BASAPPA
Central Food Technological Research Institute,
Mysore-570 013, India.

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Several cooking methods used in the preparation of porridge, roti, balls and popcorn destroyed aflatoxin to an almost same degree of about 50%. However, when roti was not cooked crisp the destruction of aflatoxin was comparatively less. No aflatoxin B_{2a} was found in any of the extracts of the cooked products. It is suggested that this point should be considered while performing epidemiological surveys in determining the exact dose of aflatoxin ingested and its effect in man.

Nearly 345 million metric tons of maize is grown all over the world[1] as both food and feed crop[2]. Maize is susceptible to mould infection and mouldy maize toxicity was recognized as early as 1940 and a product proved to be toxic to swine and mice was isolated from it[3]. At moistures > 17.5% and temp. > 13°C, corn is susceptible to aflatoxin formation by *Aspergillus flavus* [4]. Later it was found that unfavourable growth conditions of the plant[5], moisture content of grain ranging from 19-28 per cent and geographical source of maize[6] appeared to increase the incidence of aflatoxin. Damaged kernels with the characteristic flourescence contained as high as 88,500 to 1,01,000 p.p.b. of aflatoxin B_1 in U.S.A.[7]. High levels of aflatoxin concentrations in commercial maize samples have been reported from several other countries[8-14]. However, the human aflatoxicosis was first noticed in a tribal population in India[15] and high incidence of heptatomas was also recorded due to the consumption of aflatoxin containing mouldy corn[16,17].

Several physical methods such as electronic sorting[18], hand separation of aflatoxin contaminated maize[19] and removal of outer layer by soaking and crushing[20] were found to reduce aflatoxin levels to a considerable extent. It has been observed that in milled products of maize, germ portion contained maximum aflatoxin, whereas the grits contained the minimum[21,22]. Aflatoxin from contaminated maize meal could be extracted in boiling water[23] and no aflatoxin B_{2a} was found in the extract[24]. Forty to eighty per cent reductions of aflatoxin can be attained by a single passage of corn through a continuous roaster[25]. As there is partial destruction of aflatoxin B_1 by heat, the traditional methods of cooking of maize and its products was undertaken to find out the extent to which the toxin is destroyed in the products before, they are consumed by the usually undernourished rural population in backward areas. The data are presented in this note.

Fifty g samples of corn were taken in six 250 ml Erlenmeyer flasks along with 20 ml distilled water and sterilized at 120°C for 20 min. The flasks after cooling were inoculated with *A. flavus* CFTRI strain and incubated at 28°C for 7 days and inactivated by adding 50 ml of acetone to each flask and kept for one hour. Later, the mouldy corn was air-dried under shade for 3 hr and dried in a hot air oven at 60°C for 5 hr. The mouldy maize was powdered by using a laboratory hand grinder and stored in a polythene bottle. The powder was then added at various levels to samples of whole maize flour and maize semolina free from aflatoxin at different levels. For preparing popcorn, mouldy maize was mixed with maize free from aflatoxin at different levels.

Both semolina and maize flour were obtained by roller flour milling (Buhlar Brothers, Switzerland).

Known quantities of pure aflatoxin B_1 (prepared at CFTRI, Mysore) in chloroform was added to uncontaminated samples of maize semolina and maize flour. The chloroform was removed by heating at low temperature on a water bath. These samples were subjected to various cooking methods. For preparation of popcorn, it was added to the maize before popping.

Fifty g of semolina of each sample of maize was dry-roasted in an aluminium pan. Then, 100 ml of distilled water was brought to boil in a 500 ml beaker and then the roasted semolina was added to the boiling water with constant stirring with a glass rod so that no lumps are formed, then cooked for 2 min till done.

Fifty g of each of maize semolina, flour and grain samples were cooked traditionally into porridge, balls, roti and popcorn, as the case may be. Distilled water was used in all the cases of cooking. In the cooking of porridge, the roasted semolina sample was added to 100 ml of boiling water in a beaker and stirred with a glass rod till done. Similar procedure was used in the preparation of balls from maize flour. Roti was prepared by kneading the flour with 50 ml of hot water, spreading the paste on hot iron pan and cooking crisp on both sides. The popcorn was prepared by soaking the grains in 3.5 ml water for 3 hr and air puffing at 220-230°C in a hot air puffing unit (Sunvic, U.K.).

