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Collapse and Aroma Retention During Storage of Freeze-Dried Products

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The effect of heating, humidity and composition of some sugars and lactoserums on the collapse and the retention of aroma is studied. Collapse favours the loss of aroma. The retention of aroma decreases after heating the freeze-dried products at temperatures higher than their collapse temperatures. The diffusion of the volatile compounds and the chemical composition of the substrate both influence the retention.

Changes in the rigidity of the porous structure of freeze-dried products may occur during storage. The experiment showed a decrease in volume of the product and an increase in its hardness due to the destruction of its porous structure. Factors that govern this phenomena are the temperature, humidity and chemical composition of the substrate. Each product has a fixed collapse temperature (Tc) for a given residual humidity (RH). It has been shown that an increase in the RH of freeze-dried products brings a decrease in their collapse temperature[1,2]. Tc is linearly related to moisture content, approximately up to the BET monolayer which is where a break in the curve occurs[3]. Tc decreases when the number of dextrose equivalents (DE) increases[1,2]. If the temperature and moisture are too high during storage of freeze-dried products, the destruction of the structure and loss of volatile compounds may result. This loss is greater in the case of collapse obtained by sorption of water rather than by heating. Collapse also takes place during the change from the amorphous state to crystalline state.

The loss of volatile compounds during this transformation is significant. The retention of aromas depends on residual humidity (RH). If RH is lower than 30 per cent, retention remains high, but above 70 per cent, the aroma is completely lost after few hours. At mid-way RH, aroma retention is not very low[3,4]. The effect of heating of freeze-dried products on retention was studied by Chirife[4,5] and To[6]: a linear relation between loss of aroma and degree of collapse in lactose-hexanol system has been shown.

Materials and Methods

Mono, oligo and polysaccharides and lactoserums (LS) were used as substrates. Polysaccharides are hydrolysed starch, characterized by their DE (per cent of dextrose equivalent). Lactoserum LS_1 contains (in per cent) 12.5 nitrogen and 72 lactose and LS_2 contains 36.5 nitrogen and 48 lactose. The volatiles: acetone, 2-propanol, n-hexanol, ethylacetate, benzaldehyde and diacetyl were used at a concentration of 1 per cent (W/W). The concentrations of substrate were 15 and 50 per cent (W/W). Two model solutions (water-substrate-volatile) placed in separate flasks were freeze-dried using SMJ-USIFROID freeze-dryer. Quantitative analysis of the volatile substances were carried out by GC before and after drying and collapse. The freeze-dried products were rehydrated before analysis. The gas-chromatograph apparatus used was a Hewlett-Packard, serie 5710A equipped with a flame ionisation double detector. The collapse temperature was measured under microscope as follows: particular flasks were heated at the rate of 5°C/min and photographed under microscope at various stages of heating; The heating continued until the flask area became round and constant in size. The degree of collapse Dc is defined by the following formula:

$$Dc = \frac{Ai\text{-}At}{Ai}$$

where Ai is initial area of the flask and At is final area of the flask.

The normalized collapse curve represents the degree of collapse *versus* temperature. The collapse temperature Tc is the intersection of the rapid increase in the normalized collapse curve with the temperature axis. The hardness of freeze-dried products caused by collapse is measured by a penetrometer[7] and expressed in grams.

Results and Discussion

Fig. 1 shows a linear correlation between DE and Tc of some malto-dextrins at 0 per cent RH. The effect of moisture on Tc of the freeze-dried malto-dextrin DE 32 is shown in Table 1. The Tc decreased rapidly when the RH increased. This is due to the fact that hydrogen bonds which are responsible for the main structural force in freeze-dried products[8] are weakened as the moisture content rises.

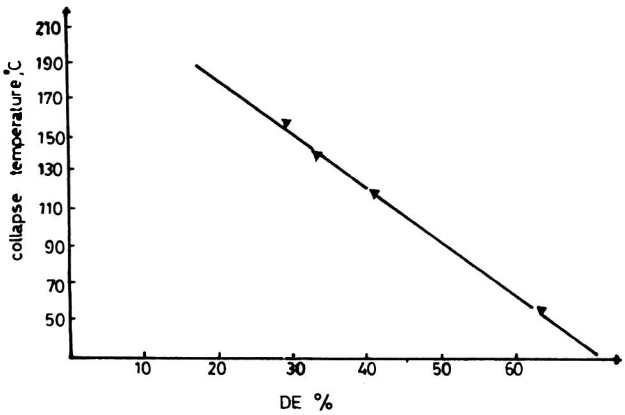

FIG. 1. Collapse temperature versus DE

TABLE 1. COLLAPSE TEMPERATURE (Tc) OF MALTODEXTRIN AT DIFFERENT RESIDUAL HUMIDITY (RH)

RH %	0	2.3	6.4	8.4
Tc (°C)	139	96	66	37

Each value is the average of five determinations

The effect on heating on retention of freeze-dried maltose at 0 and 7 per cent RH is shown in Table 2. The heating at $T < Tc$ did not show any notable loss of volatile compound whereas the heating at $T > Tc$ showed a greater loss especially for maltose at 7 per cent RH. In order to study the nature of substrate on retention, a large variety of products were used. These freeze-dried products were heated until the temperature reached their Tc. The resistance to penetration and the retention of volatile compound are shown in Table 3. From this Table, it is observed that, in the case of maltodextrins, the resistance to penetration and the retention of aroma decreased when their DE decreased. In the case of lactoserum, the retention of aroma in LS_1 was much higher than that of LS_2. This high per cent of retention in LS_1 may be due to the presence of much lactose which retains a large quantity of aroma. Recrystallisation of lactose was found to be a major cause for loss of aroma in the dry state. The retention of volatile compound during the collapse caused by heating could be explained as follows[6]: the amorphous structure holding the volatile compounds shrinks slightly and

TABLE 2. RETENTION OF VOLATILE SUBSTANCES (%) IN FREEZE-DRIED MALTOSE AT DIFFERENT RESIDUAL HUMIDITY (RH)

Temp (°C)	Acetone	Ethyl acetate	Diacetyl	2-propanol	n-hexanol	Benz-aldehyde
			AT 0% RH			
70	90	85	91	95	80	84
89	89	85	63	39	77	79
104 (Tc)	86	85	49	34	75	75
130	58	54	27	75	75	52
140	18	18	4	31	70	66
			AT 7% RH			
45	100	100	100	100	100	100
52	93	96	98	95	97	93
72	90	89	82	94	89	95
101 (Tc)	82	74	35	39	84	78
124	29	22	14	49	62	50

Each value is the average of five determinations

TABLE 3. PENETRATION RESISTANCE AND RETENTION OF VOLATILE COMPOUNDS AFTER HEATING THE FREEZE-DRIED COMPOUNDS

Substrate	Tc (°C) 0% RH	Volatile retention (%)						Pene-tration (g)	Appearance
		Acetone	Ethyl-acetate	Diacetyl	2-Pro-panol	n-hexanol	Benzal-dehyde		
DE 61. 5	56	91.8	100.0	71.2	75.7	74.6	74.2	412	collapsed
DE 32	139	26.2	84.3	6.4	44.4	100	40.4	268	collapsed
DE 28. 5	155	16.7	64.0	5.6	29.7	69.1	30.7	259	collapsed
DE 19	184	22.0	75.0	5.4	29.7	59.8	40.2	—	non collapsed
Maltose	102	100	100	30.0	97.0	100	100	—	collapsed
LS_1	34	90.0	100	14.7	92.0	100	86.0	27	slight collapsed
LS_2	34	45.5	30	16	12	97	81	—	slight collapsed

Each value is the average of five determinations

TABLE 4. COLLAPSE TEMPERATURE (°C) WITH AND WITHOUT VOLATILE COMPOUND

				0% RH		1.5% RH	7% RH
Substrate	—	0.1% acetone	1% acetone	0.1% ethyl-acetate	6 volatile compounds	0.1% acetone	6 volatile compounds
Maltose	104	82	79	84	86	75	31
Malto-dextrin (DE 61,5)	56	49	28	—	—	—	—

Each value is the average of four determinations

a large retraction in volume occurs. This may be accompanied by a notable loss of volatile compounds. Finally the product becomes liquid retaining what remains as a volatile compound. The variation in aroma retention can be explained by the destruction of micro-regions immobilising the volatiles[3,4] and by a development of the diffusion of aromas[9].

Furthermore, the interaction between the volatile compounds and substrates appeared as one of the main causes of retention.

The effect of aroma on Tc is shown in Table 4. The presence of aroma in freeze-dried products, even in small quantities, decreased the Tc. This change of Tc may be due to the weakness of hydrogen bond which maintains the amorphous and porous structure of the freeze-dried product.

Acknowledgement

The authors are thankful to Professor D. Simatos, Director, for her keen interest and guidance during the studies.

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Studies on the Preparation and Evaluation of Vermouth from Plum

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Vermouth of commercial acceptability could be prepared from plum. In the vermouth with increased levels of alcohol, TSS, pH, aldehyde, esters, phenols and per cent sediment invariably increased, while titratable acidity and vitamin C declined. However, volatile acidities, sugar contents and colour values remained unaffected. The herb/spices extract addition increased the total phenols, aldehyde and ester contents of vermouth. The physico-chemical characteristics of plum vermouth compared well with those of mango and grape vermouths. The sensory evaluation of the products showed the sweet products to be superior to the dry ones. Vermouth with 15% alcohol and sweet taste was the best product. The names, parts of the herbs/spices, their individual quantities used and the method of preparation of vermouth have been described.

Himachal Pradesh produces about 16,000 tons of Plum (*Prunus salicina* L.) annually[1]. Being a highly perishable crop, it cannot be easily transported to far off places nor can it be cold stored for long periods. Though, the fruit could be utilized for the preparation of jams, jellies etc., to accommodate the large quantities of the fruit produced during the glut periods it becomes necessary to explore alternate methods for its utilization on commercial scale. Preparation of alcoholic beverages is one of the options for this purpose and methods for preparation of plum wine of good quality are available in the literature[2]. The use of delightfully smelling and tasting herbs and spices are made in various food preparations to increase palatability. To satisfy this taste preference, wine was made for the first time by Antonio in 1786 in Italy by using wormwood and the wine was called 'Vermouth'[3].

Aperitif wines known as vermouths compounded from grape wine by adding herbs and spices mixture or their extract are popular in European countries and in U.S.A. Aromatised fruit wines are also produced commercially in U.S.S.R. and Poland[4]. Extensive studies on the recipes of the spices and herbs are reported in the literature.[4,5] Recently, Onkarayya[6] prepared commercially acceptable mango vermouth but whether plum could be used for the production of vermouth, needs investigation. Therefore, studies were carried out with the object of preparation of plum vermouth and this paper describes the results obtained.

Materials and Methods

The plum pulp was prepared by mashing the fruits of 'Santa Rosa' variety procured from University Orchard at Nauni. The must was prepared as per the method reported[2]. The culture of *Saccharomyces cerevisiae* var. *ellipsoideus* (5 per cent) was prepared in the sterilized plum pulp and was added to the must to initiate the fermentation which was carried out at a temperature of 22 ± 1°C, till the total soluble solids (T.S.S) became stable (8.0°B). It was siphoned, racked and filtered followed by maturation for a period of 6 months and was used as a base wine (PLW). A part of this base wine was distilled as per the standard methods to produce plum brandy[5].

Extract preparation: Sixteen commonly available spices and herbs (Table 1) were powdered after drying. The mixture was added to the base wine (containing plum brandy) to solubilise the active ingredients in alcohol by heating to 60°C for 5-7 min. everyday in a closed container for 10 days. It was then filtered using a muslin cloth and the residues were again extracted by using a small quantity of brandy and the filtrate was added to the original bulk. The extract was kept in refrigerator at 2 to 3°C for 24 hr followed by filtration.

Vermouth preparation: Vermouths with three alcoholic levels viz. PLV_1 original (12.2 per cent), PLV_2 (15 per cent) and PLV_3 (19 per cent) were made with plum brandy by calculating alcohol content using the formula reported[7].

After fortification and addition of spices extract, the TSS in case of sweet vermouth(S) were raised by 6 per cent with addition of sugar syrup (70°B). No additional sugar was added in the preparation of dry vermouth (D). The extract (10 per cent) and SC_2 (50 ppm) were added and products were kept for maturation for a period of 3 months.

The plum vermouths of various treatments were analysed for different physico-chemical, microbiological and sensory characteristics. T S.S. were measured by hand refractometer, while colour was compared by Lovibond tintometer as per the standard methods[8] and expressed as red (R), yellow (Y) and blue (B) units. The pH was measured with the digital

TABLE 1. SPICES/HERBS ADDED TO THE BASE WINE FOR VERMOUTH PREPARATION

Common name	Botanical name	Parts used	Quantity/l (g)
Black pepper	*Piper nigrum* L.	Fruit	0.75
Coriander	*Coriander sativum* L.	Seeds	0.70
Cumin	*Cuminum cyminum* L.	Seeds	0.50
Clove	*Syzygium aromaticum* L.	Fruit	0.25
Large cardamom	*Amomum subulatum* Roxb.	Seeds	0.50
Saffron	*Crocus sativus* L.	Flower	0.01
Fenugreek	*Trigonella foenumgraecum* L.	Seeds	0.50
Nutmeg	*Myristica fragrans* Houtt.	Seed	0.25
Cinnamon	*Cinnamomum zeylanicum* Beryn.	Bark	0.50
Poppy seed	*Papaver somniferum* L.	Seeds	1.00
Ginger	*Zingiber officinale* Rosc.	Dried root	1.00
Woodfordia	*Woodfordia floribunda*	Flower	0.25
Asparagus	*Asparagus* sp.	Leaves	0.10
Withania	*Withania somnifera*	Roots	0.20
Adhatoda	*Adhatoda* sp.	Leaves	0.25
Rosemary	*Rosmarinus officinalis*	Flowering plant	0.10

pH meter while titratable acidity was determined using standardized alkali. Volatile acidity, alcohol, esters, aldehydes, total phenols, sugars and vitamin C were measured as per the methods described[9-14]. The vermouth was analysed for standard plate count as per the routine method described[15]. Sensory analysis was carried out by getting scores for individual attributes on a prescribed proforma. Maximum score for each attribute was 20. The samples were given in the coded form to the trained panel of 10 judges in random order. The judges were allowed to rinse their mouth with water while tasting the samples. The samples were presented in separate rooms or on tables in a room.

The data on physico-chemical parameters were statistically analysed by completely randomised design and those of sensory evaluation by randomised block design as per the recommended methods[16,17].

Results and Discussion

The composition of the plum base wine used for vermouth preparation (Table 2) shows that the various characteristics are quite comparable and matched with the essential requirements of a base wine to be made into vermouth as has been reported[18]. Further, the data show that there are significant differences in total soluble solids of the wine and different vermouths. These differences are expected because of addition of sugar to make the product sweet and addition of brandy which also contributes towards the total soluble solids by effecting the refractive index. The sweet vermouths showed a slight decrease in their alcohol contents than the initial values i.e. 12.2, 15.00 and 19.00 per cent respectively. No such change in the dry products was observed.

Compared to the plum base wine, the titratable acidity registered a significant decrease with increase in the alcohol contents. As the neutral brandy was used to fortify the base wine, it might have diluted the acid contents in the respective vermouths. Titratable acidity in our products is slightly more than those reported[19] which is due to the inherent character of plum fruit. The pH values of the products were corroborating with the respective acidity values. There was no significant difference in colour values of the vermouths of various treatments and is desirable.

TABLE 2. PHYSICO-CHEMICAL CHARACTERISTICS OF PLUM BASE WINE AND VERMOUTHS OF VARIOUS TREATMENTS

Treatments	TSS (°B)	Alcohol (%v/v)	Titratable acidity (% MA)	Volatile acidity (% AA)	pH	Colour			Sugars		Vitamin C(mg/100 ml)	Sediment (%)	Total phenols (mg/l)	Aldehyde (mg/l)	Ester (mg/l)
						R	Y	B	RS (%)	TS (%)					
Plum Vermouth (original alcohol, PLV_1)															
PLV_1(D)	8.1	12.3	0.86	0.02	3.35	9.0	10.6	0.1	ND	ND	4.5	0.2	429	104	189
PLV_1(S)	11.5	11.5	0.78	0.03	3.33	10.0	10.6	0.1	4.7	5.0	4.1	0.3	399	106	212
Plum Vermouth (15% alcohol, PLV_2)															
PLV_2(D)	9.2	15.0	0.81	0.03	3.38	10.0	10.1	0.1	ND	ND	3.5	0.4	417	111	204
PLV_2(S)	15.0	14.5	0.79	0.04	3.34	10.0	10.1	0.1	4.6	4.8	3.2	0.5	390	112	219
Plum Vermouth (19% alcohol, PLV_3)															
PLV_3(D)	10.7	19.0	0.77	0.03	3.44	10.0	9.1	0.1	ND	ND	3.1	0.8	414	121	211
PLV_3(S)	14.1	18.5	0.72	0.03	3.52	10.1	9.0	0.1	4.7	5.0	2.7	0.9	383	123	225
Plum Base Wine (original alcohol, PLW)															
PLW	8.2	12.2	0.85	0.02	3.28	9.0	11.6	0.1	ND	ND	4.6	ND	396	87	171
CD (P=0.05)	0.25	0.22	0.02	0.05	0.06		NS			NS	0.95	0.01	10.8	1.3	3.0

MA = Malic acid, AA = Acetic acid, R = Red, Y = Yellow, B = Blue, Units of respective colour, D = Dry, S = Sweet, RS = Reducing sugars, TS = Total sugars, ND = Non-detectable, NS = Non-significant.

Neither the plum base wine nor dry products contained any reducing or total sugars. No significant differences between total sugar contents of the sweet products are observed. All of them are having volatile acidities less than the legal limit prescribed for the wine[5], and are lower than in mango vermouth[6].

Ascorbic acid in the base wine and the different vermouths indicates a decrease as the alcohol content increases. But sweet and dry vermouths of various treatments did not show any difference. Vitamin-C contents of wines are normally negligible[20] though appreciable amounts of it are present in plum base wine or vermouths. Increasing alcohol levels increased the sedimentation. Further, the sweet vermouth had more sedimentation than dry in the respective treatment. Dry vermouth contained significantly more phenolic content than the respective sweet-vermouths or the base wine possibly due to the contribution made by the extract of spices and herbs used to flavour it[5]. But overall results are similar to those reported[19]. Comparatively, lower phenolic contents in sweeter vermouths than the dry vermouths may have been the result of precipitation during maturation as indicated by more sedimentation in the former than in the latter (Table 2).

The results indicate a significant increase in aldehyde and ester contents over that of base wine and invariably sweeter product contained more aldehydes. The increase in these components is the effect of addition of herbal extract which is known to contribute various compounds including esters. Increase in aldehyde content of mango vermouth has also been reported[6]. The aldehyde content of grape wine has been found to range between 100 and 125 mg/l, but data on aldehyde contents of grape vermouth are lacking[5]. The effect of maturation could be another factor for such levels of ester contents which normally range from 200 to 400 mg/l as ethyl acetate in wines[5].

The plum wine and vermouths of various treatments were found to be free from any microbial contamination indicating soundness of the products.

Sensory analyses of the vermouths (Table 3) show that there were significant differences in all the parameters except the body. PLV_2 (S) obtained the highest score in colour and appearance, astringency and aroma. With respect to the taste, the data showed the sweet products in all the treatments were preferred to dry, possibly due to the balancing of the bitterness and strong aroma in the vermouths contributed by the addition of herbal extract. In the overall quality, PLV_2 (S) product scored significantly higher than the other products. PLV_2 (S) could be designated as of commercially acceptable quality as has been proposed by Ough and Baker[21].

It can be concluded from this study that the sweet vermouth with 15 per cent alcohol of commercial acceptability could be prepared by the proposed method.

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TABLE 3. SENSORY ANALYSIS OF PLUM VERMOUTH OF VARIOUS TREATMENTS

Treatments	Sensory attributes/scores*					
	Colour and appearance	Body	Aroma	Taste	Astringency	Overall quality
Plum Vermouth (original alcohol, PLV_1)						
PLV_1(D)	13.0	15.6	13.0	12.6	13.6	11.8
PLV_1(S)	14.0	14.0	15.6	15.4	13.6	13.0
Plum Vermouth (15% alcohol, PLV_2)						
PLV_2(D)	14.2	15.2	14.4	13.8	15.8	13.0
PLV_2(S)	17.6	14.0	16.8	17.4	17.8	16.6
Plum Vermouth (19% alcohol, PLV_3)						
PLV_3(D)	16.8	15.0	12.8	11.8	15.0	11.0
PLV_3(S)	17.2	14.5	15.4	15.8	14.0	14.4
$(CD_{P=0.05})$	3.6	NS	3.6	3.6	3.6	3.6

* Maximum 20 for each attribute. D= Dry S = Sweet

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Jambal Wine Making: Standardisation of a Methodology and Screening of Cultivars

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Acceptable dry table wines were prepared from the three cultivars viz., 'Pharenda'. 'Jamun' and 'Kathjamun' but, wine prepared from 'Jamun' was adjudged the best followed by 'Kathjamun'. In the preparation of must, dilution of whole fruit in the ratio of 1:1 with water was found suitable. Use of pectic enzyme was found to be more beneficial in getting a clear product.

The Jambal fruit (*Syzygium cumini* Linn) is liked for its refreshing pink to greyish juicy flesh, having a very balanced sugar, acid and tannin blend. It is generally consumed fresh and is known to have therapeutic value. It is considered useful in preventing diabetes[1-3]. Information regarding the area and total production of this fruit is not available because it is seldom planted in the form of orchard. It is widely grown in larger parts of India from the Indo-Gangetic plains in the north to Tamil Nadu in the South, scattered in fruit plantations, in parks, on road sides, avenues and as wind break. The average yield of fruit from full grown seedling Jamun tree is about 80-100 kg and from a grafted one 60-70 kg per year[4]. Very little information is available on processing of this fruit except its use in making vinegar and spicy, ready-to-serve beverages[5-7]. The attractive colour due to anthocyanin pigment is a major quality attribute in Jamun beverages[5,8] and this with other characters can be used in making various products. Wine is one such product. Though there are no named or standard varieties of this fruit under cultivation, the three cultivars of Jambal available in Uttar Pradesh were tested for their suitability and devising a methodology for making quality red wine.

Materials and Methods

Mature fruits of three cultivars, viz, 'Pharenda', 'Jamun' and 'Kathjamun' were collected from the avenue trees of Kalidas Marg, Lucknow and used in the present study. Ten Kg fruits of each cultivar were washed in water, crushed with hand without damaging the seeds and divided into two lots. Boiling water in the ratio of 1:1 and 1:2 was poured over the Ist and 2nd lot respectively to kill naturally occurring strains of yeasts and bacteria on the fruits and also to reduce the high acid and tannin contents of the fruits (by dilution effect), in order to produce a palatable wine, as in a preliminary experiment wine produced by undiluted jamun must was found too acidic and astringent. The hot must was quickly cooled and mashed again. Sufficient sugar (2 kg in Ist and 3.10 kg in 2nd lot) was then added to raise the total soluble solids to 23° Brix. Yeast nutrient in the form of diammonium phosphate (0.2 per cent) and sulphur dioxide (150 p.p.m.) in the form of potassium metabisulphite (2.35 g in Ist and 3.655 g in 2nc lot) were added. Starter was prepared by making a malt solution by adding (in per cent) 20 sugar, 0.03 tartaric acid, 2.0 malt extract, 2.0 yeast extract, 0.2 citric acid 0.2 ammonium phosphate in distilled water. The medium was brought to boiling, cooled and inoculated with seven days old culture of *Saccharomyces cerevisiae* variety *ellipsoideus* Burgandy (No. 3281) collected from National Chemical Laboratory, Poona and maintained on M.G.Y.P. medium. A 24 hr old starter was inoculated to the must at 2 per cent level and allowed to ferment at room temperature (33-35°C). When vigorous fermentation had set in, both the lots were divided into two sub-lots. In one sub-lot of each lot was added 0.25 per cent pectic enzyme, prepared from *Aspergillus wentii*[9] and other sub-lot was left as such without adding pectic enzyme. After four days of fermentation, all the lots were pressed through muslin cloth and the seeds and pomace were discarded. All the four sub-lots of each cultivar were fermented till almost all the sugar was consumed (to dryness). After settling, the wines were racked into clean glass bottles of 2.5 and 1.0 L capacity and filled upto the brim. Sulphur dioxide in bottles was maintained at 150 p.p.m. level. The air-tight sealed bottles were stored at room temperature (20-35°C) for maturation. The wine samples were racked at intervals of two months for a period of six months.

Fruits, musts and wine samples were analysed for chemical composition. Total soluble solids (TSS) were determined with a hand refractometer and pH using a pH meter. Total acidity (as citric acid), total sugars (as invert sugar) and tannins (as tannic acid) were estimated by AOAC methods[10]. Sulphur dioxide, volatile acidity (as acetic acid), ester (as ethyl acetate), ethyl alcohol and extract (alcohol-free soluble solids) were analysed as described by Amerine and Cruess[11]. The total anthocyanin pigment was measured by the method of Fuleki and Francis[12] with slight modification as suggested by Khurdiya and Roy[1,5]. Measurement was taken at 515 nm and

results expressed as mg cynanin per 100 g/ml must or wine. Pectin was estimated as calcium pectate by the method of Carre Haynes[13]. The rate of fermentation was compared by the weight loss of the musts in flasks, i.e. by the amount of CO_2 formed and released during the fermentation[14]. Sensory evaluation was done by a panel of seven judges as recommended by Amerine and Cruess[11].

Results and Discussion

The composition of fresh fruits varied with the variety (Table 1). Seed to pulp ratio was highest (1:10.2) in 'Pharenda' and lowest (1:1.8) in 'Kathjamun'. Total sugars ranged from 8.55 to 9.22 per cent and acidity from 1.06 to 1.30 per cent. Anthocyanin pigments ranged from 157 to 182 mg/100g. tannins from 302 to 345 mg/100 g and pectin from 0.48 to 0.63 per cent. As the acidity and tannin contents were high, water was added in 1:1 and 1:2 ratio into the crushed fruits to get a palatable wine and the diluted musts were ameliorated with cane sugar to 23° Brix before setting for fermentation in order to get the desired level of alcohol. Schanderl and Koch[15], also recommended the amelioration of berries and plum with water and cane sugar to produce a palatable wine. Dilution and addition of cane sugar resulted in decrease of all the constituents in the musts to an appreciable level (Table 1), except the pH which increased from 3.02-3.15 to 3.40-3.56. The acidity decreased to 0.30-0.49 per cent, tannins 93-162 mg/100 g, anthocyanins 51-82 mg/100g and pectin 0.13-0.22 per cent.

The yield, composition and organoleptic evaluation of the aged wines are presented in Table 2. Having very little sugar (traces to 0.40 per cent), the alcoholic contents ranged between 10.26 and 11.61 per cent and hence these wines could be classified as dry wines. Alcohol yield in 1:1 dilution of all the three cultivars was little higher than that of wines made from 1:2 dilution as the dilution may affect the conversion of sugar into alcohol. This aspect was investigated by comparing, the weight loss of the musts of 1:1 and 1:2 dilutions of 'Jamun' cultivar in flasks, i.e. by the amount of CO_2 formed and released during the fermentation. The average per cent weight loss for 24 hr in 1:1 dilution was found

TABLE 1. COMPOSITION* OF FRESH FRUITS AND MUSTS OF DIFFERENT CULTIVARS OF JAMBAL

Test conducted	Pharenda			Jamun			Kathjamun		
	Fruit	Must		Fruit	Must		Fruit	Must	
Dilution	—	1:1	1:2	—	1:1	1:2	—	1:1	1:2
Seed to pulp ratio	1:10.2	—	—	1:3.9	—	—	1:1.8	—	—
TSS (°Brix)	11	23	23	12	23	23	11	23	23
pH	3.08	3.40	3.52	3.15	3.46	3.56	3.02	3.40	3.48
Acidity (as citric acid %)	1.23	0.46	0.38	1.06	0.38	0.30	1.30	0.49	0.40
Reducing sugars (%)	8.32	—	—	8.82	—	—	8.05	—	—
Total sugars (%)	8.74	—	—	9.22	—	—	8.55	—	—
Pectin (as calcium pectate %)	0.63	0.22	0.16	0.55	0.20	0.14	0.48	0.18	0.13
Tannins (as tannic acid mg/100g)	320	150	108	302	142	93	345	162	113
Anthocyanins (ml/100g)	168	74	58	157	64	51	182	82	61

— Not analysed.
* Each value is an average of 4 replicates.

TABLE 2. YIELD, CHEMICAL COMPOSITION AND SENSORY EVALUATION OF JAMBAL WINES AFTER 6 MONTHS MATURATION*

Test conducted	Pharenda		Jamun		Kathjamun	
	Without PE	With PE	Without PE	With PE	Without PE	With PE
Yield (l/2.5 kg fruits)	3.80	3.92	3.62	3.70	3.52	3.62
Alcohol (%) by vol	10.93	11.16	11.23	11.61	10.71	10.86
Total acidity (as citric acid g/100 ml)	0.44	0.46	0.37	0.38	0.45	0.48
Volatile acidity (as acetic acid g/100 ml)	0.033	0.036	0.036	0.032	0.042	0.033
pH	3.40	3.40	3.50	3.48	3.42	3.40
Residual sugar (g/100 ml)	0.32	0.18	Tr	Tr	0.32	Tr
Esters (as ethyl acetate mg/100 ml)	18.48	16.22	16.12	14.24	16.46	20.48
Tannins (as tannic acid mg/100 ml)	118	104	105	94	125	115
Total anthocyanins (mg/100 ml)	46	45	44	42	48	45
Extract (g/100 ml)	3.7	3.0	3.33	2.9	3.1	2.7
Pectin (as calcium pectate g/100 ml)	0.032	Nil	0.021	Nil	0.026	Nil
*Average organoleptic score	71.0	75.0	78.5	83.0	74.3	75.4

PE = Pectic enzyme. * = significant at 5% level (ANOVA in RBD); Tr = Traces

higher (2.145) than per cent weight loss in 1:2 dilution (1.795). Total acidity in matured wines ranged from 0.29 to 0.48 g/100 ml which is slightly less than the acidity of the musts. Volatile acidity (as acetic acid) ranged from 0.032 to 0.040 g/100 ml and ester contents (as ethyl acetate) ranged from 14.23 to 20.48 mg/100 ml. An appreciable decrease was found in tannin and anthocyanin contents which ranged from 70 to 125 mg/100 ml and 29 to 48 mg/100 ml, respectively. The possible reason of decrease of these polyphenolic compounds may be due to their combination with aldehydes, to precipitate with added or natural proteins and to other reactions[11]. The pectin contents were found very much decreased and varied from nil in lots treated with pectic enzyme to traces - 0.032 g/100 ml in lots not treated with pectic enzyme. The decrease in pectin in lots not treated with pectic enzyme was due to the precipitation of pectin with alcohol produced[11]. Pectic enzyme treated lots were found slightly higher in total acidity as these enzymes raise the galacturonic acid content which is the ultimate compound produced due to the hydrolysis of pectic substances.

Clarity, colour, bouquet and taste are the characters responsible for acceptance or rejection of any wine. In sensory evaluation, the wines secured 70.3 to 83.0 per cent marks and all the 3 cultivars were found suitable for making wines. Among three cultivars, 'Jamun' was found the best followed by 'Kathjamun'. Between 1:1 and 1:2 dilution, 1:1 was adjudged superior as it secured more marks than 1:2 dilution in all the three cultivars. It gave wines of more balanced acid, tannin and colour contents and also produced higher alcohol yield. Though lots not treated with pectic enzyme also produced clear wines, the enzyme treated lots were found more clear and secured more marks than untreated ones. Thus, pectic enzyme treatment was found beneficial in making jambal wine.

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Evaporative Cooling of Potatoes in Small Naturally Ventilated Chambers

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Short duration (1-3 days) experiments were carried out on evaporative cooling (EC) of potatoes using metallic EC chambers covering the outer walls with wet cloth, with a view to finalise design details for scaling upto 10-ton capacity. The results showed that the rate of cooling during 24 hr period increased in proportion to the increase in the surface area of evaporation upto 80%, but further increase in the surface area of evaporation to 100% did not show any additional cooling due to direct solar radiation. At the end of 24 hr cooling, the temperature dropped to 22.1°C from 38.0°C (with a drop of 15.9° C) and the amount of heat removed was 2658 kJ as against wet bulb temperature of 21.1°C. Air circulation inside the EC chamber increased the cooling rate to 3.0 kJ/kg/hr as compared to 1.65 kJ/kg/hr in non-circulating chamber. The cooling efficiency in terms of heat removed (kJ/kg/hr) increased with the increase in load (1/3, 2/3 and full capacity).

Cold storage of potatoes, besides having other limitations, is capital intensive and therefore, can not be expanded rapidly to meet the need of the farmer. Hence, a farm level storage system, which is less capital intensive and extends the storage life sufficient to realise better prices is very much needed. In this context, evaporative cooling storage of potatoes is considered to meet the much desired need and hence work has been initiated in the early eighties[1]. At about the same time, work on evaporative cooling storage of fruits and vegetables was also carried out in R&D institutes of Indian Council of Agricultural Research. A zero-energy evaporative cooling double walled chamber, filling in between the two brick walls with wet sand was successfully employed for small scale domestic level storage of fruits and vegetables[2]. A potato store run on passive evaporative cooling, employing evaporative cooling pads, having a 20 ton capacity has been reported by the Central Potato Research Institute[3]. The system studied in this laboratory for storage of apples, oranges and potatoes[4,5], is based on the proportion of evaporative surface to the volume of the chamber, in which the metallic walls of the chamber cooled evaporatively, serve as heat exchangers. However, for the evaporative cooling and storage of fresh fruits and vegetables, the efficacy of the system in terms of quick removal of field heat from the produce thus cooled is very essential, as in tropical countries, the field heat of fruits and vegetables is high (>30°C) and unless it is removed quickly, there will not be sufficient extension of their storage life. Hence, keeping rapid cooling of the commodity as the principal objective of this study, the effects of (i) variation in the surface area of evaporation, (ii) forced circulation of air inside the EC chamber, and (iii) the extent of loading on the cooling of commodity were studied, to fix parameters for designing a 10 ton evaporative cooling storage structure.

Materials and Methods

Potatoes (cv 'Kufri Jyoti') weighing between 80 and 120 g (medium size), without any visible damages were used in these studies. The experiments were carried out in October/November months when the ambient dry bulb temperature ranged between 20 and 32°C and the wet bulb temperature between 17 and 22°C. Ambient conditions, actually recorded for each of the short duration experiment were used for the calculation of cooling and heat removed.

Description of the evaporative cooling chamber: The evaporative cooling chamber used in this study was similar to that reported in earlier studies[4], but differs in dimensions (100 × 25 × 50 cm). The EC chamber was made of 28 gauge aluminium sheet covered with tray as lid of size 100 × 20 × 7.5 cm made of Galvonized iron sheet. The chamber was placed in a tray of size 105 × 30 × 7.5 cm. A wire mesh (2.5 cm weld mesh) basket of 85 × 20 × 45 cm was used to load potatoes, leaving an air gap of 2.5 cm between the basket and the chamber.

Loading and operation of the chamber: Potatoes were filled in the wiremesh basket and kept inside the chamber from the top. The chamber was closed with the tray filled with water to serve as a reservoir. The evaporative surfaces of the chamber were covered with cotton cloth and kept always wet. The chambers were kept in shade allowing free wind movement. About 45 kg potatoes were stored in the chambers. In some experiments, the commodity was allowed to heat overnight (in Gallon Kamp humidity oven maintained at 40°C) to raise the temperature to near 40°C in order to facilitate

cooling studies. The chambers, with the narrow sides (ends) facing east-west direction were insulated (from out side) with 10 cm thick polystyrene sheet ('U' value: 682 J/m^2.°C hr/cm thickness).

Temperature, humidity and evaporation recording: The air temperatures at different points and core temperature of potato placed at the centre of the chamber were recorded with a six channel digital temperature indicator fitted with probes (PT-100) having an accuracy of 0.1°C. Ambient temperatures were recorded with a wet and dry bulb thermometer. The evaporation rate of water was measured by exposing cured clay bricks saturated with water, to the same experimental conditions and recording the weight loss at hourly intervals. The relative humidity inside the EC chamber was recorded by attaching a wick (suspended in water) to the tip of the probe placed in the chamber.

Determination of quantity of heat removed and cooling efficiency: The heat removed (Q) from the commodity during evaporative cooling was determined using the equation given by Rastovski[6].

$$Q = m\ T\ C$$

where m is the mass of potatoes loaded, C is the specific heat of potatoes (3.6 kJ/kg/°C) and T is the change in temperature. The per cent cooling efficiency (CE), which indicates the extent of cooling of commodity achieved in relation to ambient wet bulb, was calculated using the formula

$$C.E.\ (\%) = \frac{T_i - T_f}{T_i - T_w} \times 100,$$

where T_i and T_f are the initial and final temperatures of commodity and T_w is wet bulb temperature.

Effect of circulating air inside the evaporative cooling chamber: Two chambers with 80 per cent surface area of evaporation were used one with and the other without a battery operated fan (wing span 7.5 cm) placed at the centre of the potato heap. The velocity of air at a distance of 15 cm in front of the fan was 0.01 m/sec.

Results and Discussion

The dry bulb temperature fluctuated between 21.0 and 30.5°C, and the wet bulb temperature between 17 and 21°C. Under these conditions, the evaporation occurred for over 18 hr in a day but maximum evaporation rate of about 0.2/m^2/hr was observed between 12 noon and 5.00 p.m., when the wet bulb depression was highest.

Relationship between surface area of evaporation and evaporative cooling: An increase in surface area of evaporation from 0 to 80 per cent resulted in a faster and greater fall in the core temperature, attaining 22.1°C in the chamber with 80 per cent surface area of evaporation. The quantity of heat removed was also correspondingly higher (Table 1). The cooling efficiency increased from 58 per cent for the chamber with 0 per cent evaporative area, probably due to sensible heat loss to 93 per cent for the chamber with 80 per cent evaporative area. Further increase in the evaporative surface area to 100 per cent did not increase the quantity of heat removed from the commodity or the cooling efficiency, probably because of the simultaneous effects of solar radiation during day time. It was not possible to protect the chamber with shade from sun rays in the morning and evening without hindering free wind movement. The actual fall in temperature and the cumulative removal of heat (Fig. 1) also show that 80 per cent evaporative area is optimum.

TABLE 1. EFFECT OF EVAPORATIVE SURFACE AREA ON COOLING OF POTATOES IN MODEL EVAPORATIVE COOL CHAMBERS AND HEAT REMOVED DURING COOLING (24 hr)

Data particulars	Evaporative area (%) of the container					
	0	20	40	60	80	100
Load (kg)	43.89	47.00	44.70	46.44	46.44	44.77
Initial core potato temp (°C)	38.1	37.9	37.7	39.0	38.0	38.4
Core potato temp after 24 hr of evaporative cooling (°C)	28.2	27.5	25.5	25.3	22.1	22.6
Cooling efficiency (%)	58	62	73	76	93	91
Heat removed from potatoes in 24 hr (KJ)	1564	1760	1963	2290	2658	2547

Effect of load on evaporative cooling: The temperature fall was found to reduce with the increase in the quantity of potatoes loaded from one third (15 kg), two-thirds (30 kg) and full capacity (45 kg) due to high specific heat and mass effect of the commodity (Fig. 2). But the quantity of heat removed was progressively higher with increase in load.

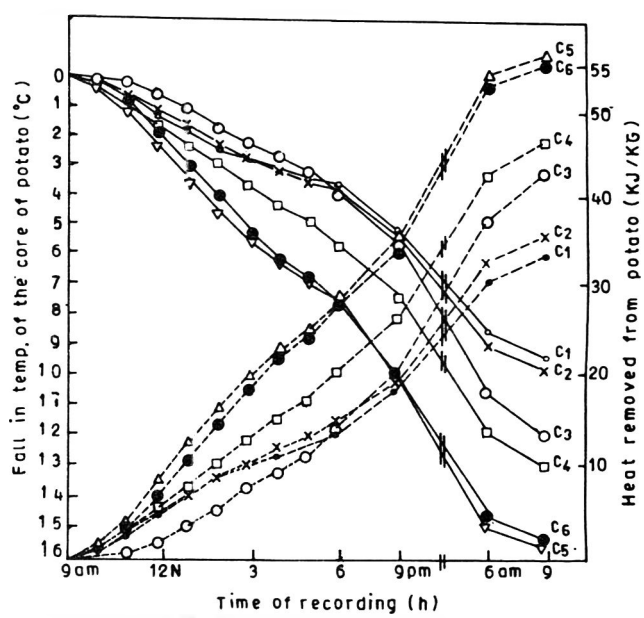

Fig. 1. Effect of variation in the area of evaporative surface on the fall in temperatures (full lines) of potato and the heat removed (broken lines). Evaporative cooling chamber with C_1 — 0%; C_2 — 20%; C_3 — 40%; C_4 — 60%; C_5 — 80% and C_6 — 100% surface area of evaporatior calculated on the total surface area of the 4 sides of EC chamber.

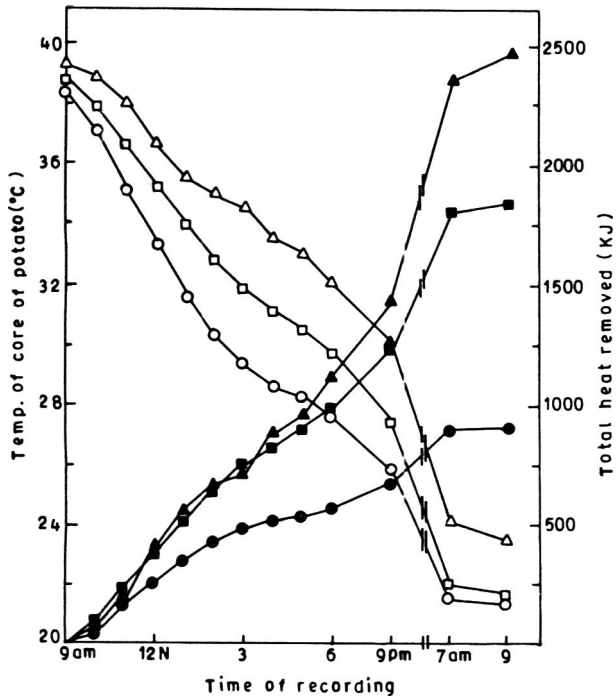

Fig. 2. Effect of loading the evaporative cocling chamber on the extent of cooling of potatoes and total heat removed (cumulative). 0, ● 15 kg; □, ■ 30 kg and ▽, ▼ 45 kg (full capacity) of potatoes. Open symbols temperature of potato and closed symbols heat removed from potatoes.

Effect of circulating air inside the chamber on cooling: The fall in air temperature at the centre of the EC chamber with and without air circulation was 3.2°C and 2.1°C respectively (Fig.3). The air temperature near internal surface of evaporative wall with air circulation was initially 27°C, rose markedly then fell by 4°C in 3 hr as against 1°C in the chamber without air circulation. Thus. air circulation resulted in a 3°C drop in the core temperature as compared to 1.3°C in the chamber without air cirulatior. (Fig. 3 a,b). Further, the heat removed was 251.5 kJ (at the rate of 3.0 kJ/kg/m) with air circulation as against 149.0KJ (at the rate of 1.65 kJ/kg/hr) without air circulation.

Mechanism of heat transfer from commodity to evaporative cooling surface: The temperatures near the evaporative wall inside the EC chamber at top and bottom (peripheral region), and also at the top and bottom at the centre of the potato heap (central region), the core of potato at the centre of heap and the ambient wet bulb were recorded during the evaporative cooling and storage in the EC chamber (with full load and without internal forced air circulation). Fig. 4, shows that there was a temperature gradient between top and bottom layers which caused convective movement of air from commodity to evaporative surface and thereby cooling.

The study revealed that the efficiency of the system can be improved by orienting the insulated sides of the chamber to East-West direction, with evaporative surface area optimum (80 per cent in this study) and maintaining air circulation within the chamber, during cooling, while the depth of loading

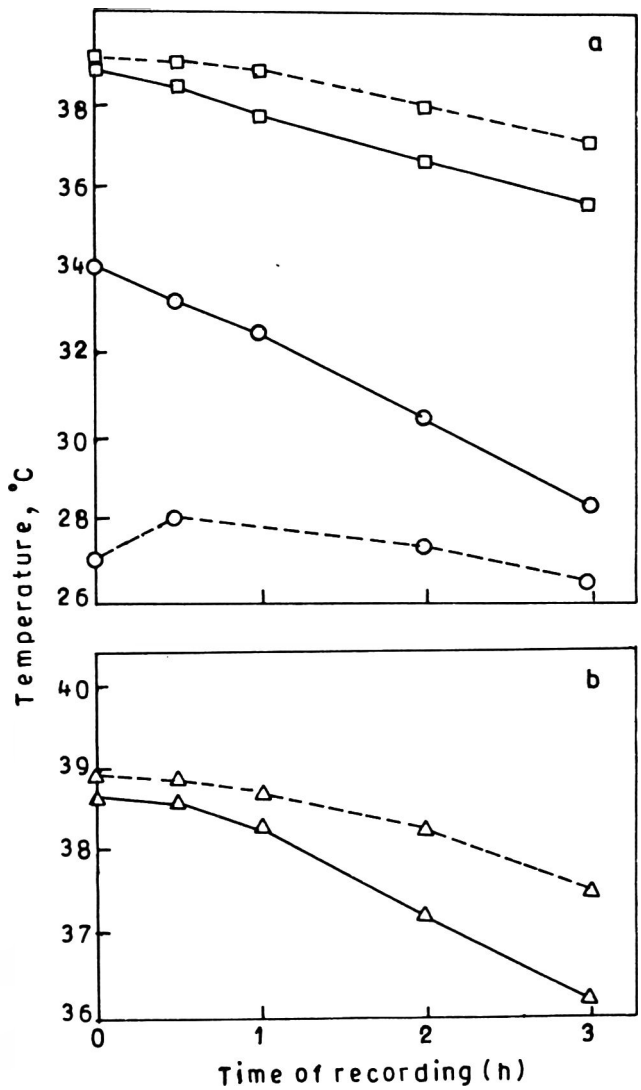

Fig. 3. Effect of forced air circulation inside the evaporative cooling chamber (45 kg load of potatoes) on the evaporative cooling. (a) Changes in temperatures of air: near the wall of the evaporative surface (0, -0-) and at the centre of the evaporative cooling chamber (□, -□-). (b) Changes in temperature of core of potato placed at the centre of the chamber (▽, -▽-). Full lines with and broken lines without forced air circulation.

does not alter it. Further, the temperature of potatoes remained equal to or less than that of the ambient wet bulb temperature[5] which is a significant advantage. Moreover, fluctuations in ambient wet bulb temperature are not rapidly reflected in the commodity temperature because of differences in specific heats of air and commodity. The water consumption in the system (0.2 l/m^2/hr) developed by us[1,5] is lesser than in pad systems (6.5 l/m^2/hr) used by Thompson and Kasmire[7,8] under Californian conditions.

This system which can cool potatoes rapidly to temperatures nearer to wet bulb with an RH of 90-95 per cent offers great scope for the storage at farm level upto about 4 months during summer. These results are useful in scaling up of evaporative cooling chambers.

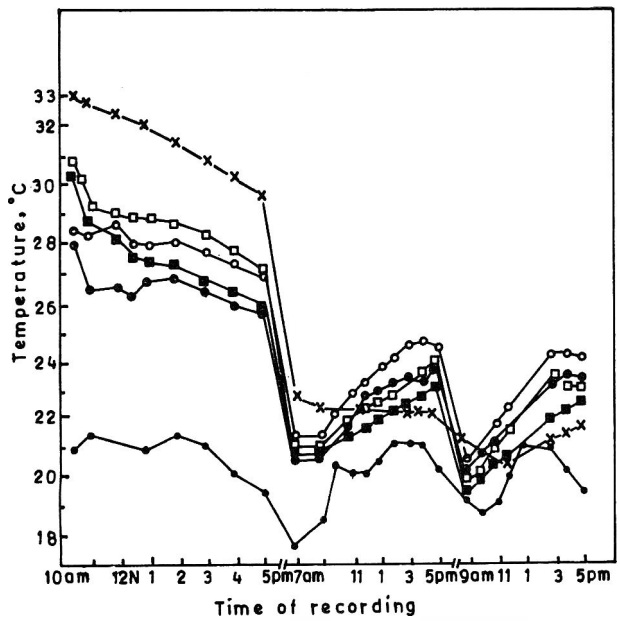

Fig. 4. Air temperatures in the different regions of the evaporative cooling chamber (with full load and without forced air circulation inside the chamber) during evaporative cooling and 2 days of storage of potatoes. 0 top peripheral region; ● bottom peripheral region; □ top central region; ■ bottom central region; X potato core; ● ambient wet bulb.

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Studies on Invert Syrup for Use in Biscuits

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Studies were carried out on the standardisation of method for preparation of invert syrup for use in biscuits. Inversion carried out at 100°C for 30 min. using 1:3 water to sugar ratio with different acids showed maximum inversion of 97.7% with 10% hydrochloric acid followed by tartaric acid (84.8%) and citric acid (74.1%). Increasing the temperature from 80°C to 110°C increased the degree of inversion from 50.2 to 97.7%, while increasing the period of inversion from 15 to 30 min. and acid level from 0.1 to 0.3% the inversion increased from 79.7 to 97.7% and 12.4 to 97% respectively. The degree of inversion decreased from 84.8 to 70.7% as the water to sugar ratio increased from 33:100 to 60:100. The maximum inversion of 97.8% was obtained at 0.5% of 10% hydrochloric acid when the inversion method was followed as done in a commercial way. The optimum level of invert syrup to be used to obtain desired biscuit colour was 2% as compared to 4% for liquid glucose.

Liquid glucose produced from corn starch is one of the ingredients used for obtaining the desired golden brown crust colour in short dough biscuits[1]. However, several small scale biscuit manufacturing units in the country use invert syrup obtained by hydrolysing sucrose instead of liquid glucose, as it works out cheaper and convenient. The inversion of sucrose to invert sugar is required as only reducing sugars in the presence of amino acids, peptides and proteins, on heating produced dark brown melanoidins due to Maillard reaction. This reaction is most important for the production of brown hues on the surface of baked biscuits[2].

Generally in bakery units, invert syrup is prepared by boiling the sugar solution with small quantity of citric or tartaric acid to bring about the inversion and allowed to cool before it is used in the biscuit formulation. The method of preparation varies among different bakery units with respect to type of acids and their levels used, temperature of inversion, ratio of sugar to water, etc., thereby changing the degree of inversion which would naturally lead to variation in the colour of biscuits.

Hence, the studies were undertaken to standardise the method of preparation of invert syrup under the conditions prevailing in the industry and study the influence of various factors like pH and degree of inversion on the colour of biscuits. The results of these studies are presented in this paper.

Materials and Methods

Refined wheat flour (maida) was procured from the local market for use in the preparation of biscuits. Commercially available liquid glucose was used in biscuits for comparison with invert sugar syrup.

Inversion of sugar: Inversion was carried out using 100 g sugar and dispersing it in the prescribed volume of water and holding it in a glycerol bath maintained at a desired temperature for a particular period. Variables tried were the ratio of water to sugar (33:100, 40:100, 50:100 and 60:100) type of acid used (10 per cent hydrochloric acid, tartaric acid and citric acid) and their level (0.1 to 0.5 per cent), inversion period (15 to 90 min) and inversion temperature (80 to 110°C). The invert sugar in the resultant mixture was determined after appropriate dilution using Lane and Eynon method[3].

Inversion of sugar by commercially used method: Saturated sugar solution made by mixing 5 kg sugar was heated by boiling and then added half a teaspoon (2.5 g) of citric acid. Boiling was continued for 3 min. and the syrup was allowed to cool. The degree of inversion was determined[3].

Determination of moisture and pH: Moisture in the invert syrup and commercial liquid glucose was determined using the method of Standard Analytical Methods of Member Companies of the Corn Refiners Association[4], pH of the syrup was determined using the pH meter (Model: Digital pH meter APX 175). pH of the biscuit dough was determined in 10 per cent dough suspension in water.

Preparation of biscuits: Short dough biscuits were prepared using invert syrup of varying inversion and pH, as per the recipe and method described by Haridas Rao *et al.*[5] pH of the syrup was varied (1.14 to 11.00) by adding either dilute hydrochloric acid or sodium hydroxide. The visual colour was evaluated by a panel of ten semi-trained judges.

Determination of colour: The colour of biscuits was determined after powdering biscuits in a pestle and mortar to a particle size of about 170 microns. The colour was measured in a photovolt reflectance meter using an amber

filter. The colour was also measured by extracting the colour with 50 per cent alcohol and measuring the percentage transmittance in Bausch and Lomb spectrophotometer at a wave length of 440 nm[6].

Storage of biscuits: The biscuits were stored at room temperature (27°C ± 2°C and 60-65 per cent RH) after wrapping it in wax coated papers (23 gsm) and polypropylene pouch (37 μ) and the colour was measured at regular intervals. All the above experiments were carried out in triplicates.

Statistical analysis: Statistical analysis of the data was carried out using Duncan's New Multiple Range Test[7].

Results and Discussion

Effect of sugar to water ratio: The degree of inversion decreased from 84.8 to 70.7 per cent as the water used to prepare the sugar solution increased from 33 to 60 ml for 100 g sugar (Fig. 1). This may be possibly due to increase in the pH (from 2.09 to 2.2) of the sugar solution due to increased dilution.