Pons' method[26] was modified and adopted for the analysis of aflatoxin B_1 and B_2 in both cooked and uncooked samples of maize. In cooked samples, the weight difference over and above 50 g was taken as the amount of water absorbed by the materials. Based on this, the volume of acetone was adjusted to give the required concentration of 70 per cent acetone for extraction. The sample extracts were taken in known quantity of chloroform and estimated on silica gel-G thin layer chromatographic plates by dilution to extinction method[27].

Standards of aflatoxin B_1 (CFTRI, Mysore) and B_{2a} (SRRL, New Orleans, USA) were used during the estimations.

In order to assess the amount of destruction of aflatoxin in maize and its products by various Indian traditional cooking methods, viz., the preparation of (a) thick porridge, (b) flour balls, (c) roti, and (d) popcorn, the following experiments were conducted.

In the first set of experiments, mouldy maize powder (or mouldy maize) was added to maize flour, maize semolina and maize grain, as the case may be, to get an aflatoxin content ranging from 130 to 330 p.p.b. The samples were subjected to different traditional cooking methods. On analysis, the cooked products showed 52-53 per cent mean aflatoxin destruction. There was no significant difference in per cent mean aflatoxin destruction between the products, namely porridge, balls, roti and popcorn (Table 1).

In the second set of experiments, pure aflatoxin B_1 was added to maize flour, maize semolina and maize grain to a level ranging from 40 to 400 p.p.b. and subjected to the preparation of different traditional cooked products. This also showed a similar trend. The per cent mean destruction of aflatoxin in these products ranged from 48 to 51 as presented in Table 1.

Similar results were also obtained, when naturally mould infested maize flour, maize semolina and maize grain were traditionally cooked as above. The aflatoxin content was reduced on cooking to a mean level of 48-50 per cent from the initial levels of 20-60 p.p.b. as shown in Table 1.

Hence, different traditional methods of cooking destroy aflatoxin to almost the same degree irrespective of the type of maize sample. Similar results were observed in rice except that pressure cooking yielded more destruction[28]. The other methods like autoclaving moist peanut meal[29], heating at 100°C for 2 hr[30] or roasting peanut at 150°C for 30 min[31,32] have been found to lower the aflatoxin content in groundnut and its meal. Similarly, roasting of pecans at 191°C for 15 min has been found to reduce aflatoxin by 80 per cent[33].

These data give an exact picture of the extent to which aflatoxin is destroyed by traditional methods of cooking of maize and its products and suggest that this point should be considered while performing epidemiological surveys in determining the exact dose of toxin ingested and its effect in man.

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TABLE 1. EFFECT OF TRADITIONAL METHODS OF COOKING OF MAIZE ON AFLATOXIN B_1*

Traditional product	Maize flour/semolina/maize + mouldy maize powder (A)		Maize flour/semolina/maize + aflatoxin B_1 (B)		Mould infested maize flour/semolina/maize (C)	
	Residual aflatoxin (ppb)	Mean destruction (%)	Residual aflatoxin (ppb)	Mean destruction (%)	Residual aflatoxin (ppb)	Mean destruction (%)
Maize semolina cooked as porridge	60—160	53	20—210	49	11—30	48
Maize flour cooked in hot water and made into flour ball	60—170	52	20—190	51	10—31	50
Maize roti	60—160	53	20—220	48	10—31	50
Maize popcorn	60—170	52	20—190	51	10—31	50

*No. of samples analysed in each case was 5
Initial aflatoxin (ppb) was 130-330, 40—440, 20—60 for A, B, C respectively.

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QUALITY CHARACTERISTICS OF CAKES PREPARED FROM DIFFERENT FATS AND OIL

N. SHRESTHA S.A. VALI; AND P.N. CHOUDHARY
P.J.T.D. Home Science College, Nagpur University,
Amravati Road, Nagpur-10, India.

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The effect of different fats and oil on specific gravity and viscosity of cake batters and consequently the volume and tenderness of baked cake was studied. Batters with low specific gravity and high viscosity produced cakes with greater volumes. No correlation could be established between specific gravity of cake batters and tenderness of baked cakes. Sensory evaluation showed variation in the condition of top crust, tenderness and flavour of cakes prepared from different fats and oil.

Fat plays an important role in the structure of cake batters and the quality of cakes is greatly affected by the amount and type of fat used. A significant function of fat in cake baking is imparting tenderness to cake crumb which has been found to be the most important characteristic governing consumer preference[1]. This work was initiated to study the quality characteristics of cakes prepared from different fats and oil viz. margarine (S_1), pure ghee (S_2), Amul butter (S_3), refined ground nut oil (S_4), bakers shortening (S_5), home made butter (S_6), and hydrogenated fat (S_7).