Effect of using different acids: The inversion of sucrose carried out by using varying levels of different types of acids (Table 1) indicated that at lower levels (less than 0.2 per cent) the inversion was more with tartaric acid (70.0 per cent) and citric acid (52.6 per cent) than hydrochloric acid (51.0 per cent). However, at 0.3 per cent, level maximum inversion of 97.7 per cent was observed with hydrochloric acid as compared to other weak acids used. With further increase in the level of acids, inversion increased in case of tartaric acid and citric acid.

The study indicated that maximum inversion of about 95 to 98 per cent was obtained with only 0.3 of 10 per cent hydrochloric acid or 0.03 ml of concentrated hydrochloric acid, whereas it required 0.4 and 0.5 per cent of tartaric acid and citric acid respectively. However, degree of inversion was negligible (< 1 per cent) when inversion was carried out without using any acid. pH of the invert syrup prepared by using the above 3 acids at different levels, ranged from 1.91 to 2.38.

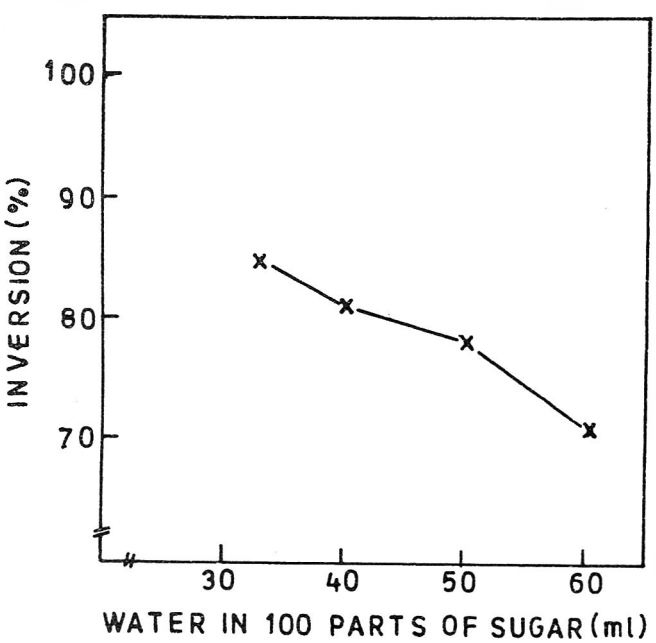

Fig. 1. Effect of water to sugar ratio on the inversion of sucrose

TABLE 1. EFFECT OF DIFFERENT ACIDS ON THE INVERSION* OF SUGAR

Acid level+ (g/100 g sugar)	Inversion (%) 10% HCl	Tartaric acid	Citric acid
0.0	0.8	0.8	0.8
0.1	12.4	52.6	31.3
0.2	51.0	70.0	52.6
0.3	97.7	84.8	74.1
0.4	97.8	95.0	86.2
0.5	97.8	96.5	95.2
	Commercial Method**		
0.3	75.8	70.4	64.1
0.4	91.7	82.6	74.6
0.5	97.8	87.0	—
0.6	—	90.1	78.1
0.7	—	95.1	—
0.8	—	—	90.1
0.9	—	—	95.3

*Carried out at 100°C for 30 min;
**Carried out by boiling sugar solution for 3 min;
+ml in case of HCl acid.

Effect of inversion period: The extent of inversion of sucrose at 100°C for different periods (Fig. 2) indicated more rapid inversion with hydrochloric acid as compared to tartaric or citric acid. Maximum inversion of 97.7 per cent was obtained within 30 min in case of hydrochloric acid while it required over 60 min in case of citric acid or tartaric acid. This again indicates that hydrochloric acid is a better acid for inversion of sucrose.

Effect of temperature: The percentage inversion evidently increased with increase in the temperature of inversion (Table 2). At any temperature of heating, higher inversion was found in the hydrochloric acid followed by tartaric and citric acids. Maximum inversion of 97.7 per cent was observed at a holding temperature of 100°C with hydrochloric acid, while in tartaric and citric acids the inversion was only 84.8 and 74.1 per cent respectively. Even at 110°C, the inversion was only 85-92 per cent in citric acid or tartaric acid.

Sucrose inversion by commercial method: The percentage inversion obtained when inversion was carried out using different acids by normally used method in the industry again confirmed (Table 1) higher inversion with hydrochloric acid as compared to citric or tartaric acid. The maximum inversion of 97.8 per cent was obtained at 0.5 ml/100g sugar of 10 per cent hydrochloric acid while the level of other acids

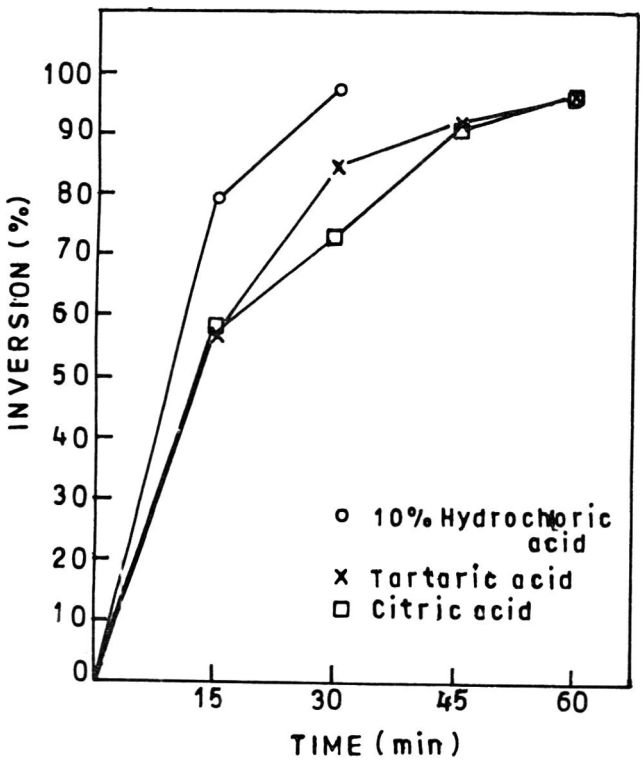

Fig. 2. Effect of holding time on inversion of sucrose.

TABLE 2. EFFECT OF TEMPERATURE ON PER CENT INVERSION OF SUGAR

Temp (°C)	% inversion*		
	10% HCl	Tartaric acid	Citric acid
80	50.2	22.3	20.3
90	64.9	57.1	29.0
100	97.7	84.8	74.1
110	—	93.4	85.4

*Using 0.3% of acid (on sugar basis) for 30 min.

required to invert sucrose to the same extent was higher. For obtaining 95 per cent inversion, the levels of tartaric and citric acids required were 0.7 and 0.9 per cent respectively. The pH values of the resultant syrup containing hydrochloric, tartaric and citric acids were 1.70, 1.88 and 1.88 respectively, indicating that a lower pH may be responsible for the observed higher inversion with HCl.

Effect of invert syrup and corn syrup on the colour of biscuits: The colour of biscuits evidently became darker with increase in the level of invert syrup or corn syrup as indicated by both visual observations and instrumental methods (Table 3). The surface colour changed gradually from cream to brown in corn syrup and to dark brown in invert syrup when used at 5 per cent level. The intensity of colour of biscuits was higher with invert syrup than with corn syrup at equal levels of incorporation possibly due to higher amounts of reducing sugars present in the invert syrup[8]. The

TABLE 3. EFFECT OF DIFFERENT LEVELS OF INVERT SYRUP AND CORN SYRUP ON THE COLOUR OF BISCUITS

Level (%)	Invert syrup		Corn syrup	
	% R[1]	% T[2]	% R[a]	% T[a]
0	50.57	86.33	50.57[NS]	86.00[NS]
1	37.80	73.67	47.67***	84.00*
2	36.87	69.00	46.50***	83.00***
3	32.00	65.33	43.53***	80.00***
4	30.17	59.67	40.33***	76.00***
5	28.47	54.33	37.03***	68.67***
	± 0.13 (12 df)	± 0.30 (12 df)	± 0.26 (12 df)	± 0.27 (12 df)

Each value is mean of three observations. Significant differences were observed between the different levels of invert and corn syrup for both % reflectance and transmittance.

a: NS, *, *** indicates non significance, significance at 5% and significance at 0.1% level from their respective invert syrup levels.

1. % Reflectance of biscuit powder measured in photovolt reflectance meter.
2. % Transmittance of the alcoholic extracts measured in spectrophotometer at 440 nm.

reflectance as well as transmittance values decreased gradually with increase in the level and at equal levels, the values for biscuits containing invert syrup remained always higher. Significant relationship was also found between colour measured visually and by instrumental methods.

The level of invert syrup required to obtain biscuits of desired colour was 2 per cent, while 4 per cent corn syrup was required to obtain similar colour.

Effect of pH: In normal practice, the invert syrup made by mixing acid is directly used without neutralisation in biscuits. This is likely to affect the colour of biscuits. Studies carried out using invert syrup of pH ranging from 1.14 to 11.00, indicated slight increase in pH from 6.56 to 7.00 of dough possibly due to its reaction with sodium bicarbonate and other chemicals. This was substantiated by the fact that pH of biscuit dough made without these chemicals was 5.41 instead of 7.28 as observed in normal dough. The pH of biscuits ranged from 6.26 to 6.70. This indicated that the variation in pH of the invert syrup did not affect the pH of biscuits much, possibly because of the small quantity used.

The colour of biscuits changed only slightly, with increase in the pH. Use of invert syrup of pH above 11.0 imparted slightly darker shades in biscuits (Table 4).

Changes in biscuit colour during storage: Storage studies on biscuits carried out indicated that colour of biscuits gradually faded with increase in the storage period (Table 5). The decrease in the colour intensity as indicated by per cent reflectance was more in biscuits wrapped in polypropylene pouches (36.6 to 47.1) than that wrapped in wax coated paper (36.6 to 42.1). This could be attributed to the transparent nature of polypropylene pouches enabling bleaching of colour

TABLE 4. EFFECT OF pH OF INVERT SUGAR SYRUP ON BISCUIT COLOUR

pH of invert sugar syrup	Reflectance (%)	Trans-mittance (%)
1.14	35.1[a]	63.0[a]
3.10	35.0[a]	64.3[a]
7.40	35.3[a]	64.1[a]
9.30	35.5[a]	64.0[a]
11.00	32.7[b]	57.0[b]
SEm	± 0.47 (10 df)	± 0.24 (10 df)

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

TABLE 5. CHANGES IN THE COLOUR OF BISCUITS** DURING STORAGE*

Storage period (days)	Reflectance (%)		Transmittance (%)	
	Wax coated	Polypro-pylene film	Wax coated	Polypro-pylene film
Initial	36.6	36.6	64.5	64.5
15	38.8	38.7	65.0	70.0
30	38.4	39.6	67.0	75.0
45	40.0	40.7	68.0	76.0
60	40.0	41.8	68.0	76.0
75	41.5	44.5	68.0	76.4
90	42.7	47.1	68.7	77.5

*At room temperature; **2% invert syrup is used in biscuit preparation

due to diffused sunlight with some possible oxidative changes[1].

The studies indicated that maximum inversion of surcrose could be obtained by using HCl instead of tartaric acid or citric acid. The level of invert syrup required to get the desired colour of biscuit was only 2 per cent as compared to 4 per cent of liquid glucose.

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Changes in the Pasting, Rheological and Baking Qualities of Flour During Short Term Storage

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Changes in the chemical pasting, rheological and baking quality during storage of flour for 4 months at 4, 27 and 37°C indicated deterioration in flour, particularly at 37°C as indicated by decrease in gluten content from 10.02 to 7.6%, sedimentation value from 21 to 17 ml, and dough raising capacity from 140 to 77%. However, no change was observed in the amylolytic activity of flour. The farinograph i.e. water absorption decreased from 58 to 56.0%, while stability increased gradually during storage. The above changes were negligible at 27° and 4°C. Pasting characteristics indicated increase in peak viscosity at all temperatures of storage, which was higher in flour stored at 37°C (580 AU) as compared to 27°C (390 AU) and 4°C (200 AU). The breakdown values also increased from 640 to 820, 860 and 990 AU for flour stored at 4, 27 and 37°C, respectively. The set back values considerably decreased in all the flour samples.

Wheat flour has been reported to undergo several functional changes during storage depending on the duration[1,2], moisture content[3] and the packaging material used[4]. The changes reported are with respect to physico-chemical characteristics[1,5,6], farinographic characteristics[2,7] and bread making quality[1,3,8]. Those studies indicated, deterioration in gluten quality, decrease in the extractable lipids and increase in the acidity and maltose value over a long storage period.

Larmour *et al.*[7] observed reduction in the farinographic water absorption, while Bell *et al.*[2] reported no change. Arya *et al.*[4], on the other hand, observed slight increase in water absorption in stored whole wheat meal. Fisher *et al.*[1] observed initial improvement in baking quality of stored flour but the bread volume decreased later as reported by Bell *et al.*[2] and Cuendet *et al.*[3]. No information is available on the pasting characteristics of stored wheat flour and the effect of storage temperature on various quality characteristics.

This study reports the changes in the pasting, rheological and bread making qualities of flour during storage at different temperatures.

Materials and Methods

Freshly milled refined wheat flour (maida) was procured from the International School of Milling Technology Mill of 20 M. ton capacity, located at the premises of the Institute.

Storage studies: Five kg lots of flour after fumigation were packed in airtight tin containers and stored at 4 (refrigerated) 27 and 37°C. Tin container was selected as the moisture changes during storage of flour were reported to be minimum[4]. The samples were drawn at monthly intervals and analysed for various quality characteristics.

Analytical methods: Estimation of moisture, gluten, colour, alcoholic acidity, diastatic activity, falling number and farinographic characteristics were carried out, as per the standard AACC methods[9]. Pasting characteristics of flour were determined using Brabender Amylograph as per the method of AACC[9] using 75 g flour. Amylograms were evaluated for gelatinisation temperature, peak viscosity (P), viscosity at 95°C and after holding time of 30 min at 95°C (H), viscosity after cooling to 50°C (C), breakdown (P-H) and setback values (C-P) as per the method of Bhattacharya and Sowbhagya[10]. Dough raising capacity of flours was determined as per the ISI methods[11].

Baking characteristics: Bread making quality of stored flours was assessed by using remix baking test[12] with the inclusion of 2 per cent fat and 0.5 per cent malt in the recipe. Sensory evaluation of breads was carried out by a panel of semi-trained judges for various crust and crumb characteristics. Loaf volume was measured in loaf volume meter using rapeseed displacement method. All the tests were carried out in triplicate and the average values are reported.

Results and Discussion

The flour used for the study had the following quality characteristics (expressed on 14 per cent moisture basis) moisture 12.80, dry gluten 10.02 per cent, sedimentation value 21 ml, colour grade value 3.1. The reasonably high value of gluten as well as sedimentation value indicated its suitability for breadmaking.

Physico-chemical characteristics: Physico-chemical characteristics of flour as affected by storage temperature over a period of 4 months are given in Table 1. The results

TABLE 1. EFFECT OF STORAGE AT DIFFERENT TEMPERATURES FOR FOUR MONTHS ON PHYSICO-CHEMICAL PROPERTIES OF FLOUR

Flour characteristics	0	Storage at 4°C				Storage at 27°C				Storage at 37°C			
		1	2	3	4	1	2	3	4	1	2	3	4
Moisture (%)	12.80	12.76	12.74	12.70	12.59	12.70	12.54	11.86	11.78	12.66	12.21	11.77	11.36
Dry gluten (%)	10.02	10.02	10.01	10.00	9.92	9.80	9.70	9.40	9.00	9.50	9.30	8.50	7.60
Colour grade value	3.10	3.10	3.20	3.10	3.10	3.40	3.80	3.90	3.90	3.60	4.10	4.30	4.40
Sedimentation value (ml)	21	21	20	20	19	19	19	18	18	19	18	17	17
Dough raising capacity (%)	140	—	140	—	130	130	125	—	115	120	120	—	77
Alcoholic acidity (% H_2SO_4)	0.06	—	—	0.06	0.06	—	0.07	0.09	0.12	0.08	—	0.10	0.15
Diastatic activity (mg maltose/10 g flour)	250	—	245	—	247	245	—	243	240	240	—	245	245
Falling no.	407	—	—	—	413	—	—	—	405	—	—	—	412

*All Values are expressed on 14% moisture basis

indicated a slight but gradual decrease in the moisture content on storage at 27° and 37°C but the extent was higher at 37°C. Arya *et al.*[4] in their studies on storage of *Atta* in tin containers observed negligible changes in moisture. Colour of flour as measured with Kent-Jones flour colour grader darkened with time and temperature of storage. Increase in colour value from 3.0 to 3.9 and 4.4 was observed after 4 months of storage at 27 and 37°C, respectively. This increase in colour grade value could be attributed to non-enzymatic browning, which depends on storage temperature and duration. However, no change in colour was observed when flour was stored at 4°C.

The gluten content and sedimentation value, remained unchanged upto 2 months at 27°C but thereafter slight deterioration was observed in gluten quality as well as quantity. The gluten content decreased from 10.0 to 7.6 per cent and sedimentation value from 21.0 to 17.0 ml, at the end of 4 months storage at 37°C. Similar decrease in gluten content, during storage of flour, was also observed by Fisher[1], Barton-Wright[5] and Yoneyama[6]. The deteriorative changes in flour stored at 37°C with respect to gluten are in line with the decrease in dough raising capacity from 140 to 77 per cent.

No change in the amylase activity was observed in the stored flour as indicated by insignificant change in Falling Number values and diastatic activity. However, Arya *et al.*[4] observed slight change in diastatic activity during storage of *atta*, for 1 year at 37°C.

Alcoholic acidity increased with increasing storage time and temperature. It increased 2 fold and approximately 3 fold after 4 months of storage at 27 and 37°C respectively. However, the values were below the maximum limit of 0.1 per cent specified under the Prevention of Food Adulteration Act. The increase in acidity during storage of flour was also reported by Larmour *et al.*[7] who observed a 3 fold increase in the fat acidity after 3 months of storage at 75°F.

Farinographic characteristics: Farinographic water absorption gradually decreased with storage period (Table 2). The decrease at the end of 4 months was 0, 1 and 2 per cent in flour stored at 4, 27 and 37°C respectively. Larmour *et al.*[7] also observed reduction in the farinograph water absorption for flour stored at 75°F for 5 years. On the contrary, Bell *et al.*[2] reported insignificant change during prolonged storage of 5 years at 12 and 25°C.

Stored flour recorded a gradual increase in dough stability and decrease in mixing tolerance index similar to the observations made by Bell *et al.*[2]. After 4 months of storage at 27 and 37°C the dough stability improved from 6 to 7 and 9 min, respectively. Storage at 4°C had no effect on the dough stability. Flour stored at 4 and 27°C nearly maintained its dough development time, while it decreased from 2.5 to 1.5 min at higher temperature of storage. Larmour *et al.*[7] also noticed decrease in dough development time, as storage progressed, while Bell *et al.*[2] observed changes in dough development time in an apparently random manner.

TABLE 2. EFFECT OF STORAGE AT DIFFERENT TEMPERATURES ON THE FARINOGRAPHIC CHARACTERISTICS OF FLOUR

Farinograph characteristics	0	Storage at 4°C				Storage at 27°C				Storage at 37°C			
		1	2	3	4	1	2	3	4	1	2	3	4
Water absorption (%)	58	58	58	58	58	58	57.4	57.4	57.0	57.4	57.2	56.8	56.0
Stability (min)	6	6	6	6	6	6	6	6.5	7.0	6.5	7	9	9
Dough development time (min)	2.5	2.5	2.5	2.5	2.0	2.5	2.0	2.5	2.0	2.5	2	1.5	1.5
Mixing tolerance index (BU)	40	40	50	40	40	54	40	20	20	40	40	20	10

Pasting characteristics: Gelatinization temperature of flour during 4 months of storage at different temperatures varied from 58.5 to 60°C in a random manner. Peak viscosity increased with temperature and duration of storage (Fig. 1) in accordance with the observations made by Yoneyama *et al.*[6] and Bell *et al.*[2]. It increased from 1780 to 1980, 2170 and 2360 AU after 4 months at 4, 27 and 37°C, respectively. Considering the insignificant change in α-amylase activity, the viscosity changes could be attributed to changes in starch during storage as earlier reported in case of rice flour[10]. Breakdown value increased with the increase in period and temperature of storage. (Fig. 2). Initial breakdown value of 640 AU increased to 820, 860 and 990 AU respectively for flours stored at 4, 27 and 37°C. This indicated that starch became more fragile during storage. Setback values followed a reverse trend. As the storage period progressed, the flour paste recorded gradual decrease in setback value (Fig. 3). The flour stored at 27°C had setback values of 580, 500, 390 and 300 AU after 1st, 2nd, 3rd and 4th month of storage, while paste from flour stored at 37°C

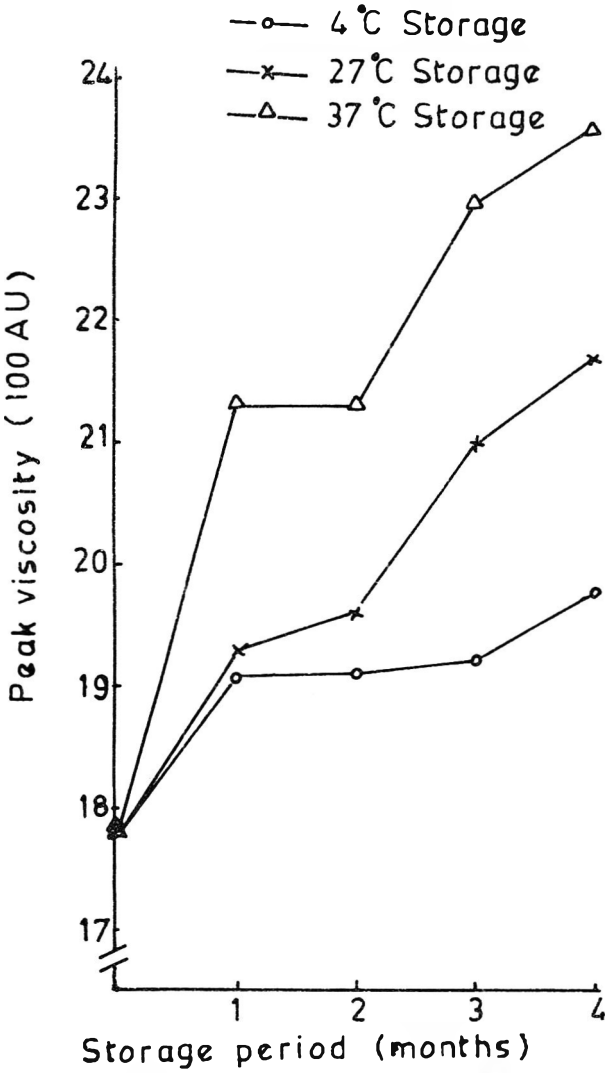

Fig. 1. Effect of storage on the peak viscosity of flour.

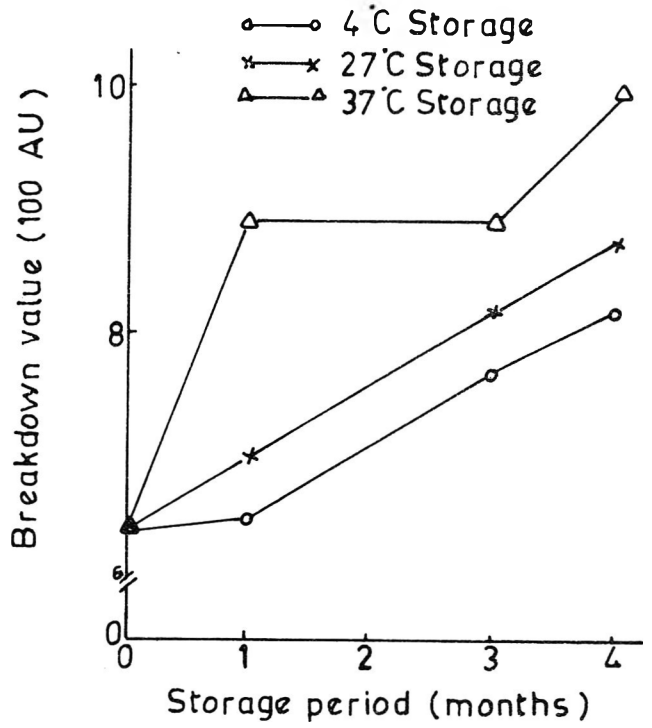

Fig. 2. Effect of storage on the Amylograph breakdown values of flour.

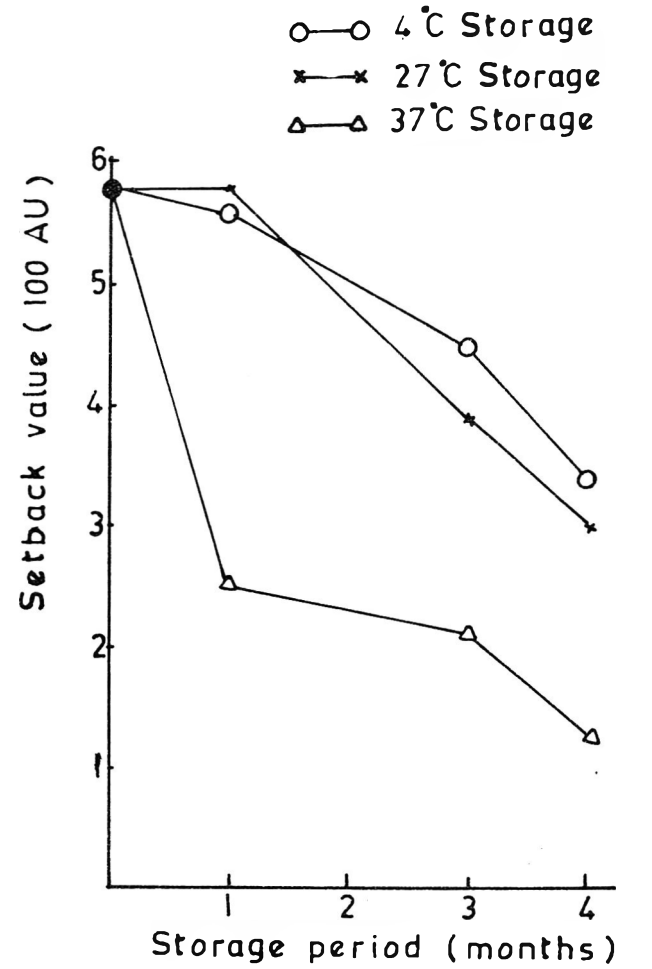

Fig. 3. Effect of storage on the Amylograph setback values of flour.

recorded still lower setback values of 250, 200, 210 and 120 AU respectively. This indicated lower retrogradation of starch in stored flours. Thus bread made from such flours is likely to stale at a slower rate, as it has been reported to be associated with retrogradation of starch[13].

Baking quality: Flour stored at 4°C recorded a gradual increase in specific volume, throughout the storage period, while improvement was observed only upto third month in flour stored at 27°C (Fig. 4). However, in flour, stored at 37°C, gradual reduction in specific volume was observed, with increase in storage time, McCalla *et al.*[8] also observed initial improvement in baking quality which maintained upto 22 months in flour stored in sealers at 2°C, while it deteriorated significantly within 3 months at room temperature. Fisher *et al.*[1], reported that the improvement in baking quality of flour during storage reaches the maximum after which progressive deterioration sets in. Cuendet *et al.*[3] have reported sharp decrease in loaf volume after 10 weeks storage of flour at 37.8°C. Deteriorative changes in baking quality at higher temperature of storage were reported by Larmour *et al.*[7] to be dependent on initial flour moisture.

The crust colour, symmetry and smoothness of loaf did not change during storage of flour except in the sample stored for 3 months at 37°C which produced pale brown crust colour and rough surface. Bread made from flour stored at 4 and 27°C maintained whitish crumb colour, soft texture with fine and uniform grain during the entire storage period while darker and harder crumb with denser and open grains was observed when bread was made from flour stored for more than 3 months at 37°C. Very little change was observed in the taste of bread.

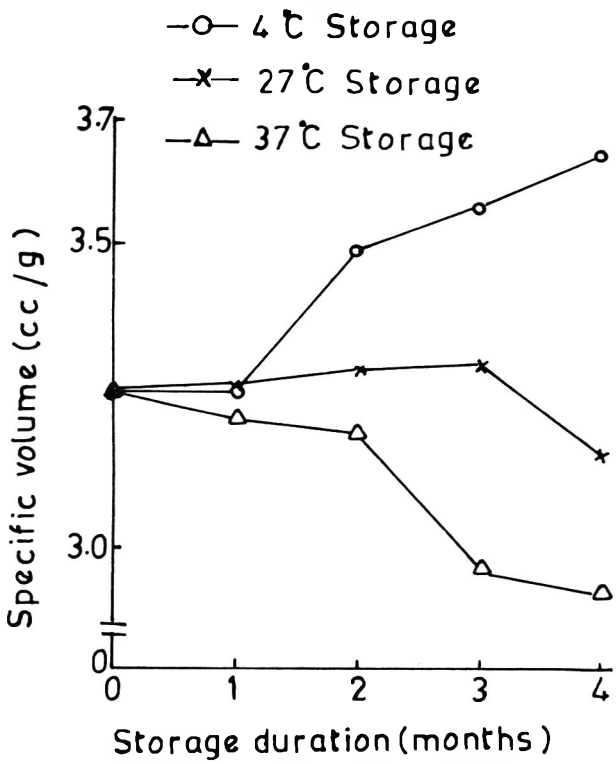

Fig. 4. Changes in bread specific volume made from stored flour.

In conclusion, the study indicated changes in flour quality stored at all temperatures of storage. Flour quality at 37°C deteriorated after 2 months of storage. This is relevant to the hot climate in many parts of our country. Results also indicated changes in wheat starch, during storage of flour and higher setback values observed indicate likely delay in staling of bread made from stored flours.

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Effect of Fat and Phosphate on the Quality of Raw and Precooked Buffalo Meat Patties

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Effects of buffalo fat, soybean oil and polyphosphate blend (PB) on quality of raw (emulsion) and precooked buffalo meat patties during refrigerated storage (2-3°C) were evaluated. The PB significantly increased emulsification of fat globules, emulsion stability, yield, moisture retention and sensory attributes of cooked patties. Soy bean oil was found to disperse in fine droplets whereas buffalo fat in relatively larger globules and encircled by thick protein matrix. The PB significantly stabilised the flavour of precooked patties for 18 days and for about a week in case of raw (emulsion) patties by inhibiting oxidative rancidity as indicated by lower TBA values. PB did not inhibit growth of bacteria during refrigerated storage of the products.

A major cause of deterioration in the quality of stored muscle foods is oxidative rancidity. Warmed over flavour in refrigerated cooked meat is attributed to lipid oxidation[1]. Polyphosphates decrease the rate of discolouration in fresh and processed meat products[2] and markedly reduce the rate of lipid oxidation in many cooked and frozen meat products[1,3,4]. Pre-blending of ground buffalo meat with salt and phosphate extended the functionality of meat and reduced the lipid oxidation during refrigerated storage[5]. Phosvitin content of egg yolk is reported to contain anti-oxidant activity in food systems[6].

Most of the buffalo meat is consumed in fresh form. In addition to the processed meat products, sale of preformed emulsion mix offers a great convenience to the meat consumers to prepare variety of products such as patties, meat balls, kababs, croquettes, etc. There is a paucity of information on the quality and storage stability of buffalo meat products. In the present study, effect of incorporation of soybean oil (control), soybean oil + phosphate blend, buffalo fat + whole egg liquid + phosphate blend on the quality of buffalo meat patties was compared and evaluated.

Materials and Methods

About 12 kg of lean meat from the round portion and 1.0 kg of pelvic and kidney fat of adult female buffalo carcasses of good finish were procured within 5 hr of slaughter. After chilling, the meat and fat at 2±1°C for 20 hr, they were passed through 8 mm plate of meat grinder. Similar meat sample was used in each of the four trials of the experiment. Refined soybean oil as fat source and whole egg liquid (WEL) from fresh eggs were used. Laboratory grade sodium chloride (salt) and a polyphosphate blend (PB) consisting of 65 per cent sodium pyrophosphate (SPP), anhydrous 17.5 per cent sodium tripolyphosphate and 17.5 per cent sodium acid pyrophosphate were used[7]. The combinations (in per cent) used were: (1) Soybean oil (control) : (2) Meat 85 and soybean oil 15 and (3) Meat 82, buffalo fat 15 and WEL 3. To the above combinations, are added (in per cent) salt 2, phosphate blend 0.5 (formulations 2 & 3 only), ice cold water 10, onions 3.5, garlic 0.5 and dry spice mix 1 while making the emulsion on the weight basis of meat and fat.

Processing: For each formulation, 4.2 kg emulsion was prepared in Hobart bowl chopper and 30 patties each weighing 80 g were hand moulded using petri dish (75 × 17 mm) and placed on perforated oven trays. They were cooked for 20 min in a preheated oven at 180°C to obtain an internal temperature of about 75°C. The patties were turned over after 10 min cooking. The internal temperature was recorded from atleast 3 patties using Wahl probe thermometer. The cooked patties were weighed individually and yield was expressed in percentage.

Three cooked patties were packed in a low density polyethylene (LDPE) bag and 7 such packages from each formulation were kept in a refrigerator (2-3°C). For raw patties, aliquots of 5 × 300 g emulsion from all the formulations packed in LDPE bags were stored in a refrigerator to make patties periodically for evaluation.

Composition of raw (emulsion) and cooked patties, emulsion stability (ES); sensory attributes and yield of cooked patties were determined. Further, they were evaluated for pH, 2-thiobarbituric acid values, aerobic plate count and sensory attributes at 3 days' interval upto 18th day.

pH values of the emulsion and patties were measured (ELICO pH meter Model LI-120) by homogenizing 10 g

sample with 50 ml distilled water. Moisture, protein and fat contents[8], 2-thiobarbituric acid (TBA value)[9] and aerobic plate count[10] at 30°C for 48 hr were determined. Emulsion stability was estimated by cooking emulsion samples (25 g) in LDPE bags at 80°C in a water bath for 20 min. After draining out the exudate, cooled samples were weighed and loss in weight was expressed as percentage. Stability of the emulsion was inversely related to its cooking loss. Samples of emulsion and cooked patties were evaluated histologically.

Patties made from the refrigerated stored emulsion and precooked were warmed in a preheated oven (80°C) prior to evaluation of their sensory attributes. They were tested by experienced panelists using 8-point structured scale wherein 8 = extremely desirable in appearance, flavour, juiciness, texture and overall palatability and devoid of mouth coating; 1 = extremely undesirable appearance, dry, crumbly texture and unpalatable with abundant mouth coating respectively. Warm patties were cut across their centres to obtain 8 similar size and shape (triangular) pieces from each patty and served to the taste panelists for evaluation. Although 4 trials were conducted, the storage stability of the products was evaluated in 3 trials. The data were subjected to analysis of variance and critical difference (CD) was calculated[11].

Results and Discussion

Addition of phosphate blend (PB) resulted in significant increase in ES as indicated by lower cooking loss, yield and moisture retention in cooked patties (Table 1) due to significant improvement in functional properties of meat by phosphate as found by other workers[7,12,13]. Even though raw emulsion composition was not different significantly, cooked patties containing soybean oil (SO) alone had significantly lower moisture and markedly lesser fat due to poor ES which has resulted in separation of moisture and fat during cooking. Raw emulsion containing PB had significantly greater pH (0.3). This is in accordance with the results of other researchers[7,12]. The increase in pH of cooked patties was about 0.25 in control and about 0.15 in phosphate containing products which is in agreement with the findings of Trout and Schmidt[12]. The pH of the raw and precooked patties increased from 5.83 to 6.02 and 6.02 to 6.13, respectively during refrigerated storage.

The shrivelled appearance and crumbly texture of control patties were due to poor emulsion stability and higher cooking loss. Histological studies of raw and cooked control patties also revealed the fat coalescence by continuous phase of fat with more aggregation of protein matrix. When soybean oil was incorporated along with phosphate, oil was dispersed in fine droplets in emulsion. However, buffalo fat was distributed in a relatively larger globules and encircled by thick protein matrix, and chances of emulsion breakdown and coalescence appeared to be relatively less. Addition of polyphosphate increased the solubility of protein to form fine protein matrix, fine fat dispersion and greater emulsification of fat globules thus contributing better appearance and smooth cohesive-texture to the product. These findings are in agreement with other workers[14,15].

TBA numbers were significantly lower in raw patties (emulsion) containing buffalo fat (BF), whole egg liquid (WEL) and PB followed by control than in patties containing SO and PB (Table 2). TBA numbers increased significantly with period of storage. Similar findings have been reported in beef, pork, and processed products[3,5,16]. However, changes in TBA numbers were non-significant during entire period of storage in the raw patties (emulsion) containing BF, WEL and PB. It might be due to highly saturated BF which is less prone to oxidation compared to soybean oil and antioxidant effects of added phosphate and phosvitin from egg yolk[6].

Precooked patties containing SO with PB had markedly lower TBA numbers (0.29 ± 0.05) than patties with SO alone (0.35 ± 0.06) but the differences were non-significant. The former had significantly ($P < 0.01$) better sensory scores for

TABLE 1. EFFECT OF FAT TYPE AND PHOSPHATE ON YIELD AND COMPOSITION OF BUFFALO MEAT PATTIES

Treatment	pH	Yield (%)	Emulsion stability (%)	Moisture (%)	Protein (%)	Fat (%)
Raw emulsion						
No. of samples	3	—	18	4	4	4
Soybean oil	5.63 ± 0.04[a]	—	27.20 ± 0.27	68.04 ± 0.27	15.46 ± 0.19	11.56 ± 0.22
Soybean oil +PB	5.92 ± 0.06[b]	—	8.70 ± 0.39[b]	67.57 ± 0.36	15.55 ± 0.06	11.88 ± 0.47
Buffalo fat +WEL +PB	5.94 ± 0.07[b]	—	9.70 ± 0.34[b]	68.08 ± 0.55	15.61 ± 0.19	11.31 ± 0.47
Cooked patties						
No. of observations	3	40	—	4	4	4
Soybean oil	5.88 ± 0.01[a]	74.80 ± 0.76[a]	—	58.17 ± 1.51[a]	19.25 ± 1.14	12.68 ± 0.14
Soybean oil +PB	6.07 ± 0.04[b]	91.50 ± 0.33[b]	—	63.51 ± 0.43[b]	17.05 ± 0.56	13.24 ± 1.17
Buffalo fat +WEL +PB	6.10 ± 0.03[b]	91.60 ± 0.28[b]	—	64.42 ± 0.74[b]	16.66 ± 0.33	14.04 ± 0.76

PB = Polyphosphates blend; WEL = Whole egg liquid.
Means with same superscript under each column do not differ significantly ($P < 0.01$).

TABLE 2. EFFECT OF FAT TYPE AND PHOSPHATE ON QUALITY OF RAW (EMULSION) AND PRECOOKED BUFFALO MEAT PATTIES DURING REFRIGERATED STORAGE

	Storage period (days)							
	0	3	6	9	12	15	18	Average
				TBA*				
Soybean oil	0.29^{a}	0.26^{a}	0.29^{a}	0.37^{a}	0.99^{b}	—	—	0.37 ± 0.09^{1}
Soybean oil + PB	0.32^{a}	0.25^{a}	0.58^{ab}	1.05^{b}	2.46^{c}			0.94 ± 0.24^{2}
Buffalo fat + WEL + PB	0.19	0.10	0.07	0.19	0.26			0.16 ± 0.03^{1}
Mean	0.27^{a} ± 0.06	0.20^{a} ± 0.06	0.31^{a} ± 0.12	0.53^{a} ± 0.11	1.16^{b} ± 0.38			
				Aerobic plate counts**				
Soybean oil	3.28	3.05	2.90	2.71	2.49	3.08	2.84	2.92 ± 0.07
Soybean oil + PB	3.16	2.85	2.76	2.73	2.59	2.96	2.79	2.84 ± 0.05
Buffalo fat + WEL + PB	3.09	2.89	2.71	2.66	2.60	3.05	2.64	2.81 ± 0.06
Mean	3.18^{a} ± 0.06	2.93^{ab} ± 0.06	2.79^{b} ± 0.08	2.73^{b} ± 0.08	2.56^{c} ± 0.06	3.03^{ad} ± 0.05	2.76^{b} ± 0.11	

PB = Polyphosphate blend; WEL = Whole egg liquid.
Means with the same superscript in each row (alphabets) in each column (numericals) do not differ significantly ($P \leq 0.05$).
* mg malonaldehyde/kg raw emulsion/patties ** log/g precooked patty.

flavour which decreased from 6.89 to 6.06 out of 8 point scale over a period of 18 days refrigerated (2-3°C) storage whereas the latter had acceptable sensory scores (5.64) only upto 9th day of storage. This has clearly shown that PB had retarded the rate of lipid oxidation and consequent development of warmed over flavour in precooked patties during refrigerated storage. Similar observations have been reported[1,4,5]. Precooked patties containing BF, WEL and PB had significantly lower TBA numbers (0.14±0.02) compared to other treatments and the trend was similar to that of raw patties (emulsion) during storage.

Irrespective of the storage period, the average aerobic plate counts (APC) were log 5.34±0.15, 5.46±0.18 and 5.50±0.18 for raw patties (emulsion) made with soybean oil (control), soybean oil + PB; and buffalo fat + whole egg liquid + PB respectively. There was marked increase of APC during refrigerated storage of raw emulsion. However, the growth of APC of precooked patties during storage was not consistent. Phosphate blend did not significantly retard the growth of bacteria during refrigerated storage of precooked patties (Table 2) which is in accordance with the findings of others[17,18].

Phosphate has significantly improved the sensory attributes of patties (Table 3) which is in accordance with the results of others[2,3,19]. In general, patties containing SO and PB were rated significantly better for sensory attributes than patties

TABLE 3. EFFECT OF FAT TYPE AND PHOSPHATE BLEND ON SENSORY ATTRIBUTES OF RAW AND PRECOOKED BUFFALO MEAT PATTIES DURING REFRIGERATED STORAGE

Sensory attributes*	Patties	Formulations		
		Soybean oil (Control)	Soybean oil + PB	Buffalo fat + WEL + PB
Appearance	Raw**	5.52 ± 0.09^{a}	6.99 ± 0.07^{b}	6.91 ± 0.06^{b}
	Precooked***	5.14 ± 0.08^{a}	6.91 ± 0.05^{b}	6.75 ± 0.06^{b}
Flavour	Raw	5.59 ± 0.13^{a}	6.29 ± 0.15^{b}	6.20 ± 0.11^{b}
	Precooked	5.29 ± 0.11^{a}	6.49 ± 0.06^{b}	6.17 ± 0.08^{c}
Juiciness	Raw	5.20 ± 0.09^{a}	6.67 ± 0.12^{b}	6.49 ± 0.08^{b}
	Precooked	4.90 ± 0.41^{a}	6.54 ± 0.06^{b}	6.28 ± 0.07^{c}
Texture	Raw	4.88 ± 0.10^{a}	6.84 ± 0.09^{b}	6.64 ± 0.07^{b}
	Precooked	4.68 ± 0.08^{a}	6.79 ± 0.06^{b}	6.49 ± 0.07^{c}
Overall acceptability	Raw	5.43 ± 0.13^{a}	6.48 ± 0.15^{b}	6.15 ± 0.11^{c}
	Precooked	4.99 ± 0.10^{a}	6.72 ± 0.07^{b}	6.13 ± 0.07^{c}

*Sensory scores: 8 = extremely desirable and 1 = extremely undesirable.
**Mean of 71 sensory scores of 3 trials and 4 periods of storage (0, 3, 6 and 9th day).
***Mean of 112 sensory scores of 3 trials and 7 periods of storage (0,3,6,9,12,15 & 18th day)
Means with the same superscript in each row do not differ significantly ($P < 0.05$).

containing BF. Sensory scores for flavour and overall palatability decreased significantly from 6.4 to 4.8; 6.3 to 4.8 based on 8 point scale respectively on 9th day of refrigerated storage of raw emulsion. Flavour scores for pre-cooked patties containing soybean oil alone decreased on 12th day of refrigerated storage with corresponding increase in TBA number. The flavour changes in precooked patties containing phosphate were non-significant during 18 days storage as reflected by small changes in TBA numbers. Tarladgis *et al.*[9] reported that TBA numbers were highly correlated with sensory scores of trained panelists for rancid odour in pork and found the threshold range of TBA numbers for detecting off-odour in pork as approximately 0.5 - 1.0.

This study indicated that PB has significantly improved the ES, yield, moisture retention and sensory attributes of cooked patties and inhibited the rate of oxidative deterioration in raw and precooked patties. The raw patties (emulsion) should be cooked preferably within 3-6 days of refrigerated (2-3°C) storage, whereas precooked patties containing PB were well acceptable upto 18 days of refrigerated storage.

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Toxicity of Peroxidized Oil and Role of Vitamins: Effect on Osmotic Fragility of Erythrocyte Membranes and Membrane Bound Enzymes

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Feeding of peroxidized oil (≃90 meq/kg) at 10% level has been found to suppress the growth, protein efficiency ratio and food efficiency ratio. It also increased osmotic fragility of erythrocytes leading to decreased haemoglobin. The activities of membrane bound enzymes like Na^+K^+ ATPase, and acetylcholine esterase were also altered. Supplementation of diets with extra doses of vitamin A (2500 IU), vitamin C (500 mg) or vitamin E (25 mg) per 100 g of the diet prevented the effects of peroxidized oil to a great extent. Vitamin A and vitamin E were more effective in this regard than vitamin C.

Oils and fats constitute one of the most important components of the diet. In addition to high calorific value, they supply essential fatty acids, carry fat soluble vitamins, and add flavour and texture to the food articles. Most food articles are consumed after cooking or frying in oil. Because of the high temperature used during frying or processing, the oils may undergo a number of undesirable chemical changes. The main adverse effects are denaturation of nutrients and development of lipid peroxides[1]. When such food articles are consumed, they may initiate the lipid peroxidation of membranes and finally alter the membrane function. Vitamins A, E or C have been reported to have protective role against lipid peroxidation[2-4] but their protective role as antioxidants has also been disputed in certain studies[5,6].

Thus, keeping in view the damaging role of peroxidized oils and the protective role of vitamins, the present study is made to compare the role of these vitamins in preventing the damaging effects of peroxidized oil.

Materials and Methods

Male albino rats (Wistar strain, 4 week old weighing about 35-42 g) were procured from the Small Animal Colony, Punjab Agricultural University, Ludhiana. The rats were housed individually and diet and water were given *ad libitum*. Groundnut oil was purchased from the local market and oxidized by irradiating with UV radiations for about 96 hr to achieve peroxide value of approximately 90 meq/kg using UV tube emitting light of 240-260 nm, the normal oil had peroxide value of approximately 8 meq/kg.

Rats were divided into 5 groups (A to E) of 5 animals each and were given the respective diets and water *ad libitum*. The basal diet consisted of starch (65 per cent, normal or peroxidized oil (10 per cent), casein (15 per cent), salt mixture[7] (4 per cent) and vitamin mixture[8] (1 per cent). Animals of group A received the basal diet containing normal oil and served as control. Animals of group B were given the diet containing peroxidized oil containing diets supplemented with vitamin A (2500 IU/100g), vitamin C (0.5 per cent), or vitamin E (0.025 per cent), respectively. The animals were sacrificed after 4 weeks of feeding. Their blood and tissue were removed for analysis.

The haemoglobin content and membrane fragility were determined by the method of Dacie and Lewis[9]. The erythrocyte membranes were prepared[10] and the activities of Na^+-K^+ ATPase[11] and acetylcholinesterase[12] were assayed. The results were compared statistically using student's 't' test.

Results and Discussion

The toxic effects of peroxidized oil are evident from the significantly lower growth of rats inspite of almost equal food intake and initial weights (Table 1). This decreased growth matches well with the reports of Yagi[1]. This could be due to the greater requirement of fats soluble vitamins[13] as feeding of peroxidized oil is reported to lower the absorption of vitamin A and also its storage in liver[14]. This is well supported by the observed lower degree of growth depressing effect of peroxidized oil on vitamin A or E supplementation. Even vitamin C prevented this effect to a considerable extent. Moreover, protein efficiency ratio (PER) and the food efficiency ratio (FER) significantly decreased with peroxidized oil feeding and vitamin fortification rectified the effect of peroxidized oil to a great extent. Peroxidized oil feeding also resulted in increased relative weight of liver ($p < 0.05$), heart ($p < 0.01$) and brain ($p < 0.05$). The weights of other organs were not affected significantly (Table 2). Supplementation of diets with vitamins prevented the increase in relative organ weights to a considerable extent. Increase

TABLE 1. GAIN IN BODY WEIGHT, PER AND FER VALUES IN RATS FED ON DIETS CONTAINING PEROXIDIZED OIL AND EFFECT OF VITAMIN SUPPLEMENTATION

Group	Initial wt (g)	Gain in body wt (g)	PER*	FER
Normal oil	37.25 ± 1.88	43.36 ± 3.55	2.50 ± 0.13	0.214 ± 0.021
Peroxidized oil	42.00 ± 1.74	29.81 ± 1.57[b]	1.85 ± 0.07[b]	0.168 ± 0.019
Peroxidized oil + Vit.A	38.33 ± 1.89	51.04 ± 4.05[d]	2.82 ± 0.12[d]	0.250 ± 0.008[d]
Peroxidized oil + Vit.C	40.25 ± 2.62	39.86 ± 2.28[c]	2.28 ± 0.25	0.204 ± 0.016
Peroxidized oil + Vit.E	41.00 ± 2.87	49.56 ± 5.06[c]	2.70 ± 0.33[b c]	0.237 ± 0.013[c]

Mean ± SEM
*Corrected to standard PER value of 2.5 for casein
Significantly different from normal oil group b $p < 0.01$
Significantly different from peroxidized oil group c $p < 0.05$ d $p < 0.01$

TABLE 2. RELATIVE ORGAN WEIGHTS OF RATS FED ON DIETS CONTAINING PEROXIDIZED GROUNDNUT OIL AND DIETS SUPPLEMENTED WITH VITAMINS

Group	Liver	Heart	Brain	Kidney	Spleen
Normal oil	3.35 ± 0.17	0.36 ± 0.01	1.38 ± 0.13	0.79 ± 0.05	0.24 ± 0.02
Peroxidized oil	4.47 ± 0.29[a]	0.49 ± 0.03[b]	1.77 ± 0.02[a]	0.91 ± 0.05	0.30 ± 0.02
Peroxidized oil + Vit.A	3.58 ± 0.19	0.45 ± 0.02[a]	1.48 ± 0.05[d]	0.91 ± 0.06	0.29 ± 0.02
Peroxidized oil + Vit.C	4.44 ± 0.23[a]	0.45 ± 0.02[a]	1.63 ± 0.23	0.89 ± 0.04	0.28 ± 0.02
Peroxidized oil + Vit.E	3.83 ± 0.28	0.42 ± 0.02	1.48 ± 0.03[d]	0.82 ± 0.06	0.25 ± 0.02

Mean ± SEM
*Significantly different from the normal oil group a $p < 0.05$ b $p < 0.01$
Significantly different from the peroxidized oil group c $p < 0.05$ d $p < 0.01$

in relative organ weights especially of liver and heart could be due to the accumulation of lipids[14,15]. As brain is a stable organ least affected by extraneous agents, slightly higher relative brain weight observed could be the result of decreased body weight of rats receiving peroxidized oil.

Feeding of peroxidized oil increased the osmotic fragility of erythrocyte membranes resulting in the decreased haemoglobin content of blood (Table 3). This effect was marginal or prevented altogether when the diets were supplemented with vitamin A, C or E (Table 3). The peroxidized oil feeding has been reported to result in the loss of phospholipids from erythrocytes[16] which can lead to the reduced membrane fluidity. This reduced fluidity might contribute to the peroxidative haemolysis of the erythrocyte. Protective role of vitamin E in erythrocyte haemolysis caused by peroxidation has also been reported by other workers[17,18]

Furthermore, the peroxidized oil feeding affected the activities of erythrocyte membrane bound enzyme acetylcholinesterase and Na^+—K^+ ATPase differentially. The activity of acetylcholinesterase decreased and that of Na^+—K^+ ATPase increased (Table 4). Membrane bound enzymes must possess the correct conformation and normal distribution in the membrane to perform its enzymatic function. The acetylcholinesterase is a peripheral protein[19] with its active site exposed to the outside[20]. The inhibition of acetylcholinesterase can be explained by considering the fact that it is a peripheral enzyme and is accessible to the lipid peroxides present in the plasma. Na^+—K^+ ATPase is

TABLE 3. HAEMOGLOBIN CONTENT AND OSMOTIC FRAGILITY OF ERYTHROCYTE MEMBRANES IN RATS FED ON DIETS CONTAINING PEROXIDISED OIL AND EFFECT OF SUPPLEMENTATION OF VITAMINS IN THE DIET

Group	Haemoglobin content (g %)	Osmotic fragility (% haemolysis)
Normal oil	13.45 ± 0.67	68.75 ± 2.72
Peroxidized oil	8.41 ± 0.63[b]	87.59 ± 2.45[b]
Peroxidized oil + Vit.A	10.22 ± 0.67[a]	65.37 ± 2.79[d]
Peroxidized oil + Vit.C	10.31 ± 0.38[b,c]	70.84 ± 3.08[c]
Peroxidized oil + Vit.E	11.01 ± 0.26[b,d]	60.60 ± 1.43[a d]

Significantly different from normal oil group
a $p<0.05$
b $p<0.01$
c $p<0.05$
d $p<0.01$
Significantly different from the peroxidized oil group

TABLE 4. ACTIVITIES OF ERYTHROCYTE MEMBRANE BOUND ENZYME (mU/mg PROTEIN) IN RATS FED DIETS CONTAINING PEROXIDIZED OIL AND DIETS SUPPLEMENTED WITH VITAMINS

Group	Acetylcholin-esterase	Na^+-K^+-ATPase
Normal oil	498.40 ± 4.05	24.43 ± 1.07
Peroxidized oil	438.80 ± 12.20[a]	31.16 ± 1.26[a]
Peroxidized oil + Vit.A	453.58 ± 12.30	26.03 ± 1.80
Peroxidized oil + Vit.C	449.61 ± 19.50	25.36 ± 0.61[c]
Peroxidized oil + Vit.E	487.52 ± 22.25	24.94 ± 1.00[c]

Significantly different from normal oil group a $p<0.05$
Significantly different from peroxidized oil group c $p<0.05$

an integral protein[19] with its active site exposed to the cytoplasmic side[21]. The enhanced activity of $Na^+—K^+$ ATPase could be due to the lipid modification in the environment of the enzyme. These effects of peroxidized oil were of considerably lower degree when the diets were supplemented with vitamins.