Cakes were prepared using the recipe given by Stevenson and Miller[2] with slight modification in the quantities of egg and fat. The amount and proportion of ingredients and the procedure were standardised.

Volume, viscosity and specific gravity of cake batters were measured. Viscosity of batter was measured using Brookfield viscometer with spindle 4,5,6 at 100 speed. Using seed displacement method volume of cake was measured.

The sensory properties of cakes were evaluated using a descriptive cum numerical score card by a selected panel of 12 judges who had undergone training on the test sample by conducting three consecutive trials.

The objective measurements of batter are shown in Table 1. Specific gravity of cake batters ranged between 0.87 and 1.15. Specific gravity measure indicates the amount of air in the batter. Greater the amount of air in the batter lower is the specific gravity. Cake batter with margarine showed low specific gravity (0.87) while that with bakers, shortening and oil showed high specific gravity of 1.00 and 1.15, respectively. According to Griswold[3], batters containing oil do not hold air well.

Batters with low specific gravity showed high viscosity. Cake batters with bakers, shortening and oil showed low viscosity of 3914 and 1965 respectively. Charley[4] stated that batters containing emulsifiers are thinner and have a high specific gravity and greater mobility.

The weights of the baked cakes did not differ much. The percentage loss of weight ranged between 5 and 7 per cent. Percentage loss of weight during baking is related to the amount of evaporation of water besides loss of carbon dioxide[5]. A striking difference was, however, observed in the cake volume. Cakes prepared with margarine and pure ghee showed larger volumes while those with amul butter, refined oil and hydrogenated fat had smaller volumes. Fat is the only ingredient in shortened cakes which is active in entrapping air and has a considerable effect on volume and grain of the product[4,6]. During baking, the fat melts releasing air cells to give expansion to the product[7]. Specific volume of cakes ranged between 1.58 and 2.11. Cakes prepared from margarine showed higher specific volume while that made of oil and hydrogenated fat had minimum specific volume.

The acceptability scores of cakes for each of the external and internal characteristics, the total scores and ranks are shown in Table 2.

Cakes prepared from margarine had a total score of 79.33 ranking first amongst all the experimental cakes. Cake made from oil though showed high specific gravity, low viscosity

TABLE 1. OBJECTIVE MEASUREMENTS OF BATTER AND CAKE

Sample code	Creaming time (min)	Batter		Cake		
		Sp gr	Viscosity*	Weight (g)	Volume (cc)	Sp vol (—cc/g)
S_1	12	0.87	7860	415	880	2.11
S_2	13	0.95	7350	416	811	1.95
S_3	6	0.98	7125	427	691	1.61
S_4	—	1.15	1965**	427	676	1.58
S_5	13	0.96	6520	419	760	1.81
S_6	8	0.97	5210	425	736	1.73
S_7	14	1.00	3910***	413	653	1.58

*Speed.—100 **Spindle.—4, ***Spindle.—5. Spindle.—6.

TABLE 2. ACCEPTABILITY SCORES OF EXTERNAL AND INTERNAL CHARACTERISTICS, TOTAL SCORES AND RANKS OF CAKES

Samples	External top crust	Top crust colour	Internal characteristics						Total score	Rank
			Grain	Moist-ness	Tender-ness	Aroma	Flavour & taste	Done-ness		
S_1	9.33±0	10±0	10±0	10±0	10±0	10±0	10±0	10±0	79.33	I
S_2	8.00±0	10±0	10±0	10±0	9.94±0.05	10±0	10±0	10±0	77.94	III
S_3	8.55±0.09	10±0	10±0	10±0	9.60±0.05	10±0	8.66±0	10±0	76.81	IV
S_4	9.21±0.09	10±0	10±0	10±0	10±0	10±0	10±0	10±0	79.21	II
S_5	7.33±0	10±0	9.33±0	10±0	9.71±0.05	10±0	9.66±0.2	10±0	76.03	V
S_6	6.00±0	10±0	10±0	9.94±.04	9.66±0	10±0	8.99±0.13	10±0	74.59	VII
S_7	8.66±0	10±0	8.55±.1	10±0	9.88±0.10	10±0	8.77±.10	10±0	75.86	VI

and smaller volume, nevertheless showed higher acceptability as judged by its total score of 79.21. Based on total scores, cakes prepared from pure ghee (77.94), amul butter (76.81), bakers, shortening (76.03), hydrogenated fat (75.86) and home made butter (74.59) were ranked in that order. Individual scores for external and internal characteristics revealed that condition of top crust, tenderness and flavour and taste were the major characteristics that were affected when the fat was varied in cake preparation. Margarine was found to be the most suitable fat while home made butter the least. Acceptable cakes could be prepared from oil as evident from the observations of this study.