Thus, it can be concluded from the present study that peroxidized oil feeding causes growth depression, tissue enlargement and oxidative damage of erythrocyte membranes leading to the increased osmotic fragility and altered activities of erythrocyte membrane bound enzymes. The supplementation of vitamin A, C or E in the diet prevented the effect of peroxidized oil and vitamins A and E are more effective in preventing the damages caused by peroxidized oil, as compared to vitamin C.

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Successive Complexometric Estimation of Iron and Copper in Milk by Constant Potential Amperometry

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A rapid and sensitive procedure has been developed for the successive determination of micro quantities of iron and copper by directly titrating against (ethylenedinitrilo) tetracetic acid (EDTA) and by employing the technique of constant potential amperometry. The difference between the stability constants of the EDTA complexes of iron and copper at pH 2.0 is taken advantage of in this method and the first end point as shown by a steep and sudden fall in the meter reading is for iron and a second similar end point indicates copper. Estimations are carried out on trichloroacetic acid (TCA) serum of milk and the results of the recovery tests on the serum agree closely with the theoretical amounts added.

Many specific analytical techniques have been developed for use in the analysis of trace elements in milk and other foods, and of these, the two methods which have been extensively utilised and reported in recent times are atomic absorption spectrometry (AAS) as a single-element technique and neutron activation analyses (NAA) as a multi-element technique. Apart from these methods, the well established x-ray techniques and electrochemical techniques as well as chemical methods based on spectrophotometric determination of metal complex in solution are still used as standard methods for determination of trace elements in many laboratories[1].

The spectrophotometric method of Sweetser and Bricker[2] for the determination of iron and copper with EDTA was further modified by Underwood[3] for the simultaneous estimation of iron and copper with EDTA at a single pH 2.0, and wavelength 745 nm, taking advantage of the different stability constants of the metal EDTA complexes and their absorption coeffecients. Since biological materials like milk contain metals only in micro amounts, and of these, iron and copper are of considerable importance in dairy industry, it was felt desirable to evolve a rapid and sensitive method which would avoid the tedious dry ashing or wet ashing procedure requiring large amounts of mixtures of acids. Following Underwood's modified procedure[3], a method was developed where iron and copper were determined successively at pH 2.0 by directly titrating against EDTA by constant potential amperometry[4,5] using a transistorised titrimeter[5,6] and the results of the same are presented in this paper.

Materials and Methods

A transistorized titrimeter[5,6] was used. An a/c operated electronically stabilized "polarizer" source was used to apply the required constant potential across the platinum and silver amalgam electrodes[7]. The pH was measured using a Polymetron pH meter, type 42 B- Disodium salt of (ethylene dinitrilo) tetracetic acid, dihydrate, (EDTA) 0.001 N solution. This solution was prepared from a 0.1 N stock solution. Mercuric nitrate solution 0.004 M 1.38 g of analytical reagent mercuric nitrate monohydrate was dissolved in deionized water containing sufficient nitric acid to give a clear solution and diluted to 1 litre[8]. Trichloroacetic acid (TCA) 20 per cent, 200 g of analytical reagent TCA was dissolved in deionized water and made upto 1 litre.

Standard mixture solution of iron (III) and copper (II): A stock solution containing iron (0.2N) and copper (0.1N) prepared by dissolving 9.64 g of "Analar $Fe(NH_4)(SO_4)_2 12H_2O$ and 2.50 g of "Analar" $CuSO_4 5H_2O$, in 100 ml of deionised water. Other concentrations were prepared from the stock solution by appropriate dilution and a standard curve for the ml of EDTA against the amounts of the Fe (III) and Cu(II) was obtained.

TCA — milk serum: One hundred ml of whole milk previously heated to 50°C was taken in a stoppered conical flask, 50 ml of TCA (20 per cent) were added and boiled in a water bath with constant swirling for about 10 min. After cooling in ice water, the contents were filtered to remove fat and proteins and the first 10 ml portion of the filtrate was discarded[9,10].

Seventy five ml of the standard solution or the TCA serum obtained from milk, containing about 3.75 μ g of copper and 15 μ g of iron were taken in a 150 ml beaker and the initial 1.2 pH was adjusted to 2.0 by adding sufficient amount of 6N NaOH. The beaker was placed on a magnetic stirrer 0.2 ml of 0.004 M mercuric nitrate solution was added to increase

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the sensitivity of the end point[8] and the platinum and silver amalgam electrodes were dipped into the solution. The silver amalgam electrode was connected to the positive terminal and the platinum electrode to the negative terminal on the transistorized titrimeter and constant potential of 100 mv was applied to the electrodes from the "polarizer" unit. The solution was titrated against 0.001 N EDTA from a micro burette. At the first end point, when all the iron had been chelated, there was a sudden and steep fall in the meter reading. The titration was continued and again there was a similar sharp fall in the meter reading, at the second end point when all the copper had been chelated.

Results and Discussion

Twenty one samples obtained from Institute's Dairy Farm were analysed for iron and copper and the results are presented in Table 1. Recovery tests were performed on the milk samples, the results of which are presented in Table 2. Fig. 1 shows a typical curve for the titration of a ferric cupric mixture with EDTA. There was quite a large drop in the amount at each end point and the curve conformed to the theoretical considerations[5,11].

The first end point was for Fe (III) and the difference between the first and the second end points was for Cu (II). In each case, the volume of the titrant (EDTA) was multiplied by two to obtain the amount of the metal contained in 100 ml of the original sample.

Each ml of 0.001 N EDTA was equivalent to 27.93 μ g Fe (III) or 31.77 μ g Cu. (II).

TABLE 1. SUCCESSIVE COMPLEXOMETRIC ESTIMATION OF IRON AND COPPER IN COW AND BUFFALO MILK

Cow		Buffalo	
Iron (μg/l)	Copper (μg/l)	Iron (μg/l)	Copper (μg/l)
113.1	58.3	129.9	22.3
146.7	30.0	146.7	82.9
150.9	39.6	140.5	128.6
134.1	79.4	176.0	124.0
175.9	120.8	162.0	88.9
170.4	117.5	198.3	82.6
173.2	45.6	170.3	50.8
159.2	50.8	178.7	66.7
159.6	88.9	173.1	63.5
181.5	73.1	257.0	66.7
184.4	34.9	162.0	57.1
195.5	41.3	251.3	84.4
164.8	50.8	242.9	123.7
189.8	80.5	209.4	79.3
206.7	93.8	255.7	71.1
169.7	95.2	179.2	45.2
206.2	84.3	170.7	84.4
194.4	49.9	160.3	123.7
244.5	76.8	209.4	33.8
217.1	45.9	255.7	51.4
171.5	49.9	181.4	67.4

TABLE 2. RECOVERY TESTS

Iron (μg/l)			Copper (μg/l)		
Added	Found	Error	Added	Found	Error
100	93.6	6.4	8.58	8.47	1.98
100	96.2	3.8	6.70	6.40	4.48
104	104.5	0.48	6.50	6.40	1.54
25	25.4	1.60	6.52	6.36	2.46
25	25.3	1.20	1.90	2.10	10.50
25	24.9	0.40	1.63	1.43	10.50
25	24.0	4.00	1.63	1.70	4.29
Mean diff	1.4429		0.0857		
Std. Dev.	$\pm$ 2.6563		$\pm$ 0.1687		
"t" value	1.4367		1.3426		

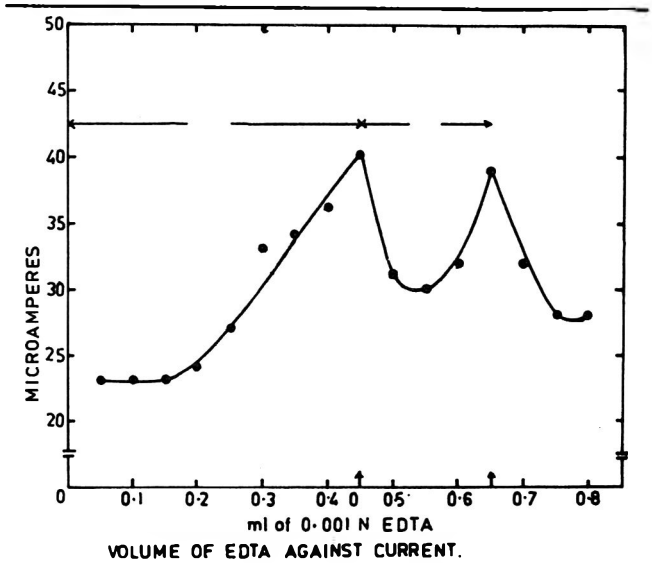

Fig. 1. A typical curve for the titration of ferric cupric mixture with EDTA.

Usually wet ashing procedure with sulphuric and perchloric acids is followed for the determination of iron and copper present in biological materials. Since this method has certain drawbacks and is beset with practical hazards, a clarification method using 20 per cent TCA for the removal of fat and proteins in milk has been proposed[9,10] and this method has been used in the present study with advantage.

The technique of constant potential amperometry[4] employed in the successive estimation of calcium and magnesium in a single sample[5] was successfully adopted in the present method (Tables 1 and 2). Previous values reported[12] for these metals in milk were copper 30-170 μ g/l, Iron about 300 μ g/l and the present values copper 30-95 μ g/l Iron 113 to 257 μ g/l agreed closely with the reported values. These estimations were compared with standard colorimetric methods for iron and copper and the values are reported in Table 3. The values obtained by the standard colorimetric methods are generally higher because of the inherent drawbacks mentioned earlier.

The fixing of the pH at 2.0 was quite satisfactory for the successive determination of copper and iron because the

TABLE 3. COMPARISON OF ELECTROTITRIMETRIC METHOD AND PHOTO COLORIMETRIC METHODS OF ANALYSIS FOR IRON AND COPPER IN MILK

Iron (μg in 1000 ml milk ashing)			Copper (μg in 1000 ml milk ashing)		
Electro titri-metric	Photo-colori-metric	Error (%)	Electro-titri-metric	Photo colori-metric	Error (%)
281.6	280.0	0.57	106.9	110.0	2.81
250.2	280.0	10.64	133.8	140.0	4.42
265.3	280.0	5.25	104.6	108.0	3.15
248.0	253.8	2.16	78.8	80.0	3.15
241.5	280.0	13.75	89.4	93.7	4.58
103.5	109.3	5.30	117.7	130.0	9.46
224.8	280.0	19.71	66.9	67.5	0.89
286.2	306.2	7.18	161.6	163.0	0.86
290.1	350.0	16.88	139.0	141.0	1.42
271.1	280.0	2.46	164.0	168.0	2.36
245.3	280.0	12.39	99.7	111.0	10.78
Mean diff	24.7	4.54			
Std. Dev.	± 20.65	± 3.93			
"t"	3.9674	3.8244			

difference in the stability constants of these two metal — EDTA complexes are quite large for convenient and sensitive detection of the respective end points. The applied constant potential of 100 mV was also sufficient for obtaining sharp deflections. The use of the combination of platinum electrode and silver amalgam electrode helped in getting sharp, stable and sensitive end points[4,5]. Though most of the EDTA titrations are accurate only for milligram amounts of the metals[13], the application of the constant potential amperometric technique enabled the determination of the metals Fe(III) and Cu(II) present in micro-quantities. The stability constants of EDTA - complexes of iron, copper and manganese differ sufficiently and so it is possible to determine these metals in presence of each other at different pHs. For example, iron has log K 25.1 at pH 2-3, copper has log K 18.4 at pH 5 and manganese has log K 13.5 at pH 10. The determinations of these trace elements are done both by EDTA - indicator and EDTA - amperometric method and comparable results have been obtained by both these methods.

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Manufacture of Lactose — Effect of Processing Parameters on Yield and Purity

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Method for the manufacture of lactose was evaluated using fresh paneer whey and deproteinized paneer whey. Deproteinized whey afforded higher recovery and purity of lactose. Deproteinized whey was concentrated to 65% total solids to obtain maximum yield. Intermediate cooling temperature of 60°C with a final cooling temperature of 30°C over a 12 hr period was necessary during crystallization. The product had a lactose content of 97%.

Whey represents approximately 80-85 per cent of the initial milk volume used for cheese, casein and paneer manufacture. It poses an effluent disposal problem as its biological oxygen demand (BOD) value ranges from 32,000 to 60,000 p.p.m. It contains approximately 6 per cent total solids of which more than 70 per cent is lactose and about 0.7 per cent are whey proteins. Thus, whey problem to a large extent, is a lactose problem. Manufacture of lactose from whey solves both the problems of improving economics of whey utilization and of pollution as lactose itself can reduce BOD of whey by about 70-80 per cent as compared to 20 per cent reduction by manufacture of whey protein concentrate alone[1].

Lactose has various applications in food and pharmaceutical industries due to its multiple functional properties[2]. The manufacture of lactose is basically a four-step process, i.e. deproteinization of whey, concentration, crystallization and recovery of crystals. Many problems have been encountered during concentration of whey in conventional evaporators. In order to overcome all these problems, an application of thin film scraped surface heat exchanger was suggested which is having unique performance characteristics[3].

The purity of recovered lactose depends on the degree to which proteins and salts are separated from whey. However, on the other hand, adding processing materials to clarify the whey may result in loss of lactose through occlusion with the extraneous matter being removed and added to the cost. Subsequently, it yields a less useful mother liquor for feed use[4]. Hence, a compromise has to be made between yield and purity of lactose while manufacturing.

This paper presents the effect of various parameters in the manufacture of lactose.

Materials and Methods

Fresh paneer whey was obtained from Experimental Dairy, NDRI, Karnal. It was separated to remove the residual fat and suspended particles using a cream separator. It was then deproteinized[5]. Forty kg of whey was taken for each trial. To standardize the method for the preparation of crude lactose, a basic general procedure as outlined in Fig. 1 was followed. Whey (fresh and deproteinized) was concentrated to 55, 65 and 75 per cent TS in thin film scraped surface heat exchanger (SSHE) employing the combination of various process variables.

Crystallization was done in a fabricated crystallizer of capacity 10 kg. The lactose in concentrated whey was allowed to crystallize at two cooling rates: (1) 4°C/hr and (2) intermediate cooling temperature of 60°C and final temperature of 30°C over a 12 hr period. The concentrated

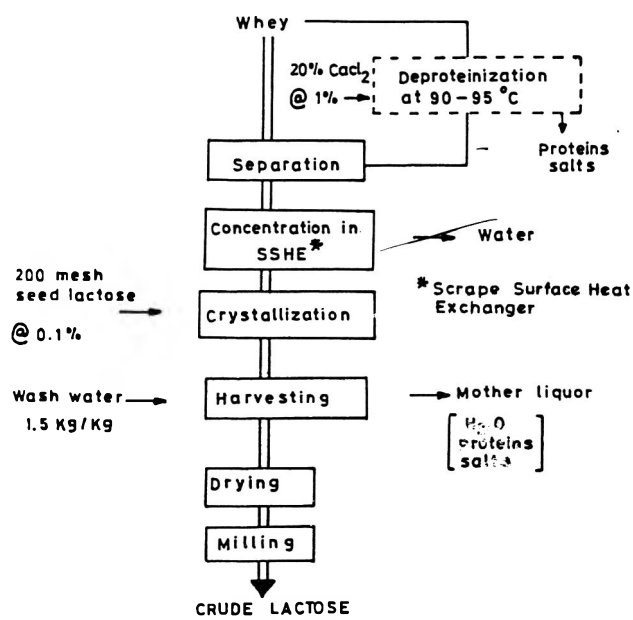

Fig.1 Flow Diagram For Crude Lactose Manufacture

whey was introduced in an insulated jacketted crystallizing tank to provide cooling rate of 4°C/hr. The temperature of whey was measured at 1 hr interval using sensors of digital temperature measuring device. When a temperature of 60°C was attained the seed lactose of 200 mesh size was added at the rate of 0.1 per cent of whey concentrate. The seed lactose was thoroughly mixed with the concentrate with the help of agitator provided with the crystallizing tank. The agitator was run at 10 r.p.m. for 5 min to ensure proper mixing. Crystallization was allowed to continue until a final temperature of 30°C was attained.

The mass of crystallized lactose was harvested at 4,000 r.p.m. for 15 min in a basket centrifuge with a cheese cloth lining. The harvested crystals were washed with 1.5 kg of water per kg of crystallized mass. Then, they were further subjected to centrifugation in the basket centrifuge at 4,000 r.p.m. for 10 min to drain off the washed water.

The washed lactose crystals were tray-dried at 70°C for 4 hr. They were then ground to 200 mesh and further subjected to tray-drying at 70°C for 2 hr to obtain the final crude lactose with less than 2 per cent moisture.

Analytical grade reagents were used for chemical analysis, unless stated otherwise. All determinations were made in duplicate. A standard solution of 0.1 N NaOH was used for titration with phenolphthalein indicator to determine acidity[6]. pH was determined at 20°C using Metroohm pH meter (ELTOP-3030) with combined electrode. Total solids were determined gravimetrically by drying at 100 ± 1°C for 3 hr[6]. Fat in whey was determined by Gerber method using skim milk butyrometer. Fat in crude lactose was determined by Mojonnier method[6]. Protein in whey was determined using the colorimetric method given by Lowry *et al*[7]. Lactose in whey was determined using the colorimetric method given by Ibrahim H. Abu Lehia[8]. Ash determination was done according to the method suggested by McKenzie[9]. The data on yield and quality of crude lactose were analysed using mixed factorial experimental design for each treatment combination with three replicates.

Results and Discussion

The mean composition of paneer whey (fresh, separated and deproteinized) used for the manufacture of crude lactose is presented in Table 1.

Effect of type of whey on yield and composition of crude lactose: The data pertaining to the effect of whey on the yield and composition of crude lactose are presented in Table 2. It is evident from Table 2 that the type of whey had a highly significant ($P < 0.01$) effect on the mean per cent recovery of lactose and mean per cent loss of lactose in mother liquor. It also had a highly significant ($P < 0.01$) effect on the protein, ash, lactose and acidity (per cent lactic acid) of the recovered lactose. However, it had no significant effect on the fat per cent of the recovered lactose.

TABLE 1 GROSS COMPOSITION OF PANEER WHEY

Type of paneer whey	Total solids (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
Fresh	6.75	0.25	0.45	5.42 (80.30)	0.63
Fresh separated	6.34	0.05	0.39	5.38 (84.86)	0.52
Deproteinized	6.00	0.05	0.09	5.39 (89.83)	0.47

*Average of 36 samples; ** Values in parentheses indicate per cent lactose on dry basis.

The higher lactose yield from deproteinized whey in comparison to fresh was also reported by Haken and Hartman[10]. This may be attributed to higher level of supersaturation of lactose in deproteinized whey in comparison to that in the fresh whey at the same level of concentration[11]. The extent to which protein and salts are removed from whey prior to concentration and crystallization largely determines the purity of lactose. Protein and salts result in contamination of the crystals during crystallization and cause additional difficulties. They greatly increase the viscosity of concentrated whey and this makes separation of the crystallization of lactose exceedingly difficult in extreme instances even preventing its crystallization[4,12]. The high mean protein content (on dry basis) of crude lactose obtained using fresh whey (2.57 per cent) in comparison to that obtained using deproteinized whey (0.92 per cent) may be attributed to the removal of proteins from whey by heat coagulation in deproteinized whey. The removal of certain degree of ash along with the protein in deproteinized whey might have resulted in a lower ash and higher lactose contents of crude lactose obtained from deproteinized whey (1.77 and 97.12 per cent respectively) in comparison to that obtained from fresh whey (2.33 and 94.91 per cent, respectively). Since separated whey was employed for lactose manufacture, the type of whey had no significant effect on average fat content (0.19 per cent) of crude lactose. The lower protein and mineral content of deproteinized whey in comparison to fresh whey might have resulted in corresponding lower protein and mineral contents in crude lactose and therby resulted in low acidity.

The analysis and foregoing discussions on the effect of type of whey on yield and purity of recovered lactose lead to the conclusion that deproteinized whey resulted in a greater recovery of crude lactose as well as higher purity in comparison to fresh whey.

Effect of level of concentration on yield and composition of crude lactose: The data pertaining to the effect of level of concentration on the yield and composition of crude lactose are presented in Table 3. It is evident that the level of

TABLE 2. EFFECT OF TYPE OF WHEY ON YIELD AND COMPOSITION OF RECOVERED LACTOSE

Type of whey	Yield (%)	Lactose loss in mother liquor (%)	Fat (%)	Protein (%)	Ash (%)	Lactose (%)	Acidity (% lactic acid)
Fresh	58.97	41.03	0.19	2.57	2.33	94.91	0.86
Deproteinized	63.85	36.15	0.19	0.92	1.77	97.12	0.71
F-value	129.53**	129.66**	0.28	8509.75**	503.63*	3889.05**	84.98**

** F value highly significant at 1 per cent level of significance.
Results are on % dry basis except yield and loss of lactose.

TABLE 3. EFFECT OF LEVEL OF CONCENTRATION ON YIELD AND COMPOSITION OF RECOVERED LACTOSE

Concn. level (%)	Yield (%)	Lactose loss in mother liquor (%)	Fat (% d.b)	Protein (% d.b)	Ash (% d.b)	Lactose (% d.b)	Acidity (% lactic acid d.b)
55	55.53	44.47	0.23	2.12	3.08	94.58	1.15
65	69.97	30.33	0.18	1.65	1.72	96.45	0.65
75	59.03	40.97	0.16	1.47	1.35	97.02	0.56
F-value	392.53*	392.60**	48.64**	466.53**	1811.09**	1748.63**	514.47**

* F value significant at 5 per cent level of significance.
** F value highly significant at 1 per cent level of significance.

concentration had a highly significant ($P < 0.01$) effect on the yield and composition of lactose. The mean per cent recovery of lactose crystals increased with increase in concentration from 55.53 per cent at 55 per cent total solids to 69.67 per cent at 65 per cent total solids. Increase in the concentration of total solids from 65 to 75 per cent resulted in a drop in the mean lactose yield to 59.03 per cent. This may be attributed to the decrease in mean crystal size with increase in total solid content, resulting in greater losses in the mother liquor during harvesting of lactose crystals[13]. The decreasing trend observed in fat, protein, ash and acidity, and an increasing trend in the lactose with increasing concentration might be the result of formation of agglomerates of the lactose crystals at 55 per cent level of concentration. These agglomerates entrapped impurities during crystallization and thus were difficult to wash and the wash water used could not remove the impurities to the desired extent[13].

The above study on the effect of level of concentration on the yield and composition of recovered lactose leads to the conclusion that concentrating whey to 65 per cent total solids (TS) gave a higher yield whereas a higher degree of purity of crude lactose was obtained when it was concentrated to 75 per cent TS.

Effect of cooling rate on yield and composition of recovered lactose: The data pertaining to the effect of cooling rate on the yield and composition of crude lactose are given in Table 4. It is evident that the cooling rate employed had a highly significant ($P < 0.01$) effect on protein and lactose and a significant ($P < 0.05$) effect on ash and acidity of the recovered lactose. However, it had no significant effect on the fat of recovered crude lactose. Employing cooling rate of 40°C/hr gave a lower mean lactose recovery of 58.58 per cent in comparison to 64.24 per cent recovery obtained on employing intermediate cooling temperature of 60°C and final cooling

TABLE 4. EFFECT OF COOLING RATE ON THE YIELD AND COMPOSITION OF RECOVERED LACTOSE

Concn. level (%)	Yield (%)	Lactose loss in mother liquor (%)	Fat (% d.b)	Protein (% d.b)	Ash (% d.b)	Lactose (% d.b)	Acidity (% lactic acid d.b)
4°C/hr	58.58	41.42	0.19	1.71	2.02	96.08	0.77
Intermediate	64.24	35.76	0.19	1.78	2.08	95.95	0.81
F-vaiue	173.52**	173.55**	0.77	15.40**	5.95*	14.75**	7.07*

* F value significant at 5 per cent level of significance;
** F value highly significant at 1 per cent level of significance.

TABLE 5. ANOVA TABLE FOR YIELD AND COMPOSITION OF CRUDE LACTOSE

Source of variation	d.f.	Yield	% loss of lactose in mother liquor (%)	Fat (%)	Protein (%)	Ash (%)	Lactose (%)	Acidity (as % lactic acid)	pH
Replicates	2	2.69	2.69	0.001	0.009	0.001	0.0106	0.004	0.000
Type of whey (A)	1	214.76**	214.77**	0.000	24.371**	2.745**	43.604**	0.198	0.027**
Level of concn (B)	2	650.83	650.84**	0.017**	1.336**	9.870**	19.606**	1.199*	0.014**
Cooling rate (C)	1	280.70**	287.69**	0.000	0.044**	0.032*	0.1654**	0.016	0.007**
Interactions:									
A × B	2	33.22**	33.21**	0.000	0.729**	0.418*	2.222**	0.014**	0.002*
A × C	1	5.18	5.17	0.000	0.019*	0.037*	0.113**	0.001	0.003*
B × C	2	19.86**	19.86**	0.001	0.016*	0.081**	0.072*	0.001	0.002*
A × B × C	2	10.15**	10.15**	0.001	0.009	0.060**	0.085**	0.000	0.001
Error	22	1.66	1.66	0.000	0.003	0.005	0.0112	0.002	0.000

*F value significant at 5% level of significance; ** F value highly significant at 1% level of significance.

temperature of 30°C over a 12 hr period (intermediate cooling rate). This may be attributed to increase in the growth rate of the crystals on increasing the temperature[14] and production of large lactose crystals on employing gradual temperature reduction, which are easy to separate by centrifugation[15].

Thus, it can be inferred that the effect of cooling rate on yield and composition of recovered lactose, employing intermediate cooling rate, resulted in higher recovery of crude lactose. Whereas on employing 4°C/hr cooling rate, a high degree of purity of crude lactose should be obtained. The samples of crude lactose prepared using a combination of different treatments had a mean moisture content of 0.98 per cent (range 0.79 to 1.23 per cent). The samples of mother liquor obtained by using a combination of different treatments had a mean pH of 5.47 (range 5.4 to 5.59) which is well above the standard pH of more than 5.4. The statistical analysis of all variables is shown in Table 5.

The foregoing discussions lead to the conclusion that in order to obtain maximum yield of lactose, deproteinized whey having a concentration of 65 per cent total solids and intermediate cooling rate should be used for lactose manufacture.

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FATTY ACID COMPOSITION OF HIMACHAL OLIVE OIL

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Oleic acid, palmitic acid and linoleic acid were the major fatty acids in the oil of all the olive cultivars. The content of oleic acid ranged from 68.6 - 75.5, palmitic acid, 10.2 - 18.5 and linoleic acid, 3.9 - 13.2%.

Olive oil is used for edible purposes and application on human skin. It is also known to have some medicinal properties[1]. Olive oil is characterized by the presence of high percentage of oleic acid (upto 93 per cent) in the natural lipid fraction[2]. Some Spanish and Italian cultivars of olive have been introduced in Himachal Pradesh during 1976. Since not much information is available on fatty acid composition of olives grown in India, an attempt has, therefore, been made to study the fatty acid composition of olive oil from seven cultivars of olive grown in Himachal Pradesh.

Seven cultivars of olive (*Olea europaea* cv. 'Cornicobra', 'Ascoitrena', 'Pendulino', 'Frantoio', 'Corotina', 'Leccino' and 'Alagandeu'), growing in the orchards of the Department of Fruit Culture and Orchard Management, Solan were selected based on their yield potentiality and performance under the agro-climatic conditions of Himachal Pradesh. Fruits were harvested at fully ripe stage. Horticultural practices like pruning and manuring were done at regular intervals.

One hundred g. fruits of each variety were dried in an air oven at 70°C and the moisture-free oil was extracted in Soxhlet apparatus using petroleum ether (40-60°C). Fatty acid methyl esters were prepared by the method of Luddy *et al.*[3] and analysed following the method of Ahuja *et al.*[4] by gas-liquid chromatography using an AIMIL 5700 Series gas chromatograph with flame ionization detector having 6 mm × 2 m column packed with 15% (W/W) diethylene glycol succinate (DEGS) on 80 to 100 mesh chromosorb W. The instrument was operated at 190°C with a N_2 flow of 60 ml/min and H_2 flow of 30 ml/min. The peak area and the relative percentage of each fatty acid were calculated with data processor Chromatopac Model EIA. Determinations were carried out in triplicate and the data were analysed statistically.

Table 1 shows the fatty acid composition of olive oil of different cultivars on percentage relative weight basis. Data show that all the cultivars contained only palmitic, stearic, palmitoleic, oleic and linoleic acids. Among the saturated fatty acids, highest proportion was that of palmitic acid followed by stearic acid. Olive oil showed varietal differences with respect to palmitic acid and stearic acid. Eckey[5] has reported 1.5 - 3.3 per cent stearic acid in the olive oil. Gracian[2] reported that oil obtained from Tunisia and Spain had relatively higher palmitic acid than stearic acid.

Oleic acid was the major fatty acid followed by linoleic and palmitoleic acids, among unsaturated fatty acids. All samples contained a small percentage of higher fatty acids.

Oleic acid range in different oil samples from Italy, Greece, Spain, Argentina, Tunisia, and California was 63-80, 57-93, 65-79, 54-79, 55-70 and 62-83 per cent, respectively[2]. The range of oleic acid in Himachal olive oil was relatively narrower. However, Raina *et al.*[6] have reported oleic acid ranging from 10.19 - 39.30 per cent in Himachal olive oil. The Codex[7] limits for principal fatty acids in olive oil are oleic 56-83 per cent, palmitic 7-20 per cent and linoleic 3-20 per cent.

The results presented in this paper indicate that the olive oil obtained from Himachal olives had low linoleic and palmitic acids and high oleic acid content. Therefore, elaborate plans can be made for commercial exploitation of this crop.

TABLE 1. FATTY ACID COMPOSITION OF OLIVE OIL

Cultivar	< 16 carbon	Palmitic (16:0)	Palmitoleic (16:1)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	> 20 carbon
Cornicobra	0.2	15.8	3.0	1.5	71.5	5.2	1.6
Ascoitrena	0.1	14.4	2.3	1.7	75.5	3.9	1.9
Pendulino	0.1	18.5	0.8	1.0	72.1	4.6	1.7
Frantoio	0.1	12.9	1.9	1.1	68.6	13.2	2.2
Corotina	0.1	14.2	0.6	1.5	72.6	10.1	1.5
Leccino	0.1	10.2	1.3	1.3	73.3	12.5	1.2
Alagandeu	0.2	15.9	—	1.1	75.1	6.6	1.1
CD at p = 0.05		0.227	0.325	0.197	1.225	0.762	0.548

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VARIATION IN QUALITY TRAITS OF PIGEON PEA (*CAJANUS CAJAN* L. MILL SP.) VARIETIES

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Physico-chemical composition and nutritional quality of six promising varieties of pigeon pea were investigated. The ranges of values were : test weight 66.10-73.50g, dhal in grain 66.45-68.50%, yield 13.95 - 21.85 q/ha, moisture 10.00-11.00%, protein 18.20-19.80%, protein yield 2.54-4.33 q/ha, digestibility of proteins 32.10-38.10%, fat 1.19-1.37%, total carbohydrates 65.88-67.32%, energy 312.30-326.75 kcal, tryptophan 0.37-0.46g/16g N, tryptophan yield 0.94-1.99 kg/ha, methionine 0.98-1.10g/16gN, methionine yield 2.49-4.63 kg/ha, chemical score, 29.17-32.74%, biological value 57.28-59.38, total minerals 2.30-2.95%, calcium 115.05-122.30 mg/100g, iron 8.00-9.20 mg/100g, available iron 1.90-3.41 mg/100g, ascorbic acid 2.35-3.40 mg/100g, vitamin A 108.00-118.70 I.U., thiamine 0.40-0.47 mg/100g and riboflavin 0.09-0.12 mg/100g. 'Type-21' was found to be superior over other varieties analysed.

Pulses are known to form the important sources of protein and other dietary constituents in Indian diet. Information regarding the quality constituent of the pulses is of great importance to breeders, agronomists, nutritionists and consumers. Literature evidence indicates varietal variations in nutrient composition of foodstuffs[1]. Pigeon pea is one of the important pulse crops of India. However, detailed systematic information on biochemical constituents and biological value of pigeon pea varieties is still scanty. The present communication describes the variations in nutritional attributes of promising pigeon pea varieties of the State.

Seeds of six pigeon pea varieties, namely, 'Type-21', 'Pusa Ageti', 'Prabhat', 'Type-17', 'Type-7' and 'UPAS-120', were obtained, from G.P.B. Farm. Four replicate samples were selected from each variety and all the determinations were carried out in duplicate. After observing their colour, size, test weight and moisture, they were cleaned and dehusked and ground to powder to pass through 40 mesh sieve. Dhal in grain was found out in relation to the total weight of grains.

Moisture content was calculated by subtracting dry weight from fresh weight and expressed as per cent of fresh weight. The methods as described in A.O.A.C.[2] were used to analyse protein, fat, ascorbic acid, total minerals, vitamin A and thiamine contents. Digestibility of proteins was determined by the method of Akeson and Stachman[3]. Total carbohydrates were obtained by difference[4]. Riboflavin was estimated according to De Ritter[5]. Iron and calcium were analysed as described by Ranganna[6]. The *in vitro* method of Narasinga Rao and Prabhavathi[7] was followed to assess the available iron. The method of Spies and Chambers[8] was followed for tryptophan whereas methionine was estimated by following the procedure of Horn *et al.*[9] Chemical score and biological value of methionine were computed by the method of Block and Mitchell[10] and Rao and Subramanian[11], respectively. Yield data were recorded for calculating protein, tryptophan and methionine yield.

Data presented in Table 1 show slight variations in colour and size of grains (seeds). There was a significant difference in test weight and yield of these varieties. However, there was no significant variation in percentage of dhal in grain. Test weight, yield and percentage of dhal in grain were found to be the highest with 'Type-21' and lowest with 'Prabhat' variety.

Considerable differences were observed in all the constituents except moisture, thiamine and riboflavin. Protein and protein yield were significantly high in 'Type-21'. The per cent digestibility of proteins exhibited appreciable variations ranging from 32.10 to 38.10 among the six varieties. The digestibility of proteins was found to be significantly low (32.10 per cent) in variety 'Type-7'. The highest values for fat (1.37 per cent) and energy (326.75 Kcal.) were observed in variety 'Type-21' having less (65.88 per cent) carbohydrate.

Amino acids like tryptophan and methionine were also analysed because the quality of protein depends on adequate amino acid make up[12]. Tryptophan and methionine contents were highest in 'Type-21' and 'UPAS-120', respectively, while yield of these amino acids was maximum in 'Type-21' variety. Both chemical score and biological value were found highest in 'UPAS-120' followed by 'Type-21'.

Total mineral contents of varieties varied from 2.30 to 2.95 per cent. Significant variations in calcium, iron and available iron were recorded in pigeon pea varieties and their highest value was observed in 'Type-21'. The total iron content of any food is relatively of little importance in nutrition as it does not reflect the actual amount of physiologically available iron. Therefore, rating the food as a source of iron, on the basis of available iron is considered as better index[13] and our findings are expressed in the light of this fact.

Variety 'UPAS-120' showed maximum ascorbic acid content (3.40 mg/100g), while vitamin A was found to be highest (118.70 I.U.) in 'Type-21'.

It is clear from these observations as a whole that wide varietal variations exist in physico-chemical composition and quality of the six tested varieties of pigeon pea and variety 'Type-21' emerged nutritionally excelling over others.

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TABLE 1. PHYSICO-CHEMICAL COMPOSITION AND QUALITY OF PROMISING PIGEON PEA VARIETIES

Traits	Type-21	Pusa Ageti	Prabhat	Type-17	Type-7	UPAS-120	C.D. at 5%
1	2	3	4	5	6	7	8
Seed colour	Light brown	Dark brown	Light brown	Light brown	Brown	Light brown	—
Seed size	Medium bold	Medium bold	Small	Medium bold	Medium bold	Medium	—
Test wt (g)	73.50	71.60	66.10	70.50	72.10	68.70	1.05
Yield (q/ha)	21.85	19.25	13.95	16.99	15.50	18.10	1.03
Dhal in grain (%)	68.50	66.70	66.45	67.20	67.50	67.90	N.S.
Moisture (%)	10.00	10.60	11.00	10.40	10.20	10.20	N.S.
Protein (%)	19.80	18.40	18.20	18.80	19.10	19.50	0.05
Protein yield (q/ha)	4.33	3.54	2.54	3.19	2.96	3.53	0.08
Protein digestibility (%)	38.10	33.70	37.25	36.90	32.10	37.60	1.60
Fat (%)	1.37	1.23	1.19	1.26	1.30	1.34	0.02
Total carbohydrates (%)	65.88	67.32	67.31	66.91	66.70	66.06	0.30
Energy (%) (Kcal.)	326.75	314.74	312.30	317.48	319.91	323.16	0.91
Tryptophan (g/16gN)	0.46	0.38	0.37	0.43	0.39	0.43	0.01
Tryptophan yield (kg/ha)	1.99	1.35	0.94	1.37	1.16	1.52	0.03
Methionine (g/16g N)	1.07	1.01	0.98	1.03	0.99	1.10	0.01
Methionine yield (kg/ha)	4.63	3.58	2.49	3.29	2.93	3.88	0.07
Chemical score (%)	31.85	30.06	29.17	30.65	29.46	32.74	—
Biological value	58.79	57.66	57.09	58.03	57.28	59.38	—
Total minerals (%)	2.95	2.45	2.30	2.63	2.70	2.90	0.03
Calcium (mg/100 g)	122.30	116.10	115.05	117.30	118.60	120.20	0.64
Iron (mg/100 g)	9.20	8.00	8.25	8.40	8.60	8.95	0.08
Available iron (mg/100g)	3.41	2.50	3.17	2.95	1.90	3.29	0.05
Ascorbic acid (mg(100g)	3.10	2.41	2.35	2.70	2.94	3.40	0.04
Vitamin A (I.U.)	118.70	110.40	108.00	111.80	115.80	117.40	1.01
Thiamine (mg/100g)	0.47	0.40	0.42	0.43	0.42	0.44	N.S.
Riboflavin (mg/100g)	0.12	0.09	0.10	0.09	0.10	0.11	N.S.

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STUDIES ON NITROGEN REQUIREMENTS OF SOME LIGNICOLOUS EDIBLE FLESHY FUNGI OF NORTH EASTERN HILL INDIA

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Four edible fleshy fungi namely *Pleurotus djamor, P. platypus Lentinus sajor-caju* and *L. connatus* were studied for their utilization of nitrogen compounds. Among the inorganic nitrogen sources, *Pleurotus* and *Lentinus* spp. preferred tri-ammonium citrate and ammonium tartrate respectively whereas urea and peptone supported the maximum mycelial production among the organic sources.

Edible species of *Pleurotus* and *Lentinus* occur quite commonly in the forests of North Eastern Hill States of India and very much preferred by local people. In an attempt to standardize their artificial cultivation, their physiological and nutritional requirements were studied. In the present communication, requirement and utilization of nitrogen sources by two species each of *Pleurotus* and *Lentinus* viz *Pleurotus djamor* (Fr.) Boedjin, *P. platypus* (Cooke & Massee) Sacc, *Lentinus sajor-caju* (Fr.) and *L. connatus* Berk are being reported.

Twenty three nitrogen sources (16 organic and 7 inorganic) were tested singly for their effect on the mycelial growth of the test fungi using modified Humfeld and Sugihara[1] medium. The pH of the medium was adjusted to 6.0 and 5.5 for *Pleurotus* and *Lentinus* spp. respectively. A series with no nitrogen source served as control. All the N-sources were sterilized by steaming for 30 min for 3 consecutive days. The culture flasks inoculated with 3mm diameter mycelial discs were incubated for 15 days as still cultures at 25 ± 1°C for *Pleurotus* spp. and 30 ± 1°C for *Lentinus* spp. before the mycelial mats were harvested, dried and final pH of the culture filtrates were recorded.

The data in Table 1 indicate that the test fungi preferred different nitrogen sources for their maximum biomass production. Among inorganic nitrogen sources both *Pleurotus* and *Lentinus* spp. attained maximum growth on ammoniacal nitrogen. Tri-ammonium citrate was the best for the *Pleurotus* spp. and ammonium tartrate for *Lentinus* spp. This is in conformity with the findings of several earlier workers on other mushroom species[2,3]. Ammonium chloride supported only poor growth of all the test fungi. Similar findings have also been reported for *P. flabellatus*[4] and *P. sajor-caju*[5]. Nitrates of calcium and magnesium supported moderate growth of all the test fungi, the least in case of *L. connatus*. Nitrate nitrogen utilization by different edible fungi has been reported by earlier workers also[6,7]. However, sodium nitrite was better N source than nitrate for *Morchella* esculenta[6]. In the present study, three test fungi failed to grow on nitrites, and *L. connatus* could make only feeble growth on sodium nitrite which is in agreement with earlier reports on *Macrolepiota procera*[8]. The growth inhibition of the test fungi may be attributed to the toxic effect of nitrite at acidic pH[9].

Among the various organic sources of nitrogen tried, urea was the most preferred source for *Pleurotus patypus* and *L. sajor-caju* while peptone was the best for *P. djamor* and *L. connatus*. Peptone was the second best source for the other two fungi viz. *P. platypus* and *L. sajor-caju*. These findings are in general agreement with the earlier reports on certain other edible mushrooms[1,8,10]. Among the amino acids, L. asparagin supported good to moderate mycelial growth of all the test fungi while in other amino acids different test species performed differently. Similar utilization of organic nitrogen sources have also been reported in case of *P. ostreatus*[11] and *P. sajor-caju*[5] and other mushroom species[3]. Tryptophan was unable to support good mycelial growth of *P. djamor, P. platypus* and *L. sajor-caju* although it supported good growth of *L. connatus*. Similar good growth with tryptophan was also reported for species of *Lepista, Cantharellus, Pleurotus*, and *Volvariella*[12]. None of the test fungi could make any growth in nitrogen free medium which confirmed that the test fungi lack the activity to fix the atmospheric nitrogen. This is contrary to various earlier reports[13,14]. The final pH after incubation indicated that there was a general lowering of pH by all the test fungi with *L. sajor-caju* making the maximum shift. Only in a few cases like utilization of urea by *P. djamor* and calcium nitrate by *P. platypus*, the pH was changed to alkaline side. Different growth response of the mushroom species to different organic and inorganic nitrogen sources may be attributed to factors like hydrogen ion concentration, presence of trace elements and aeration besides appropriate enzyme system which together determine whether a particular nitrogen compound can be utilized or not.

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TABLE 1. EFFECT OF DIFFERENT SOURCES OF NITROGEN ON THE MYCELIAL GROWTH OF *PLEUROTUS* AND *LENTINUS* SPECIES.

Source	*P. djamor*		*P. platypus*		*L. sajor-caju*		*L. connatus*	
	Mycelial dry wt. (mg)	Final pH	Mycelial dry wt. (mg)	Final pH	Mycelial dry wt. (mg)	Final pH	Mycelial dry wt. (mg)	Final pH
L. asparagine	44.5	5.1	37.8	4.1	48.3	5.9	55.3	4.5
Leucine	17.3	4.8	12.0	4.4	49.8	3.3	25.3	4.5
Glutamine	21.3	4.9	13.8	4.3	10.5	4.1	24.3	4.6
Alanine	27.0	4.8	11.3	4.5	55.3	3.5	14.3	4.3
Aspartic acid	24.8	5.8	33.3	5.2	x	4.7	31.0	5.3
Methionine	25.5	5.1	4.5	5.1	13.3	3.4	16.3	4.2
Cystine	23.5	4.4	13.3	4.1	27.8	2.8	23.0	3.7
Tryptophan	x	5.8	x	4.9	x	4.2	33.0	6.1
Lysine	29.0	4.8	28.0	4.4	12,3	2.3	16.0	3.6
Serine	11.5	4.9	7.8	3.9	13.3	3.5	11.8	4.4
Casein	15.8	4.2	42.8	4.7	14.3	3.1	24.5	4.2
Phenylalanine	26.3	5.1	34.3	5.1	50.8	3.3	16.0	4.2
Valine	4.8	62.2	4.2	27.0	3.2	21.03	4.2	
Histidine	21.8	4.1	32.3	5.1	24.3	4.4	33.80	4.1
Urea	61.5	7.5	134.8	4.9	134.5	5.3	38.00	5.0
Peptone	67.3	4.4	85.0	4.1	114.3	3.4	83.00	3.9
Tri. ammonium citrate	77.0	4.4	115.3	4.1	56.0	5.2	42.50	5.1
Ammonium tartrate	52.3	4.6	71.5	4.2	147.8	4.9	76.80	5.7
Ammonium chloride	14.0	3.5	13.0	3.1	8.8	2.9	16.00	3.6
KNO_3	38.0	5.1	x	5.1	19.8	4.1	51.00	5.0
$Ca(NO_3)_2$	45.5	5.2	96.3	7.3	112.0	6.1	22.30	4.8
$Mg(NO_3)_2$	28.5	4.3	76.3	5.1	116.3	4.2	23.50	4.3
$NaNO_2$	x	6.1	x	6.1	x	6.0	8.00	7.3
Control (no. N)	x	x	4.6	4.6	x	4.4	x	5.7
S.E.	1.26		1.39		1.59		1.48	
C.D. at 5%	2.49		2.75		3.15		2.94	

X — trace growth

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PRODUCTION OF 5-HYDROXYMETHYL-2-FURFURAL DURING PREPARATION OF KHOA

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The extent of browning in khoa was determined by estimation of 5-hydroxymethyl -2-furfural (HMF) during preparation of khoa. The contents of both free as well as potential HMF increased correspondingly with total solids. The rate of increase in HMF content was higher in Karahi khoa than in steam pan khoa.

Browning is a common phenomen noticed in foods during processing and storage. Maillard type of browning and caramelization are the two important types of browning reactions encountered in dairy products such as milk powder, evaporated milks and indigenous milk products like Kulfi, Khoa, etc., Khoa being the most conspicuously browned milk product amongst all. The extent of browning can be estimated by stable intermediate compound 5-hydroxymethyl-2- furfural (HMF). Several workers consider estimation of HMF as a measure of browning in heat treated milk products[1-4]. A study on production of HMF during preparation of khoa is reported in this paper.

Cow's milk obtained from the college herd was used to prepare khoa, Milk (2L) was desiccated with constant stirring over a Karahi or steam pan by controlling the heat source[5]. Samples were drawn at equal intervals from positive pat stage and analysed for free and potential HMF content. The method of Keeney and Bassette[6] for estimating HMF in milk was modified to suit for Khoa. Sample corresponding to one g dry matter weight was taken for analysis and the values were expressed as micromoles per 100 g dry matter weight of khoa. Total solids in the samples were estimated by ISI method[7].

Considerable amount of HMF was produced during preparation of khoa. The production of both free and potential HMF increased proportionately with the increase in total solids in both karahi and steam pan made khoa (Table 1). rate of increase in free and potential HMF with respect to total solids was maximum in khoa samples taken out just before partially dried khoa like material (Pat) formation. The rate of increase in HMF content was higher in karahi made khoa than in that of steam pan made khoa for the same amount of total solids content. The increase in potential HMF was higher than that of free HMF at all stages. The proportion of free HMF to potential HMF increased with increase in total solids, particularly above 60 per cent.

TABLE 1. INCREASE IN THE HMF CONTENT DURING PREPARATION OF KHOA

Total solids (%)	Potential HMF (μ/100g)	Free HMF (μm/100 g)	Potential HMF (%)
	Steam pan made khoa		
11.25	9.50	2.21	22.30
30.50	11.50	2.60	22.60
37.00	12.41	2.85	22.96
44.90	14.09	2.98	21.14
49.63	15.35	3.27	21.30
53.09	16.67	3.71	22.25
59.32	18.46	4.56	24.70
65.51	21.25	6.17	29.03
70.14	24.16	7.82	32.36
70.80	26.69	9.55	36.78
74.85	29.22	11.39	38.98
78.92	32.20	11.70	36.33
——— r = 0.86* ———			
———— r = 0.93* ————			
	Karahi made khoa		
11.25	9.50	2.12	22.31
31.56	15.35	2.32	15.11
36.52	16.89	2.69	15.92
46.72	18.76	3.23	17.21
47.16	19.93	3.75	18.81
51.33	21.38	5.26	24.60
57.15	23.35	6.53	27.96
62.00	26.25	8.26	31.46
65.76	28.62	9.19	32.11
69.78	32.89	11.37	34.56
74.15	35.92	12.52	34.85
79.00	39.12	13.69	35.35
——— r = 0.91* ———			
———— r = 0.96* ————			

*Significant at 1% level.

A linear relationship Y=0.21 × +5.585 for steam pan and Y=0.33x +4.990 for karahi, where Y=HMF content, x=total solids was derived. However, beyond 60 per cent of total solids, the increase in HMF was rapid. The HMF estimated in khoa was very much higher when compared to that estimated in any other dairy products, such as UHT milk[4] (23.5 MM/l) and dried skim milk powder[4] (8.74 MM/m)[3].

The potential HMF is the amount of intermediary compound which can give rise to HMF during browning reaction. Hence, both potential as well as free HMF increased

during the preparation of khoa. Higher HMF content in khoa as compared to other dairy products could be attributed to the higher heat treatment and processing conditions such as open pan heating and continuous stirring which together reduce the inhibitory effect of free sulphydryls on browning as incorporation of oxygen during stirring oxidises free sulphydryls. The HMF at a given percentage of total solids was less in steam pan made khoa as compared to karahi made khoa. This can be attributed to higher heat treatment and longer time in karahi because the heat source in karahi is limited to the bottom of the pan, whereas in case of steam pan, heat is provided along the complete surface of the jacketted pan. This provides higher activation energy for HMF accumulation in karahi[8,9]. Moreover, direct contact of super saturated solution of lactose with hot surface of karahi could enhance lactose caramelization resulting in the formation of HMF[10].

It was also observed that the HMF increased in skim milk powder and sterilized milk with added iron[5]. Incorporation of as much as 103 p.p.m. iron was noticed during stirring and scrapping in the preparation of khoa[6].

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EFFECT OF REHEATING AND STORAGE ON PHYSICO-CHEMICAL CONSTITUENTS OF PALMOLEIN OIL

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The effect of household method of discontinuing batch type operations involving heating-storing-reheating process on the physico-chemical characteristics of palmolein oil and the organoleptic acceptability of the food products with initial low and high moisture contents were determined. There was a slight increase in FFA, refractive index, and specific gravity but no change in Lovibond colour units and iodine value. The products fried in heated-stored-reheated oil were highly acceptable even after third frying.

The changes occurring in oils on the physico-chemical constituents due to reheating the same sample either continuously or intermittently, singly or in combination with one or more oils have been studied extensively[1-3]. Continuous replenishment of absorbed oil to attain steady state during frying at the rate of 8 per cent per hr has been expressed in mathematical terms[4]. But none of these experiments has taken into consideration the dilution effect combined with short term storage adopted by Indian housewives on the extent of chemical changes in frying oils, especially so on palmolein oil which is commonly consumed at present. The present study, therefore, was attempted to simulate the home conditions that prevail in Indian households with regard to reheating and storing of palmolein oil and determine the:

1. changes in the physico-chemical characteristics,
2. differences in these changes, if any, when food material with low and high moisture contents were fried and
3. organoleptic acceptability of the food products fried in reheated-stored oil.

The refined bleached and deodourised (RBD) palmolein oil used in the present study was procured from one of the ration shops in Anantapur. The quantity of oil used was 500 ml of fresh oil for frying a known quantity of "Vadiams" (V) and "Chips" (P) the low and high moisture foods, respectively. "Vadiams" are steamed, extruded and dehydrated cereal foods that are preserved for more than a year and deep-fried in oil as and when required. "Chips" are freshly sliced potatoes deep-fried in oil. This process of frying was repeated three times using the left over oil with a gap of three days during which time the used oils was stored in plastic containers separately (V and P). Every time the quantity of oil in the frying pan was kept constant (500 ml) by replenishing with fresh oil. Care was taken to use the same pan (thoroughly cleaned and dried after use) for frying in order to keep the size, shape and the metallic base constant throughout the experiment.

Palmolein oil samples were analysed after each frying for specific gravity, refractive index, Lovibond colour units, free fatty acids and peroxide value[5]. Viscosity was measured by the method of Lewkowitsch[6], and moisture content and iodine value by AOCS[7].