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BOOK REVIEWS

The Guidelines for Predicting Dietary Intake of Pesticide Residues, WHO, Geneva, 1989, pp 24; Price: Sw fr 8/-.

The above cited booklet has been prepared by joint UNEP/FAO/WHO Food Contamination Monitoring Programme in collaboration with the Codex Committee on Pesticide Residues. The booklet covers 1) Acceptable daily intake and maximum residue limits, 2) Predicting the dietary intake of pesticide residues, 3) Use of the guidelines, 4) References, 5) Glossary and Annexures 1 and 2. Annexure 1 describes Joint FAO/WHO consultation on guidelines for predicting dietary intake of pesticide residues, while annexure 2 provides relevent notes on commodity and residue levels etc.

As it has been rightly pointed out that even when pesticides are applied strictly in accordance with good agricultural practice, there may be still some uncertainty as to whether the levels in the diet of the members of the community are within acceptable limits. Confirmation of this can be obtained through detailed dietary intake studies, which is costly, hence a method for predicting the dietary intake has been worked out by comparing the recommended acceptable daily intake (ADI). With this in view, the present guidelines describe the proceedures for predicting the dietary intake of pesticide residues. These procedures are included to assist acceptability of Codex maximum residue limits (MRLS). These basic approaches, which are designed to provide reasonable assurance that use of Codex MRLS will not result in a dietary intake of pesticide that exceeds its ADI, are placed for consideration of relevent authorities.

It should be appreciated that the use of the MRLS in the prediction of pesticide residue intake will lead to an over estimation of actual pesticide intake. It is recommended that average daily food consumption values be used in predicting pesticide residue intake for comparison with the ADI.

The booklet serves as a useful guide for those engaged in dietary studies with respect to pesticide residues.

J. R. RANGASWAMY
C.F.T.R.I., MYSORE-13.

Dichlorvos, Environmental Health Criteria 79 WHO, Geneva, 1989; pp 157; Price: Sw fr. 16.

Dichlorvos-Environmental Health criteria 79 is published under the Joint sponsorship of the United Nations Environment Programme, the International Labour Organisation and the World Health Organisation.

This document has 12 chapters being classified as under. Summary and recommendations cover 15 pages inclusive of general aspects, environmental aspects, distribution and transformation, effects on mechanisms in the environment and experimental animals and effects on man.

The second chapter includes identity, physical and chemical properties, conversion factors, analytical methods. The physical and chemical properties are presented in Table 1 in page 18, while the analytical methods for Dichlorvos residues in food and biosystems are dealt in detail in Tables 2 and 3. The cholinesterase activity in blood and the concentration of Dichlorvos have been dealt in detail in Table 4 while determination of Dichlorvos in air, soil and water are included in Table 5. This chapter very satisfactorily covers the sampling effects and analytical methods. Chapter 3 deals with exposure of DDVP on environment and humans. Distribution, transport and transformation of DDVP in environment concerning the biotransformation and also the environment levels and human exposure are respectively presented in chapters 4 and 5. Pharmaco-kinetics of metabolism of DDVP are very well depicted in the figure presented in page 46 and also the elimination and excretion in expired air, faeces and urine have been systematically catalogued besides biological body burden. Chapter 6 describes the effects of mechanisms in the environment including micro organisms, aquatic organisms. The toxicity to fish has been elaborated in Table 11. One section deals with the effect of Dichlorvos to non-target aquatic insects and terrestrial organisms, being covered in Table 12. LD_{50} values have been presented in Table 15 to various experimental animals. The effect of short term exposure to various mammals including oral, dermal, and inhalation studies, cholinesterase activities in response to DDVP and also long term exposures, embryo toxicity and mutagenicity are dealt int chapters 7 and 8. Carcinogenicity and mutagenicity both *in vivo* and *in vitro*, mechanism of neurotoxicity and effects, factors modifying the toxicity also form part of chapter 8. Effects on man by poisoning incidents, occupational exposure are adequately covered in chapter 9. A critical evaluation has been made in Chapter 10 elucidating the human health risks and effect of DDVP on the environment.