Sensory evaluation of the fried products was done on a score card developed on the basis of a 5 point Hedonic rating scale by a panel of trained judges to assess the organoleptic acceptability of the products. The same panelists aged 20 to 35 years participated in testing the fried products V and P throughout the study. The scores assigned represented: Excellent-5, Good-4, Fair-3, Poor-2 and Unacceptable-1.

All the analyses were done in duplicate and the average values have been reported.

Table 1 gives the combined effect of heating and storing on the physical constituents of palmolein oil. The specific gravity of oil showed slight increase from 0.909 to 0.913 at the end of third frying. But there was no difference between the oils used for frying V and P. There was a simultaneous increase in the refractive index and viscosity regardless of the moisture content in the food material fried. Viscosity and refractive index increase with an increase in the average chain length of fatty acids in triglycerides.

The Lovibond colour units increased after the third frying from 12.6 to 15.0 and 13.0 to 15.5 units in oil of V and P, respectively. Though change in colour depends on the nature of the pigments present or formed, plain heating of groundnut oil and ghee with no food being fried showed a gradual discolouration or browning of oil[8].

The moisture content of oil V (low moisture product) as expected, was found to decrease on repeated heating while that of P (high moisture product) showed an increase especially after the third frying.

Free fatty acid contents increased significantly with the progressive stages as in Table 2. But the absolute values of 0.193/0.194 per cent were still much lower than that of the ghee. Further, there was no difference in oils used for frying

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TABLE 1. EFFECT OF HEATING AND STORING ON PHYSICAL CONSTITUENTS OF PALMOLEIN OIL

No. of fryings	Sp. gr. at 30°C		Ref. index at 40°C		Lovibond colour*		Viscosity (Redwood sec)		Moisture (%)	
	V	P	V	P	V	P	V	P	V	P
I	0.909	0.090	1.460	1.450	12.900	12.900	230	230	0.100	0.100
II	0.908	0.911	1.462	1.460	12.900	12.400	220	248	0.090	0.100
III	0.911	0.911	1.468	1.472	12.600	13.000	258	250	0.080	0.120
IV	0.913	0.912	1.473	1.477	15.000	15.500	258	260	0.060	0.250

*1" cell Y+5R, V=Vadiams; P = Chips.
The values are the means of three replicates
Maximum variation among replicates was <5% of the mean value

TABLE 2. COMBINED EFFECT OF HEATING AND STORING ON CHEMICAL CONSTITUENTS OF PALMOLEIN OIL

Frying stages	FFA (as % oleic acid)		Iodine value (Wijs)		PV (meq O_2/100g of sample)	
	Vadiams	Chips	Vadiams	Chips	Vadiams	Chips
I	0.104 ± 0.002	0.104 ± 0.002	56.92 ± 1.08	56.92 ± 1.08	10.40 ± 0.05	10.45 ± 0.02
II	0.187 ± 0.004	0.183 ± 0.017	55.68 ± 0.94	55.38 ± 0.96	6.80 ± 0.62	3.55 ± 0.02
III	0.193 ± 0.001	0.194 ± 0.004	55.18 ± 0.28	55.15 ± 2.15	2.40 ± 0.20	1.17 ± 0.04
IV	—	—	55.16 ± 0.54	55.15 ± 1.00	2.05 ± 0.07	2.20 ± 0.04

V and P. Mohammed *et al.*[9] who studied the chemical changes in mixtures of palm and soybean oils during deep-fat-frying found an increase in free fatty acids due to hydrolysis.

A very slight decrease in iodine value was observed in oils of V and P indicating that the degree of change in unsaturation of fatty acids is quite small even after the third heating.

Peroxide value that measures the amount of peroxides and volatile hydroperoxides present in the samples, was found to be 10.4 meq O_2/1000g of fresh palmolein oil and contrary to the expectations, lowered due to heating and storing. Such a decrease in peroxide value of Sunflower seed oil has been reported by Sulthana and Sen[10]. Khan *et al.*[11] reported a widely different peroxide values for refined groundnut oil. Gomathy[12] has also found a gradual decrease after an initial increase in the peroxide value of palmolein oil heated for 8-24 hr.

Sensory evaluation scores of the products fried in reheated-stored-palmolein oil showed that the products are highly acceptable even after the third frying. The mean scores ascribed for each of the characteristics by the panel of judges were between 4 and 5 indicating that the products were rated as 'good'. But interestingly, the scores obtained for products fried in fresh palmolein oil were also in the range of 3.6 to 4.7 for different sensory attributes. There was no difference between the products (low moisture and high moisture foods) with regard to the sensory evaluation scores ascribed by the panelists.

Thus, the present study indicated that the method of diluting-reheating-storing palmolein oil employed in Indian households does not cause any change in the physico-chemical characteristics of oil or organoleptic acceptability of the products fried in it even after third reuse.

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EFFECT OF UREA ON 7S FRACTION OF SOYBEAN

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The viscosity, gel-filtration and UV difference spectra studies showed that the 7S fraction of soybean dissociates or unfolds at 5M urea concentration. Further dissociation of 7S protein was observed at 8M urea concentration by gel-filtration and viscosity studies and even at this urea concentration, the protein is still not found to be in completely random-coil conformation.

Soybean consists of about 40 per cent protein and the major storage proteins of soybean are 7S (β-conglycinin) and 11S (glycinin) globulins. As the data on the effect of denaturants on purified 7S fraction are meagre, this study was undertaken to find out the effect of urea on gel-filtration pattern, ultra-violet difference spectra and reduced viscosity of soybean 7S protein (β-conglycinin).

The seeds of soybean (*Glycine max* cv. Bragg) were procured from Department of Plant Breeding of this University. The crude extract of 7S fraction from defatted soybean meal was prepared by the method of Thanh and Shibasaki[1] based on differential solubilities of the 7S and 11S globulins in dilute Tris buffer at pH 6.6. Since the preparation was not completely free of 11S fraction, the purification of this crude extract was carried out by differential ammonium sulphate fractionation, adopting the method of Roberts and Briggs[2] as most of the soybean 11S component is removed by fractionation with 55 per cent ammonium sulphate saturation. The 55-100 per cent ammonium sulphate precipitated 7S fraction was dispersed in standard buffer (32.5 mM K_2HPO_4, 2.6 mM KH_2PO_4, 0.4M NaCl, pH 7.6, 10 mM 2-mercaptoethanol) and purified by gel-filtration technique[3] on a 2×50 cm column of Sepharose-6B. A single peak was obtained by repeating gel filtration with Sepharose-6B. The purified sample was then completely desalted by dialysis against distilled water. This 7S fraction was equivalent to β-conglycinin. Protein concentration was measured by measuring the absorbance at 280 nm on Beckman DU-7 spectrophotometer. The purified 7S fraction was found to be homogeneous by polyacrylamide gel electrophoresis[4,5] and was used for these studies. The effect of urea was studied using the following techniques.

Gel filtration: Sepharose-6B (Agarose gel) which had been equilibrated with 0.1M phosphate buffer (pH 7.6) containing 0.4M NaCl was packed into a column (2×50 cm). The protein (100 mg) solution was loaded to the column. When the experiment was performed in the presence of urea, the column was equilibrated with buffer containing the appropriate concentration of urea and eluting buffer also contained urea. Fractions (5 ml) were collected and absorbance was measured at 280 nm.

Viscosity: The viscosity measurements were carried out in a Ubbelohde viscometer having a flow time of 288 sec with the phosphate buffer. The flow time of 7S fraction was measured in the presence of different concentrations of urea.

UV difference spectra: Difference spectra produced by the addition of different amounts of urea to 7S fraction against identical protein concentration were recorded in the range of 270 to 310 nm on a Beckman DU-7 spectrophotometer.

Effect of urea: Reduced viscosity of the protein solution (1.5 per cent) remained almost unaltered upto 2M urea concentration (Table 1). Then, a steady increase in reduced viscosity observed between 2M and 4M urea was followed by a sharp increase for 5M urea concentration. The maximum value (15.08 ml/g) was observed in the presence of 8M urea. Tanford[6] has reported a reduced viscosity of 23 ml/g for various proteins in completely random coil conformation. In the present results, a plateau was not observed for reduced viscosity even at 8M urea concentration, suggesting thereby a partial random coil conformation at this concentration.

On the basis of sedimentation analysis of acid precipitated (pH 4.8) 7S fraction of soybean, Roberts and Briggs[2] reported that this component dissociates to slower sedimenting forms, probably 1S-3S, in the presence of urea concentration greater than 4M. Thus, it appears that the sharp increase in reduced viscosity in the presence of 5M urea may arise from dissociation of 7S.

A positive difference spectra with minor troughs at 278 nm, 285 nm and 296 nm were observed in the presence of 1M

TABLE 1. EFFECT OF UREA ON REDUCED VISCOSITY AND GEL-FILTRATION PATTERN OF 7S FRACTION

Urea concn (M)	Ve/Vo peak		Reduced viscosity at 25°C (ml/g)
	Major	Minor	
0	1.47	—	5.23
1	1.47	—	5.36
2	1.47	0.93	5.40
3	1.47	0.80, 2.0	6.21
4	1.47	1.80	6.67
5	1.47	0.87, 1.60	10.77
6	1.47	0.80, 1.60	11.63
7	1.47	1.66, 1.80	11.89
8	1.27	1.60, 1.73, 2.13	15.08

*Dept. of Chemistry.

to 4M urea. Compared to 1M urea, the difference spectrum was less positive in the presence of 2M urea. The difference spectrum shifts from positive to negative at urea concentrations greater or equal to 5M, with a predominant trough at 293 nm in addition to minor trough at 278 nm and 285 nm. This suggests that 7S fraction is highly dissociated in the presence of 5M urea. With further increase in urea concentration to 8M, the intensity of trough at 293 nm increased, indicating further unfolding or dissociation of 7S fraction. The trough at 279 nm originates mainly from the exposure of tyrosine residue where the trough at 293 nm is due to the exposure of tryptophan residue and one at 285 nm originates from both tyrosine and tryptophan residues[7]. In the present study, the predominant trough at 293 nm in the presence of 5M to 8M urea indicates the perturbation of tryptophan residue in the 7S protein. Catsimpoolas *et al.*[8] have reported on the basis of ultraviolet difference spectrum that both tyrosine and tryptophan residues are exposed by treatment of soybean 11S protein with different concentrations of urea and 6M urea caused irreversible and complete dissociation of the 11S protein into subunits.

Ve/Vo (elution volume/void volume) value was 1.47 for native protein in the absence of urea (Table 1). In the presence of 2M urea, the position of the major peak at 1.47 remained unaltered while a minor peak appeared at Ve/Vo of 0.93 which suggests initiation of aggregation of 7S fraction. Two minor peaks at Ve/Vo of 0.8 and 2.0 were observed in addition to major peak at 3M urea concentration. In the presence of 4M urea, the peak with Ve/Vo = 0.8 disappeared whereas the additional peak at Ve/Vo = 1.8 indicated dissociation of aggregated fraction. Again at 6M urea concentration, minor peaks at Ve/Vo = 0.8 and 1.6 were observed along with major peak, suggesting partial aggregation of protein species. At 8M urea concentration, the major peak shifted to Ve/Vo of 1.27 and three other minor peaks were observed at Ve/Vo 1.60, 1.73 and 2.13 which suggest the dissociation of 7S fraction.

In conclusion, the concerted use of techniques like viscosity, gel-filtration and difference spectra suggest dissociation of 7S fraction of soybean at urea concentration greater than 5M.

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CHEMICAL STUDIES ON THE NUTRITIONAL VALUE OF *LAUNAEA CORNUTA* — A WILD LEAFY VEGETABLE*

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***Launaea cornuta*, a wild leafy vegetable is widely eaten by people on coastal Tanzania. Chemical analyses of *Launaea* leaves indicated high levels of protein, crude fibre, vitamin C, Na, K, Ca and Fe. The vegetable was devoid of phytic acid. In general, young and mature leaves did not differ much in their chemical constituents. Cooking the leaves (steaming or boiling) results in the loss of some minerals and vitamin C. During storage, the content of vitamin C decreased along with the loss in moisture. Phytochemical screening indicated that most of the phytoconstituents are water soluble.**

Green leafy vegetables in general, form rich sources of vitamin C,[1-4] minerals,[1,3,5] and protein[4-6]. *Launaea cornuta* Oliv. & Hiern C. Jeffrey (Asteraceae) is a widely used wild leafy vegetable by people on the coast of Tanzania. When used for culinary purpose, the leaves of *L. cornuta* are boiled and the water discarded and the procedure is repeated atleast two times mainly to reduce its bitter taste. The vegetable is then mixed with other ingredients for serving. Even when it is not required for immediate use, it is boiled and the water discarded and then sundried for future use.

Although leafy vegetables are widely eaten in Tanzania, relatively little information is available on the chemical composition of wild leafy vegetables.[4,7] The present work is aimed at determining the chemical composition of *Launaea* leaves and the effect of cooking. Phytochemical screening was carried out so as to get some idea of the chemical nature of the bitter principles present in *Launaea*.

Plant material for analyses was collected in the field normally between 9.00 and 10.00 a.m. and brought to the laboratory in poythene bags. Young (third fully formed leaf from apex) and mature (leaves lower than the third leaf from apex but not including old leaves) leaves were used for analyses.

Cooking was done either by boiling the leaves with excess water (and the cooked water discarded) or by means of improvised steaming method till the leaves are cooked.

Improvised steaming method for cooking: A liberal amount of water was heated in an aluminium pot to boiling. Then, a wire mesh of a slightly bigger diameter than the rim of the pot was put in place and plant material placed on top of the first one, mouth to mouth. A small weight was placed on top of the pots so that the system was almost steam-tight.

Moisture was determined by drying a known weight of fresh leaves at 102°C for 24 hr in an oven. The oven dried material was ashed in a porcelain crucible in muffle furnace at 500°-600°C for 16-20 hr. Total N was determined by the macrokjeldahl's method. The crude protein was calculated using the factor 6.25. Crude fat was determined by the NCFA method[8]. Carbohydrate content was calculated by difference. Iron content was estimated using method number 40-41 by AACC[9]. The contents of Na, K and Ca were estimated using EEL flame photometer.

The phosphorus and phytic acid contents were determined by the NCFA method[10]. Vitamin C was determined by the AOAC method[11]. The method described by Das and Bhattacharjee[12] was used for phytochemical screening. All the analyses were done in triplicate and the average values along with standard errors are reported in the text.

From the results given in Table 1, it is clear that both young and mature leaves of *Launaea* have similar composition. However, potassium content was lower while that of vitamin C was higher in the mature leaves than in the young ones. The ash contents of young and mature leaves were quite high suggesting that both are rich sources of minerals. This is

TABLE 1. CHEMICAL CONSTITUENTS (PER 100 G-EDIBLE PORTION) OF YOUNG AND MATURE LEAVES OF *LAUNAEA CORNUTA*

	Young*	Mature**
Moisture (g)	86.8 ± 2.30	85.7 ± 2.20
Ash (g)	2.5 ± 0.03	2.6 ± 0.07
Crude proteins (g)	3.9 ± 0.00	3.8 ± 0.03
Crude fat (g)	0.9 ± 0.00	1.1 ± 0.07
Carbohydrates (g)	4.5 ± 0.09	5.1 ± 0.04
Fe (mg)	7.2 ± 0.12	6.8 ± 0.03
Na (mg)	57.9 ± 3.30	57.5 ± 2.20
K (mg)	869.0 ± 1.10	550.0 ± 9.60
Ca (mg)	214.0 ± 0.00	215.0 ± 2.30
P (mg)	13.2 ± 0.03	12.7 ± 0.09
Phytin - P (mg)	tr.	tr.
Vitamin C (mg)	18.7 ± 0.02	24.4 ± 1.20

Mean ± S.Em
*Young leaf : Third fully formed leaf from apex
**Mature leaf: leaves lower than the third leaf from apex but not including old leaves.

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reflected in the contents of individual minerals like Na, K, Ca, P and Fe (Table 1). Likewise, the content of protein was high for both young and mature leaves of *Launaea*. The crude fat contents of leafy vegetables were reported to be generally low ranging from trace amounts to 1 g per 100 g edible portion[13]. Our results on young and mature leaves of *Launaea* (0.9 and 1.1 g respectively) generally agree with these findings.

The vegetable was devoid of phytic acid. This agrees with the findings of Taha[14] who concluded that many vegetables are devoid of phytic acid but rich in minerals. Phytic acid when present in the diet forms complexes with mineral nutrients like iron, thereby rendering them unavailable to the body. Thus, the absence of phytic acid in the vegetable is of some nutritional importance in so far as mineral absorption is concerned.

Cooking markedly decreased the contents of total ash, Na, K and Ca (Table 2). Previous reports[15,16] also indicate the loss of minerals in cooked vegetables. In general, out of the two methods of cooking employed in the present study, the loss was greater when the leaves were boiled than when they were steam cooked. Cooking seems to have no effect on iron content. There was a remarkable loss in vitamin C in the leaves cooked by boiling or steaming (Table 2). Previous reports also indicate that cooking affects the water soluble vitamins[7,15,17].

The freshly collected leaves were stored for 36 hr under two conditions, viz.. leaves kept open on the table and leaves covered with wet cloth. In the leaves kept open, decrease of moisture from 86.8 to 58.5 per cent and vitamin C from 24.4 to 10.5 mg/100 g were noticed whereas in the leaves covered with wet cloth there was only a slight decrease. The results suggest that it is advisable to cover the leafy vegetables with wet cloth (in the absence of a refrigerator) if they are not immediately used.

Majority of the phytoconstituents present in *Launaea* are hot water soluble anthocyanidin glycosides, polyronides, polyose, catecholic tannins and anthracene glycosides. It has already been reported that some of these substances are associated with bitter taste[18]. During the peculiar mode of cooking (see introduction), there would be substantial leaching of these substances accounting for the reduced bitter taste in the cooked *Launaea* leaves. In addition, methanol soluble steroidal glycosides and coumarin glycosides and ether soluble sterols and carotenoides were detected in *Launaea* leaves.

TABLE 2. EFFECT OF COOKING ON MINERAL AND VITAMIN CONTENTS OF THE MATURE LEAVES OF *LAUNAEA* (PER 100 G EDIBLE PORTION)

	Fresh	Boiled	Steamed
Ash (g)	2.6 ± 0.07	1.5 ± 0.08	1.8 ± 0.01
Fe (mg)	6.8 ± 0.03	6.7 ± 0.10	6.8 ± 0.09
Na (mg)	57.5 ± 2.20	33.0 ± 1.80	38.8 ± 1.72
K (mg)	550.0 ± 9.60	294.0 ± 8.30	433.3 ± 11.60
Ca (mg)	215.0 ± 2.30	144.0 ± 4.70	165.1 ± 3.80
P (mg)	12.7 ± 0.09	12.5 ± 0.08	10.4 ± 0.30
Vitamin C (mg)	24.4 ± 1.20	1.3 ± 0.04	1.6 ± 0.06

Mean ± S.Em

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DETERMINATION OF TRYPSIN AND CHYMOTRYPSIN INHIBITORY ACTIVITIES IN SORGHUM PRODUCTS

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In unleavend bread 55 and 45% of trypsin and chymotrypsin inhibitory activities were lost respectively. The corresponding losses in Kichadi were 45 and 54% respectively. However, there was much decrease in chymotrypsin inhibitory activity in Thalipeeth. In Idli, there were decreases of 55 and 75% of trypsin and chymotrypsin inhibitory activities, respectively. Effect of different types of grinding was also studied.

Sorghum and millets have gained importance in many parts of the world and their potentialities of use as a superior food by blending them with wheat flour have been explored[1-4]. With a wide diversity in food habits in various regions, there exists a number of recipes that could be made from sorghum[5]. Sorghum is consumed as unleavened bread, dumpling and boiled rice like products[6]. The most common product prepared out of sorghum is unleavened bread. The trypsin and chymotrypsin inhibitors in the sorghum were isolated, purified and characterized by Harishkumar *et al*[7,8]. In the present paper, the results of studies on trypsin and chymotrypsin inhibitory activities in various sorghum products are reported.

Sorghum grains were procured from the local market. Trypsin and chymotrypsin from bovine pancreas were purchased from Sigma Chemical Company, USA. Casein was purchased from Wilson Laboratory, Bombay. Products like unleavened bread, kichadi and idli were prepared only from sorghum, but in thalipeeth along with sorghum, rice, pearl millet and Bengal gram were used. They were all prepared according to conventional method[9].

Inhibitor extract was prepared by homogenizing 2 g of sample in 30 ml of 0.1 M sodium phosphate buffer of pH 7.6. Then, it was centrifuged at 3360 × g for 20 min. The supernatant obtained was dialysed against 0.05 M sodium phosphate buffer of pH 7.6 for 6 hr. This dialysed extract was used for assay of trypsin and chymotrypsin inhibitors.

The assay of trypsin and chymotrypsin inhibitor activities in sorghum products were carried out by casein digestion method[10]. This consists of taking enzyme (10 μ g) and inhibitor (0.8 ml) extract and 0.1 ml of 0.1 M sodium phosphate buffer of pH 7.6, and this mixture was set aside for 10 min at 37°C. The reaction was initiated by adding 1 ml of 1 per cent casein. The reaction was stopped after 20 min by adding 5 per cent TCA. The solution was centrifuged at 840 × g for 10 min; 0.5 ml of clear supernatant was analysed for residual trypsin and chymotrypsin activities by the method of Lowry *et al.*[11] using bovine serum albumin as standard protein. One unit of inhibitory activity is the amount of inhibitor that suppressed one unit of proteolytic activity. It was found that the original trypsin inhibitory activity values were 24.8 and 70.5 respectively while the corresponding chymotrypsin inhabitory activity values were 111.38 and 120.00 respectively.

From our data presented in Table 1, it is observed that there were decreases in the trypsin and chymotrypsin inhibitory activities in the sorghum products, when they were heat processed. It is observed that, unleavened bread, kichadi and Idli had low trypsin and chymotrypsin inhibitory activities, whereas thalipeeth had high trypsin and chymotrypsin inhibitory activities. It is observed that 55 per cent of trypsin and 45 per cent of chymotrypsin inhibitory activities are lost in the preparation of unleavened bread, while in kichadi 45 per cent of trypsin and 54 per cent of chymotrypsin inhibitory activity are lost. In Idli, 55 and 75 per cent of trypsin and chymotrypsin inhibitory activities, respectively were lost. High trypsin and chymotrypsin inhibitory activities in thalipeeth may be due to the presence of other constituents like rice, wheat, pearl millet and Bengal gram. Heat treatment is known to improve the nutritional quality of plant proteins which also causes partial inactivation of proteinase inhibitors[12-15]. Yasmin and Pattabiraman[16] observed reduction in trypsin and chymotrypsin inhibitory activities in preparation of gruels, unleavened bread and roasted millets. Decrease in trypsin inhibitory activity in soybean meal is accompained by a concomitant increase in the nutritive value of the protein[17].

From the data in Table 2 it is observed that there are decreases in the trypsin and chymotrypsin inhibitory activities by grinding sorghum in chakki and flour mill, the latter gave better reduction than the former. The losses of trypsin and chymotrypsin inhibitory activities are due to the heat generated while grinding.

TABLE 1. PERCENT LOSSES OF TRYPSIN AND CHYMOTRYPSIN INHIBITORY ACTIVITIES IN DIFFERENT SORGHUM PRODUCTS

Sl. No.	Sorghum product	Loss of trypsin inhibitory activity (%)	Loss of chymotrypsin inhibitory activity (%)
1.	Unleavened bread	55.0	45.00
2.	Kichadi	45.0	54.00
3.	Idli	55.0	75.00
4.	Thalipeeth	10.1	22.20

TABLE 2. TRYPSIN AND CHYMOTRYPSIN INHIBITORY ACTIVITIES IN SORGHUM FLOUR GROUND IN DIFFERENT GRINDERS

Type of grinding	Trypsin inhibitor units Mean ± SD	Chymotrypsin inhibitor units Mean ± SD
Pestle morter	38.25 ± 0.20	137.25 ± 0.80
Chakki	33.80 ± 0.65	128.25 ± 1.64
Flour mill	24.80 ± 0.65	111.38 ± 0.31

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CHARACTERISATION OF *PSEUDOMONAS STUTZERI* CAUSING BROWN SPOT DISEASE OF OYSTER MUSHROOM

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Pathogenicity test of six bacterial isolates from oyster mushroom substrate materials indicated that one was from 'Brown Spot' infected substrate (BSI_4) and another from Paddy straw (PSI_2) caused 'Brown Spot' disease. Based on their morphological and biochemical properties these two bacterial isolates which caused 'Brown Spot' of oyster mushroom substrate were identified as *Pseudomonas stutzeri*.

The microbial associates namely fungi, bacteria and viruses cause economic losses due to low mushroom yield, as pathogens and as associative competitors[1]. Bacterial disease of mushroom viz. mummy disease (*Pseudomonas* sp) and bacterial pit (*Pseudomonas tolaasi*) of button mushroom and 'Yellow blotch' disease (*Pseudomonas agriici*) of oyster mushroom are mainly caused by pseudomonads[2]. The present study was undertaken to characterise the bacterial isolates causing 'Brown Spot' disease of oyster mushroom.

Bacterial cultures: Six bacterial isolates namely BSI_3, BSI_4, BSI_6 (brown spot isolates), PSI_2, PSI_5 (paddy straw isolates) and RBI_1 (rice bran isolate) which decreased mushroom yield considerably by inoculating them to substrate were used in the pathogenecity test.

Pathogenecity test of bacterial isolates: In this experiment pure culture of the above six bacterial isolates which reduced oyster mushroom yield considerably in earlier studies were grown on nutrient agar medium. After 24 hr, cells were harvested in a sterile buffer solution separately and optical density was adjusted to 0.4 at 620 nm (10^8 cells/ml)[3]. Ten ml of each bacterial suspensions were uniformly inoculated by spraying on sterilized substrate contained in each mushroom bag aseptically with a sterile automizer. Sterile distilled water was used as control. There were four replications for each treatment. Brown Spot appearance was recorded 12-15 days after spawn running. The mushroom yield was recorded after usual incubation period of 26 days.