The criteria document ends in Chapter 11 by recommending that continuous exposure of young children and diseased elderly people to DDVP in poorly ventilated rooms should be avoided. As DDVP obtained from different sources varies in purity and contains varieties of impurities, attention should be paid to its composition and should confirm to FAO and WHO specifications. The previous evaluations by other international bodies have been reviewed which should be

taken into cognisence by the people and the researchers, manufacturers while synthesising this compound.

This document has 478 references starting from page 117-157.

This document on DDVP will be very useful for research personnel, formulators, environmentalists and many user organisations, in public sector for containing the insects in various locales. Since its bibliography is very vast, basic researchers on formulation development, biodegradability, biological half life and residue problems could be understood by using the information sources. The effort made by Dr. E.A.H. Van Heemstra-Lequin and Dr. G.J. Van Esch of the Netherlands are commendable.

M.K. KRISHNA KUMARI
C.F.T.R.I., MYSORE-13.

Cypermethrin-Environmental Health Criteria 82: WHO, Geneva, 1989, pp 154; Price: Sw fr.16

This monograph contains an exhaustive introduction in addition to other 11 main sections on Cypermethrin from environmental transport to effects on man. Cypermethrin was initially synthesised in 1974 and first marketed in 1977. In most substrates, the practical limit of determination is 0.01 mg/kg. Cypermethrin may cause a transient itching and/or burning sensation in exposed humen skin. It is relatively stable to sun light, may be it may undergo photodegradation. It is absorbed from the gastro intestinal tract and its eliminations are quite rapid. Cypermethrin does not cause repetitive activity in nerve fibres but it affects the sodium channels in the nerve membrane and cause a long-lasting prolongation of the transient increase in sodium permeability of the membrane during excitation. Consistent with the lipophilic nature of Cypermethrin, the highest mean tissue concentrations are found in the body fat. The oral exposure of the general population is low and negligible. No case of accidental poisoning has been reported as a result of occupational exposure.

The monograph contains 11 main sections. They are Identity, Physical and chemical properties, Analytical methods, Sources of human and environmental exposure; Environmental transport, Distribution and transformation., Environmental levels and humen exposure., Kinetics and metabolism., Effects on organisms in the environment., Effects on experimental animals and *in vitro* test systems., Effects on man., Evaluation on health risks for man and effects on the environment., Recommendations., and previous evaluations by international bodies.

The monograph provides an extensive citations on Cypermethrin running upto 341. The WHO task group on environmental health criteria under the Chairmanship of Dr. M. Mercier has done a commendable job in presenting all aspects of Cypermethrin. The monograph serves as a useful guide for all those people working on various aspects of Cypermethrin.

J.R. RANGASWAMY
C.F.T.R.I., MYSORE-13.

DDT and Its Derivatives-Environmental Aspects. Environmental Health Criteria 83 Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation and the World Health Organisation, Geneva, 1989, pp 98; Price: Sw. fr. 13.

The report contains collective views of an international group of experts and does not necessarily represent the decisions or the stated policies of the UN Environment Programme.

The review deals with the physical and chemical properties of DDT and related compounds, kinetics, metabolism, biotransformation and bioaccumulation, retention in soils and sediments, plant uptake, uptake and accumulation by plants, micro-organisms, aquatic invertebrates, fish, terrestrial invertebrates, birds, mammals, toxicity to micro-organisms, particularly bacteria and cyanobacteria, freshwater micro-organisms, marine micro-organisms, soil micro-organisms including fungi.

There is a chapter on toxicity to aquatic invertebrates, short-term and long-term toxicity, physiological effects on fish, development of tolerance, toxicity to amphibians, sub-lethal behavioural effects on fish and physiological effects on fish.

The chapter dealing with terrestrial organisms reviews short and long-term toxicity to birds, toxicity to birds' eggs, effects on bird reproduction, reproductive hormones and behaviour, reproductive effects on the male, effects on the thyroid and adrenal glands in birds and synergism with other compounds in birds.

It was made very clear in this document that the aim was to take the ecologists' point of view and consider effects on populations of organisms in the environment which is totally different from the approach of the toxicologist. This document does not consider effects on organisms in the environment but does consider environmental levels of DDT likely to arise from recommended uses. No attempt was made to reassess the human health risk.

The review is a valuable document for those dealing in environmental safety of DDT used now largely in public health and some of the derivatives being used in agriculture. The information will be useful for research workers and those who are involved in pesticide regulations.

V. AGNIHOTHRUDU
UPASI TEA RES. INSTITUTE
VALPARAI-642 127

Lead-Environmental Aspects, Environmental Health criteria 85, WHO, Geneva, 1989, pp 106; Price: Sw. fr.13.

This review has been published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation and the World Health Organisation.

The WHO Task Grants on EHC met at the Institute of Terrestrial Ecology, Markswood, UK during December 1987 and the first draft was prepared by Dr. S. Dobson and Mr. P.D. Howe. Dr. M. Gilber and Dr. P.G. Jenkins as members of the IPCS Central Unit were responsible for the overall content and editing respectively.

It starts with a short introduction in page 8 defining the aim of the document, taking the ecologist's point of view, consider effects of Lead on biological population in the environment and considerable attention has been paid to persistence in the environment and bioaccumulation and covers only the data which are considered to be essential in the evaluation of the risk posed by Lead in the environment. The physical and chemical properties of Lead have been well documented already in EHC 3: (WHO 1977). The book comprises of nine chapters followed by 250 references with a comprehensive coverage.

The first chapter presents the summary and conclusions elucidating the physico-chemical properties of Lead and sources of pollution, uptake, loss and accumulation in organisms, its toxicity to micro organisms, aquatic species, terrestrial organisms, toxic effects in the field. Physical and chemical properties and some of Lead in the environment have been very briefly dealt in chapters 2 and 3 respectively as these have been documented in EHC 3 (WHO 1977). Chapter 4 deals with uptake, loss and accumulation in organisms and these are well represented in Tables 1 and 2.

Effects of Lead salts, organic Lead on microorganisms including zoo and phytoplanktons have been dealt in chapters 5 and 6, toxicity of Lead salts are included to aquatic plants, invertebrates (described in Table 3) fishes (Table 3) Amphibians have been well documented. A comprehensive review on several effects of various forms of Lead on terrestrial organisms viz., invertebrates, birds and wild mammals forms chapter 7.

Chapter 8 includes the effect of Lead in the field - tolerance of plants to Lead, industrial sources of Lead, industrial sources of lead pollution and massive bird kills in estuaries. The last chapter (9) comprises of Evaluation of Environmental hazards of Lead, its entry to the equatic and terrestrial environment by references.

Information collected, classified and highlighted in this book on Lead Published by 160/UNEP/WHO will be helpful to all user organisations, environmental scientists, educationists and toxicologists. The task groups' painstaking effort in bringing out this essential document is noteworthy.

M.K. KRISHNAKUMARI
C.F.T.R.I., MYSORE-13.

Mercury - Environmental Aspects - Environmental Healtl Criteria 86 Published under the joint sponsorship of th United Nations Environment Programme, the International Labour Organisation and the World Health Organisation, as part of the IPCS (International Programme on Chemical Safety), World Health Organisation, Geneva, 1989, 115 pp; price: Sw. fr. 14.

The WHO Task Group on Environmental Health Criteria has done an excellent job in reviewing the various aspects, which include: Physical and chemical states of mercury in the environment, sources of mercury in the environment, including natural and anthropogenic sources and cycling, speciation, levels in the environment and methylation of mercury.

There are chapters on uptake, loss and accumulation in organisms, toxicity to micro-organisms, toxicity to aquatic life, toxicity to terrestrial organisms and effect of mercury in the field.

Effort has been made to present information in the criteria documents as accurately as possible. The document, however, is not intended to be exhaustive in the material included. Concentration figures for mercury in the environment or in particular species of organisms, have not been included unless they illustrate specific toxicological points.

The publication is valuable for agrochemical industry, research workers and those involved in regulatory affairs of pesticides.

V. AGNIHOTHRUDU
UPASI, VALPARAI-642 127, T.N.

Abrasive-disk dehullers in Africa: from research to dissemination: by Michael W. Bassey and O.G. Schmidt, International Development Research Centre, P.O. Box 8500, Ottawa, Ontario, Canada, KIG 3H9, 1989; pp 98; Price: not stated.

This book discusses the application of Abrasive-disk dehullers in processing of sorghum, pearl millet and certain grain legumes. This book can be viewed as a Technical companion to their earlier publication, an end to pounding (Eastman, 1980).