Characterisation of 'Brown Spot' causing bacterial isolates: Characterisation of two bacterial isolates BSI_4 and PSI_2 which were found to cause 'Brown Spot' disease (Table 1) during oyster mushroom production on paddy straw was done as per the methods described by Schroth *et al*[4]

TABLE 1. PATHOGENECITY OF BACTERIAL ISOLATES

Bacterial isolate	Mushroom yield/bag (g)	Bioefficiency*	Brown spot appearance
Control	431.40	107.85	Nil
BSI_3	381.52	96.88	Nil
BSI_4	198.80	49.70	Brown spot
BSI_6	422.50	105.62	Nil
PSI_2	184.20	46.05	Brown spot
PSI_5	361.60	90.48	Nil
RBI_1	392.80	98.20	Nil

C.D at 5% for mushroom yield is 6.6.

$$\text{Bioefficiency} = \frac{\text{Total yield of fresh mushrooms}}{\text{Dry weight of substrate used}}$$

Sport staining, oxygen requirement and motility tests were carried out using the methods described by Carbtree and Hinsdill[5]. For testing accumulation of poly β - hydroxy-butyrate (PHB), the isolates were grown in PHB medium and they were tested for PHB granules[6]. Fluorescent pigment production was tested on King's A and King's B media[7]. Denitrification, starch hydrolysis and growth at 4 and 41°C of these two isolates were carried out by the methods of Stolp and Godkari[8]. Gelatin hydrolysis test was carried out by streaking the isolates on nutrient agar supplemented with 1 per cent gelatin. After the growth of the bacteria, the plates were flooded with saturated solution of ammonium in 1N sulphuric acid and observed for gelatin hydrolysis.

It was observed that inoculation of all the six bacterial isolates (BSI_3, BSI_4, BSI_6, PSI_2, PSI_5 and RBI_1) decreased mushroom yield considerably compared to control which was 431.40 g per bag. It is evident from Table 1 that two isolates BSI_4 and PSI_2 reduced mushroom yield drastically. Further, these two isolates produced typical 'Brown Spot' symptom (Fig 1) on all replications. Other four isolates viz., BSI_3,

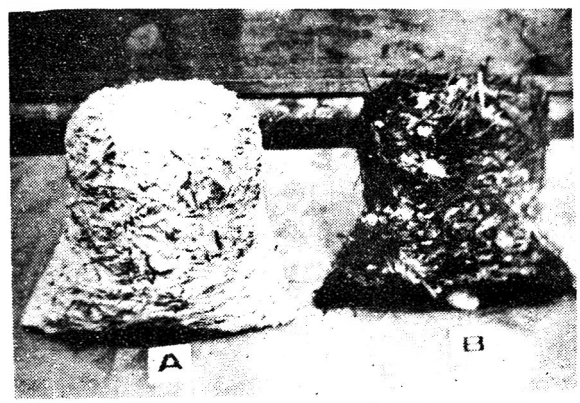

Fig. 1. Brown spot disease symptom caused by *Pseudomonas stutzeri*
A: Control - uninoculated
B: Inoculated with *Pseudomonas stutzeri*

BSI_6, PSI_5 and RBI_1 eventhough reduced mushroom yield, did not cause 'Brown Spot' symptom. Decrease in the mushroom yield may be due to competitive or antagonistic activity of these associates[1]. The isolate BSI_4 was isolated from brown spot infected substrate and isolate PSI_2 from paddy straw which suggests the source of infection.

Identification of brown spot disease causing bacterial isolates BSI_4 and PSI_2: The two bacterial isolates which caused 'Brown Spot' of paddy substrate were highly aerobic, Gram negative rods, motile and did not produce spores (Table 2). These confirm to the genus *Pseudomonas* of the family pseudomonadaceae[8].

These isolates (BSI_4 and PSI_2) were further characterised and the results are given in the Table 2. These isolates did not require growth factors, both the isolates did not produce PHB and fluorescent pigment. They also denitrified nitrate, gelatin was not hydrolysed but both hydrolysed starch, no growth at 4°C and considerable growth at 41°C.

TABLE 2. CHARACTERISATION OF BROWN SPOT DISEASE CAUSING BACTERIAL ISOLATES BSI_4 AND PSI_2.

Tests conducted	Bacterial isolates	
	BSI_4	PSI_2
Gram reaction	—ve	—ve
Shape	short rod	short rod
Spore	—	—
Motility	+	+
Oxygen requirement	Aerobic	Aerobic
Accumulation of PHB	—	—
Fluorescent pigment production	—	—
Growth on minimal media	+	+
Denitrification	+	+
Hydrolysis of gelatin	—	—
Hydrolysis of starch	+	+
Growth at 4°C	—	—
Growth at 41°C	+	+

The above characters of the isolates BSI_4 and PSI_2 were compared with the dichotomous key[8]. These characters confirm that these isolates belong to *Pseudomonas* genus and the species *Pseudomonas stutzeri;* which caused 'Brown Spot' disease of oyster mushroom substrate. Further work on effective control measures of this disease would greatly help the oyster mushroom farmers.

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INHIBITION OF AFLATOXIN BIO-SYNTHESIS BY LIGHT IN LIQUID CULTURE AND FOOD GRAINS

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Effect of different coloured light on aflatoxin biosynthesis in liquid culture (SMKY) and solid substrates (ragi and rice grains) was studied. Maximum inhibition of biosynthesis was recorded under green light on rice, and by blue light on ragi and liquid broth. Emperical relationship was also established between the different wavelength of light and inhibition of aflatoxin biosynthesis using simple polynomial equation.

Light has been shown to inhibit mycotoxin production in *Aspergillus flavus* and a mutant strain of *A. parasiticus*[1]. Detoxification of aflatoxin through light has been reported[2,3]. The aim of the present work was to investigate, whether light colour has any effect in inhibiting aflatoxin biosynthesis in liquid culture and on solid substrates.

Comparative effect of four coloured light viz., blue, green yellow and red was conducted in SMKY liquid culture[4] and two substrates viz., ragi (*Eleusine coracana*) and rice (*Oryza sativa var* 'Basmati'). Fifty ml culture medium in 250 ml Erlenmeyer flask was autoclaved. Ragi and rice grains, 50 g each were surface sterilized with 2 per cent sodium hypochlorite solution and kept in 250 ml flask. Each flask was wrapped in polythene sheet transparent to different coloured lights. Control experiments were done under the normal light. The colour of the medium or substrate changed, due to transmitted light. The changes observed are presented in Table 1.

The medium/substrates was inoculated with 0.5 ml spore suspension (1×10^8 spores/ml) of a known aflatoxigenic strain of *Aspergillus flavus* NRRL-3557. The flasks were incubated at 28 ± 1°C for 7 days. At the end of the incubation period, aflatoxins were extracted and analysed[5-7].

The results presented in Table 2 show that maximum inhibition took place in the green light on rice, and by blue light in SMKY medium and ragi. From Table 1, it is apparent that under blue light, the substrate ragi as well as the liquid culture showed green colouration. Thus, the green light appears to be the most effective light in controlling the aflatoxin synthesis. Similarly, other coloured lights also produced different degrees of inhibition. The degree and pattern of inhibition are Blue > Green > Yellow > Red respectively in SMKY and ragi, and green > yellow > blue in rice. The observations given in fourth column of Table 2 indicate that the dry weight of the fungus in SMKY liquid medium shows almost negligible changes under different coloured lights over the control. It indicates that the coloured light inhibits the aflatoxin biosynthesis without affecting the fungal growth. Green light has potentiality to inhibit aflatoxin biosynthesis. It was reported that blue colour inhibits *Alternaria* toxin production[8].

We have tried to establish an emperical relationship using a simple polynomial equation.

$$Y = a + bx + cx^2 \quad \ldots\ldots$$

where Y is per cent inhibition; x is wavelength of light in $nm\times10^{-2}$. The theoretical values obtained by the emperical relationship presented in Table 3 show per cent inhibition. The theoretical values generally are in conformity to the experimental findings.

It can be concluded that green light is in general, the most effective and plays a role in the photocontrol of aflatoxin B_1 biosynthesis upto 50 per cent. The photochemical effect can

TABLE 1. COLOUR CHANGES IN THE MEDIUM/SUBSTRATES AFTER THE APPLICATION OF DIFFERENT COLOURED LIGHT

Type of light	SMKY medium		Rice		Ragi	
	Original colour	Final colour of medium (nm)	Original colour	Final colour of substrates (nm)	Original colour	Final colour of substrates (nm)
Normal light (Control)	Deep yellow	Deep yellow	White	White	Deep yellow	Deep yellow
Blue	,,	Grass green (525-550)	,,	Blue (450-475)	,,	Grass green (525-550)
Green	,,	Light green (475-500)	,,	Green (525-550	,,	Light green (475-500)
Yellow	,,	Light orange (625-650)	,,	Yellow (575-600)	,,	Light orange (625-650)
Red	,,	Deep orange (675-700)	,,	Red (700-725)	,,	Deep orange (675-700)

TABLE 2. EFFECT OF LIGHT ON AFLATOXIN INHIBITION IN LIQUID (SMKY) AND SOLID (RAGI AND RICE) SUBSTRATES[a]

Type of light	SMKY			Ragi		Rice	
	Aflatoxin B_1 elaborated (μg/l)	Inhibition (%)	Dry wt. of fungus (g)	Aflatoxin B_1 elaborated (μg/kg)	Inhibition (%)	Aflatoxin B_1 elaborated (μg/kg)	Inhibition (%)
White (Control)	508.86	Nil	2.237	2.48	Nil	926.42	Nil
Blue	251.68	50.54	2.015 (—10)	1.49	39.92	784.68	15.30
Green	316.85	37.73	2.11 (—6)	1.91	22.98	387.58	58.16
Yellow	366.31	28.01	2.24 (0.0)	2.48	0.00	784.62	15.31
Red	385.20	24.30	2.42 (+8)	2.16	12.90	954.78	(—) 3.06

[a]The experiment was carried out thrice with three replicates.
Figures in parentheses indicate % gain or loss over control

TABLE 3. THEORETICAL PER CENT INHIBITION* OF AFLATOXIN IN LIQUID AND IN SUBSTRATES UNDER LIGHTS OF DIFFERENT WAVE LENGTHS

Wave length** (nm) × 10^{-2}	% inhibition of aflatoxin synthesis (Y)		
	SMKY	Rice	Ragi
4.25	14.14	15.30	(—) 6.93
4.75	37.73	42.85	22.98
5.25	50.54	58.16	39.92
5.75	52.57	61.22	43.88
6.25	43.83	52.04	34.88
6.75	24.30	30.61	12.90
7.25	(—) 5.98	(—) 3.06	(—) 22.05

* a,b,c values in SMKY — a = —621.4504, b = 241.153, c = —21.5533,
Rice — a = —713.2881, b = 275.515, c = —24.49,
Ragi — a = — 784.9934, b = 293.346, c = — 25.9466

** The value of x was taken after multiplying the wave length by 10^{-2}

be exploited for control of aflatoxin elaboration in storage.

Authors are thankful to Prof. K.S. Bilgrami for laboratory facilities and U.G.C. for financial assistance.

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Evaluation of Programmes to Ensure Food Safety — Guiding Principles : World Health Organization, Geneva, Price : Sw. Fr.9, pp: 47; 1989.

This publication deals mainly with guiding principles on how to monitor and evaluate programmes to ensure food safety in different countries which have different priorities for ensuring control and safety of food. Provision of safe food, free from harmful ingredients is an essential component of primary health care. One of the most important health problems in the world currently is illness resulting from eating contaminated food. This publication suggests guidelines for measuring progress on the work of national authorities in their food safety and related programmes.

The subject matter is discussed in three chapters. In Chapter I, the nature and extent of the problem of food contamination are considered. It has been estimated that foodborne diseases may be the second largest cause of morbidity in Europe during the period 1986-89, next only to respiratory tract infections, due to various factors like mass rearing of food animals and their centralized processing, resulting in greater spread of various enteric pathogens in both raw and processed foods and increasing environmental pollution resulting in contamination of a high percentage of food and feed lots. In developing countries, contaminated food is responsible for a high incidence of infantile diarrhoea. Even if diarrhoeal disease is not fatal, the associated effects such as withdrawal of food, reduced intake, nutrient losses and malabsorption may severely accentuate the effects of poor diet, thus initiating or aggravating malnutrition. Some of the most important food-borne pathogens are - *Salmonellae* in poultry and meat, *Camphylobacter* in milk and meat, *Staphylococcus aureus* in foods of high salt or sugar content, *Clostridium perfringens*, Vibrio spp, *E. coli* and *Histeria monocytogenes* in foods of animal origin and *Bacillus cereus* in rice and meat products. Apart from these pathogens, over 100 parasitic species may be transmitted to man by infected food.

The desirability is stressed of preparing a country profile/ data base that will provide information on factors that may influence food safety, in order to facilitate the planning, implementation and evaluation of policies and programmes to ensure food safety. An annex is also provided containing questions to facilitate preparation of such a data base which can serve as a basis for evaluation of food safety programmes.

In the II Chapter, the various factors faced by National authorities throughout the world are listed, that render it difficult to achieve, the development, implementation and maintenance of effective and efficient food safety and food control programmes. The methodologies that can be employed for evaluation of food safety programmes are also discussed in depth. The need for selecting ideal indicators for the conduct of evaluations to obtain valid data is stressed. Some questions that could be useful in developing suitable indicators are listed in Annex 2. Annex 3 provides examples of possible indicators that might be used.

Chapter III covers aspects of the evaluation process such as management of the evaluation, interpretation of evaluation results and use of evaluation results. In order to ensure effective management, several steps are listed that should be considered by persons charged with conducting the evaluation. As any erroneous interpretation of evaluation results could do more harm than good, it calls for appropriate care and objectivity in interpretation of evaluation data. If programme managers have been adequately consulted prior to the start of the evaluation, as well as during its execution, it can be reasonably expected that the evaluation results will contribute to positive developments in the fields of food safety, food control or community action programmes.

This publication will be most useful to officials in charge of food safety in developing countries in evaluating the progress of their programmes from time to time.

S. VENKAT RAO
C.F.T.R.I., MYSORE

Advances in Nutritional Research, Vol. 8: Edited by Harold H. Draper, Plenum Publishing Corporation, New York, U.S.A.; 1990; pp: 149; Price: US $ 55.

This book is Volume 8 in the series 'Advances in Nutritional Research' and covers many topics of prime importance in nutrition. The volume is presented into six chapters.

First chapter deals with the nutrients transfer across the perfused human placenta. The author has discussed the use of the technique of *in vitro* perfusion of human placenta to study foetal nutrition. He has chosen two nutrients namely, glutamic acid and riboflavin to illustrate experimental designs and type of information obtained about nutrient metabolism by placenta, transfer of substrates to the foetus as well as the rate of elimination of material from the foetal blood. Advantages as well as limitations of the technique have also been appropriately highlighted. Chapter 2 addresses the issue of impaired immune function associated with severe form of protein-energy malnutrition (PEM). Authors have surveyed available evidence with regard to involvement of micronutrients such as zinc, copper, certain essential amino acids in immune function, replenishment with immunological components as means of adoptive immunotherapy, manipulation of blood levels of immunologically active hormones such as thyroid hormones, gluco-corticoids and immunostimulatory drugs. On the basis of limited direct intervention studies with tri-iodothyronine, authors contend

that immunoenhancement therapy in PEM should be based on re-establishing metabolic priority to immune functions, by way of administration of hormones, their agonists or inhibitors. There is lack of convincing evidence supporting probable improvement of immune function in seovere PEM by supplementation with critical micronutrients.

Chapter 3 provides a comprehensive review of the role of several nutritional non-pharmacological factors in the control of blood pressure. These include obesity, alcohol consumption, sodium, potassium, calcium and magnesium intakes, dietary fat and further cover population studies, their mode of action on blood pressure. Authors provide comments at the end of each section.

Chapter 4 reviews various aspects of energy metabolism of the foetus and newborn infants, including energy intake, its utilization and storage together with various influencing factors. Authors rightly point out the need of long term studies in view of a possible relationship between nutrient density (expressed on the basis of energy content) and quantitative aspect of tissue growth in pre-term and low birth weight infants.

Chapter 5 discusses various methods used to assess nutritional status of hospitalized patients and includes a number of anthropometric and clinical ones. The approach of using prognostic nutritional index based on the combination of different types of measurements to classify patients in respect of the degree of post-operative complications, appears much promising since no simple accurate single method is available for nutritional assessment to day.

Chapter 6 concerns with new emerging area of pathogenicity of oxygen radicals in the tissues. Different types of oxygen radicals and lipoxy radicals arise during cellular reactions and food particularly edible oil processing and cooking. If not suitably eliminated, they may prove to be toxic. The author has reviewed the influence of micronutrients including vitamin C, vitamin E, niacin, beta-carotene, trace elements such as selenium, copper, zinc, manganese, sulphur amino acids on the generation and scavenging of these radicals. The author has discussed about the prophylactic role of these nutrietns in modulating diseases of oxygen radical pathology, besides their known nutritional role and suggested that in the event of use of any nutrient for its prophylactic activity toward any specific disease, it should be used as a supplement to the diet, rather than food fortification in terms of the compliance of the dietary recommendation for the prevention of the nutrient deficiency.

A special feature of this compilation is that there is a fair coverage of the large body of carefully chosen research literature at the end of the every chapter. In addition, a conclusive overview at the end of each chapter gives not only present status but also suggests future line of research. The editor should be complimented for this excellent compilation of research work in different areas of nutrition. The volume will serve as a valuable reference book for researchers and academicians in the field of nutrition, dietetics and medical sciences, desirous to have an overview in different areas.

A.D. DEODHAR,
N.D.R.I., KARNAL.

Food Toxicology — A Perspective on the Relative Risks: Ed. by Steven L. Taylor and Richard A. Scanlan; Published by Marcel Dekker Inc. 270, Madison Avenue, New York, N.Y. 10016, pp: 464; 1989; Price: US$ 59.75 (US and Canada), $71.50 (All other countries).

The book contains the papers presented at the 12th Basic Symposium in Food Toxicology held in New Orleans sponsored by Institute of Food Technologists and the International Union of Food Science and Technology. Much of the attention has been focussed on the chemical carcinogens such as chemicals in processed food, mycotoxins and N-nitroso compounds that find their way into our food. It is needless to say that not all food toxicological problems relate to risks of cancer. The book contains 15 articles contributed by different authors. They are 1. A perspective on Diet and Cancer, 2. Carcinogenic Potential of Mycotoxins in Food, 3. Heterocyclic Amines in Cooked Foods, 4. Relative Exposure to Nitrite, Nitrate and N- Nitroso Compounds from Endogenous and Exogenous Sources, 5. Anti-carcinogens and Tumour Promoters in Foods, 6. A case study: The Safety Evaluation of Artificial Sweeteners, 7. Glutathione and Vitamin E in Protection against Mutagens and Carcinogens, 8. Comparisor of the Carcinogenic Risks of Naturally Occurring and Adventitious Substances in Food, 9. Behavioural Disorders Associated with Food Components, 10. Food Allergies and Sensitivities, 11. Role of Lipid Oxidation Products in Atherosclerosis, 12. Regulatory Distinctions Between Naturally Occurring and Added Substances in Food, 13. Toxicological and Pharmacological Interactions as Influenced by Diet and Nutrition, 14. Strengths and Limitations of Toxicological Testing Procedures, and 15. Pros and Cons of Quantitative Risk Analysis.

The potential sources of harm associated with foods, the largest by far is microbiological contamination, followed closely by nutritional imbalance including the excessive consumption of food as well as nutritional defiencies. Cancer is a very much complex disease, the origion of which are only dimly understood. Diet and nutrition may affect it. The factor most closely linked to enhancement of carcinogensis is dietary fat, but How? Is a big question. There is a limited epidemiologica evidence that anti-carcinogens in food may provide human with some protection against cancer. Unfortunately studies todate of specific anti-carcinogens, such as beta carotene have been disappointing. Potential cancer inhibitors from foods of plant origin, such as plant phenols and calcium from foods of animal origin have been much talked off.

It has been generally accepted that initiation of the development of Cancer involves DNA modification and therefore a number of short-term *in vitro* tests for DNA modification have been used to predict the cancer-initiating activity of many fungal metabolites - Aflatoxin B_1, C_1, M_1, Patulin, Penicillic Acid, Ochractoxin A, (S) — Zearalenone and Nivalenol have been found to possess such activity in one test or the another. But can this carcinogenicity data obtained from the rat be directly extrapolated to humans? The idea that life style has an important influence on the development of cancer in human is generally accepted. Among the factors in life style, apart from tobacco and alcohol, now recognised to influence cancer development are diet and nutrition. Basic fractions of the smoke condensate from charred surfaces of boiled fish and meat were found to be mutagenic; charred amino acids, proteins and proteinous foods all contained mutagenic substances. Some heterocyclic amines isolated from pyrolysates of Proteins and amino acids were found to be mutagenic. The role of nitrite, nitrate and N-nitroso compounds might play in aetiology of cancer remains unclear.

Food allergies and sensitivities have been the subject of confusion and controversy. Numerous Physiological and sensitivities. The term food allergy should only be used to identify true immunologically based adverse reactions, although many of the individualistic adverse reactions do not involve immune system. Some extremely interesting new findings in the areas of hypercholesterolemia and thrombosis-vascular spasm have been thoroughly reviewed, with a primary focus on exposing the hypothesized link between lipid oxidation products and atherosclerosis.

Likewise many toxicological and phermacological implications or interactions as influenced by the Diet and Nutrition have been well presented. The book is a worth possessing asset for those directly or indirectly involved in Food Toxicology; and on the shelf of the library in organisations dealing with Food and Nutrition.

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

IPCS : Environmental Health Criteria. 104 : Principles for the toxicological assessment of pesticide residues in Food: WHO, U.N. 1990; pp: 117, Price; Sw. fr.15.

This monograph is published under the joint sponsorship of the United Nations Environmental Programme, the International Labour Organisation and the World Health Organisation.

JMPR meetings which are joint efforts of FAO/WHO on pesticide residues, have evaluated a large number of pesticides. The WHO expert group on pesticide residues in these meetings largely relied upon procedures developed by other expert groups such as Joint FAO/WHO expert committee on food additives (JECFA) and developed specific principles for evaluating various classes of pesticides that are used on food crops and which may leave residues on them. The publication of WHO Environmental Health Criteria 70 principles for the safety assessment of food addtives and contaminants in food summarised the assessment procedures used by JECFA. These procedures are used by the WHO expert groups on pesticide since its publication.

Following inter-country meetings held in 1985 in Canada, an IPS planning meeting was held in March 1987 at Carshalton, Surrey, UK when a first draft was prepared to consider the toxicological basis and data requirements for the estimation of an ADI or temporary ADI and to provide general guidance on relevant toxicological methodology. The final draft was considered by the WHO expert group in 1984 JMPR and the present publication, under review, therefore, reflects the views of a large number of international experts who are involved in the toxicological assessment of pesticides. It is expected that the present publication will be useful to ensure consistent decision making using up-to-date principles. It is hoped a publication of this nature involving test methodologies, choice species, strains, group size, selection of dose levels, test duration, pathological procedures on carcinogenesis, reproduction studies, neurotoxicity and genotoxicity will be of great use to the government regulatory bodies responsible for establishing safe levels of pesticide residues on food commodities and companies producing safety data on pesticides.

The more recent additions to the battery of toxicity tests available for use in safety assessment are discussed in this monograph. Some of these tests especially in the field of immunotoxicity and behavioural toxicity are not yet at a stage of development where results are consistently reproducible and therefore readily utilizable in safety assessment. It may be observed that criteria for interpretation of such studies have not yet been sufficiently developed to be of value in routine safety assessment. Therefore, only the potential of these studies is discussed in this document.

The developments in the field of toxicology in recent years have been remarkable. Decision taken by JMPR are always provisional and ADIs are subject to re-evaluation as new, significant data become available.

With microbial pest control aspects, the group suggests that two factors of primary importance to human health, *viz.*, the infectivity of the residual organism and the ability of the organism to produce toxins which occur as residues should be considered. In the case of tissues, their ability to incorporate into the cell genome should also be considered.

There are areas of special consideration for individual classes of pesticides. For example, opthalmological effects and alicsterase (carboxyl-esterase) inhibition in organophosphates. In 1980 JMPR, it was noted that OP compounds tend not to show genotoxicity *in vivo* or to induce carcinogenic responses in laboratory animals. It was recommended that careful evaluation of all available data

should be performed to determine whether carcinogenicity tests are required for individual organophosphate pesticides. It is also recorded that the possible structure-activity relationship of the non-phosphatic ester moiety of the pesticides should be considered. The monograph discusses ocular toxicity of bipyridilium compounds and there is a note on goitrogenic carcinogensis.

This is a valuable publication for environmentalists and toxicologists.

V. AGNIHOTHRUDU
COIMBATORE

Multilingual Dictionary of Fish and Fish Products: 3rd ed. Prepared by the Organisation for Economic Co-operation and Development. Fishing News Books, A Division of Blackwell Scientific Publications Ltd., U.K; 1990; pp: 442; Price: £29.50.

The stated objective of the compilation is to promote and facilitate international trade in fish and fish products that are in commercial use internationally. The Fisheries Division of the Organisation for Economic Co-operation and Development, U.K. has compiled this thoroughly revised 3rd edition.

There are 1117 items in the dictionary. The nomenclatures cover product designations, definitions and names of fish species. Each item is numbered with descriptions in English and French as also equivalents for the main heading in fourteen other languages (viz. Danish, Dutch, Finnish, German, Greek, Icelandic, Italian, Japanese, Norwegian, Portuguese, Serbo-Croat, Spanish, Swedish, Turkish), besides scientific names for species of fish, shell fish, etc. This is the main part of the dictionary. The other part comprises separate indices for each of the sixteen languages in addition to one of scientific names. The user has to find the known name in the index of the relevant language, turn to the item number indicated in the index and obtain description in English or French.

The compilation is extremely useful and handy to anyone interested in international trade in fish and fish products, as a ready reference book.

N.V. SRIPATHY
C.F.T.R.I., REGIONAL CENTRE, MANGALORE.

AFST (I) NEWS

Bangalore Chapter

The Annual General Body Meeting was held on 6th April 1991. The following office bearers were elected:

President	:	Mr. Srikanth Chatrapathy
Vice-President	:	Dr. Satish Kulkarni
Hony. Secretary	:	Mrs. B.M. Leelavathi
Hony. Treasurer	:	Dr. R.R. Mohite

Delhi Chapter

The Annual General Body Meeting was held on 12th April 1991, the following persons have been elected as office bearers for the year 1991-92.

President	:	Mr. D.S. Chadha
Vice-President	:	Dr. S.M. Ilyas
		Dr. D.S. Khurdiya
		Mr. Y.K. Kapoor
		Mr. V.B. Oberoi
Hony. Secretary	:	Mr. B.L. Kapoor
Hony. Jt. Secretary	:	Mr. S.K. Checker
Hony. Treasurer	:	Mr. P.N. Narang

On this occasion, Mr. Laljit Singh was felicitated by the members for receiving the Kashlakar Memorial Award conferred on him by the All India Food Preservers' Association.

Madras Chapter

The Annual General Body Meeting was held on 9th May 1991. The following are the office bearers for the year 1991-92.

President	:	Dr. P.G. Adsule
Vice-President	:	Mr. N. Ibrahim
Hony. Secretary	:	Mr. K. Manohar
Hony. Jt. Secretary	:	Dr. S. Gopalan
Hony. Treasurer	:	Mr. K.H. Krishnan

On this occasion, Dr. Sailesh Patel, Director (Foods) Auro Foods, Pondicherry delivered the Guest lecture on "Food Technology for Housewives".

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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