The book is divided into seven chapters. The chapter 'Introduction', deals with the food problem in Africa and inexpensive machine dehulling to save substantial energy and time of the average rural home maker. Next is a unique

chapter on 'traditional foods, user preferences and grain quality', describes several steps involved in traditional processing and food preparation and wide range of dishes prepared from both sorghum and millet. Implications of grain characteristics to obtain satisfactory functional properties of food and population preferences in use of dehullers for the local grains and foods prepared from the processed products are discussed.

The next two chapters deai on 'evolution of the dehullers, and their design features'. They describe exhaustively the evolution of several variants of large and small dehullers and their applications to a range of problems in different National contexts. The salient design and operating features of the dehullers and their various components and the grains to be dehulled are discussed.

The next chapter 'grain machine interactions', examines the action of removing the hull from a grain from two points of view: the grain structure; the nature of the abrasive material in the grinding wheel or disk; and defines relevant measures for comparing dehullers performance.

The last two chapters 'small scale milling systems' and 'Dissemination of the technology', provides information on the small scale milling installation—a single dehuller-grinder combination, requirements for planning, installing, operating, managing and evaluating in rural and urban areas for custom and trade milling. Finally, the role and responsibility of the applied researcher in wider dissemination of technology is examined.

R. SHANKARA
C.F.T.R.I. MYSORE-570 013.

AFST(I) NEWS

Jabalpur Chapter

The Annual General Body Meeting was held on 14th September 1990. The following office-bearers were elected:

President	:	Dr. Y.K. Sharma
Vice-President	:	Dr. N.J. Sawarkar
Hony. Secretary	:	Mr. S.S. Shukla
Hony. Jt. Secretary	:	Ms. L.P. Ayachi
Hony. Treasurer	:	Ms. Anubha Shukla

Nagpur Chapter

The Annual General Body Meeting was held on 10th August 1990. The elections were unanimous. The office-bearers elected for the new year are as follows:

President	:	Dr. G.D. Nageshwar
Vice-Presidents	:	Shri C.R. Patel
		Dr. Sudhir Bhiwapurkar
Hony. Secretary	:	Prof D.K. Kawadkar
Hony. Jt. Secretary	:	Shri S.V. Joshi
Hony. Treasurer	:	Dr. G.V. Mulmuley

Hyderabad Chapter

The Chapter had its Annual Generl Body Meeting on 1st September 1990 and elected new office-bearers. They are as follows:

President	:	Mr. K.S.T. Shayee
Hony. Secretary	:	Mrs. Mallika Janakiraman
Hony. Jt. Secretary	:	Mr. A. Satyanarayana
Hony. Treasurer	:	Mr. H.K. Guru Raj Rao

Karnal Chapter

The Annual General Body Meeting was held on 16th October 1990. The following office bearers were elected:

President	:	Dr. D.K. Mathur
Hony. Secretary	:	Dr. H. Abichandani
Hony. Jt. Secretary	:	Dr. Ashok Patel
Hony. Treasurer	:	Sh. R.K. Kohli

NEW CHAPTER

The Central Executive Committee of AFST(I) in its meeting held on 24th September 1990 has approved for opening a AFST(I) Chapter at Manipur. The following have been elected as office bearers for the year 1990-91.

President	:	Mr. G. Nandalal Sharma
Vice-Presidents	:	Mr. Ng. Maimu Singh
		Mr. K. Dhaneshwar Singh
		Mr. M. Tonjao Singh
Hony. Secretary	:	Mr. S. Joykumar Singh
Hony. Secretaries	:	Mr. L. Saratchandra Singh
		Mr. Th. Joychandra Singh
Hony. Treasurer	:	Mr. T. Sanajaoba Singh

ANNOUNCEMENT

As a consequence of the increase in the cost of printing, publication and postal tariff, it has now become absolutely necessary to enhance the SUBSCRIPTION RATES of the Journal of Food Science and Technology. The new rates are as follows:

Annual Subscription of Journal

India
Rs. 300/-

Foreign
Air Mail US$ 125/-
Surface Mail US$ 100/-

This will come into effect from the beginning of Volume 28, 1991. I solicit your kind cooperation.

—Editor

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The Editor expresses his gratitude to the following referees for their valuable service in the evaluation of papers in the JOURNAL during 1990

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INSTRUCTIONS TO AUTHORS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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