JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS, INDIA

VOL. 24. NO. 3. MAY / JUNE 1987

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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Volume 24 Number 3 May/June 1987

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Research Papers

Research Notes

Isolation and Properties of Linseed Mucilage

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Received 29 *May* 1986; *revised* 18 *September* 1986

Linseed mucilage was isolated by aqueous extraction and precipitation with acetone or ethanol. The variation in viscosity of aqueous dispersions of the mucilage has been studied as a function of concentration, pH and temperature. The effect of some inorganic and organic salts has been tested; iron salts were found to decrease the viscosity to a greater extent. The mucilage at about 1% concentration stabilised the foam formed by surface activity proteins against thermal disruption.

Linseed (*Linum usitatissimum*) is an annual herb of which the two types are well known. The flax is grown for fiber and linseed for a drying oil which finds use in paints and varnishes. It ranks fifth among the oilseed crops of India with an annual turnover of 500-600 thousand tons. The defatted meal has a high content of protein and has been used as an animal feed. Nutritional and protein quality have been studied in rats and chicks[1-4]. The proteins isolated after removal of the mucilage and fat have also been characterised[5-7]. The mucilagenous polysaccharide present in the outer husk of the seed has medicinal value as it has been reported to prevent bloat in ruminants fed mostly on legumes. Structural investigations have been carried out on the partially purified fractions obtained by salt or solvent precipitation of the polysaccharide and also on those purified by column chromatography on DEAE-cellulose[8-11]. The functional properties of this mucilage have not been studied. As the aqueous dispersion of this polysaccharide exhibits high viscosity, whether it can stabilize the foam formed by surface active proteins (obtained from oil seeds) has been tested and the results are reported in this paper.

Materials and Methods

Linseed ('Khategaon' variety) was purchased from M/s Flour and Foods Ltd., Indore, India. Peanut and sesame seeds were purchased from the local market. The seeds were cleaned and stored in sealed containers at 5-7°C until further use. All the chemicals and reagents used were of analytical grade. Solvents were distilled before use.

Preparation of linseed polysaccharide: The seeds when soaked in water swell considerably due to the presence of the mucilage and forms a highly viscous dispersion. Seeds were soaked in water at 5-7°C (seed: water - 1:8 w/v) for 8-10 hr with occasional stirring and filtered through a nylon cloth. This extract was added to three times its volume of acetone. The precipitated polysaccharide was washed with the solvent, redispersed in water, dialysed against water and lyophilized. The variation in the yield or viscosity of the material was marginal in the pH range of 4-8 and hence only water was used for extraction. The proximate composition of the seeds and the polysaccharide content were determined according to methods of AOAC[12].

Preparation of defatted flours: Peanut seeds were partially dried at 30-40°C for 2-3 hr and decuticled. They were flaked and solvent extracted in a soxhlet apparatus with petroleum ether (B. P. 40-60°C) to remove the fat. The residue was air dried and powdered in an apex grinder to get the flour (60 mesh). Sesame seeds were soaked in water for 10-12 hr at room temperature (22-27°C), drained and dehusked by rubbing over a gunny bag. The seeds were air dried, winnowed/aspirated to remove the husk, flaked, defatted and powdered to get sesame flour (60 mesh). These defatted flours were used as a source of surface active protein.

Determination of viscosity: The viscosity of aqueous dispersions was determined in an Ostwald viscometer. For comparison, guar gum dispersions were tested under similar conditions. Aqueous dispersions (0.1-0.6 per cent concentration) were tested at room temperature (22-27°C). Dilute HCl (0.5N) or NaOH was used to adjust the pH in the acidic or alkaline range. The effect of temperature was tested at 22, 42, 56 and 76°C. For heat treatment, aqueous dispersions were heated on a boiling water bath (95-97°C) for 30 min, cooled to room temperature and viscosity determined before

and after heating. The effect of some inorganic and organic salts (0.1-0.5M levels) was tested at room temperature with 0.3-0.4 per cent aqueous dispersions. The change in viscosity of dispersions was expressed as residual viscosity taking the viscosity of control samples as 100 per cent. The values represent average of three independent determinations in the above experiments.

Foam stabilization: This was carried out as described earlier[13]. Aqueous (1 ml) dispersions of defatted oilseed flours corresponding to 0.5-0.6 per cent were taken as a source of surface active protein using 0.5 ml of $NaHCO_3$ (5 per cent) and 0.5 ml of citric acid (5 per cent) as *in situ* source of CO_2 along with varying amounts of linseed polysaccharide or guar gum (0.25-1.5 per cent). The contents were stirred well and allowed to stand at room temperature for 10 min and then tested in a water bath at 95°C. Foam volumes were noted before and after incubation. Photographs of foam columns were taken after 1-2 min standing at 95°C.

Results and Discussion

Linseed used in the study had a density of 1.1 and the average weight of the seeds ranged between 8.5 and 10.5 mg. The proximate composition (in per cent) was: moisture 6.5, protein 23, fat 39, carbohydrate 20, ash 3.9 and crude fiber 9. Ethanol, acetone or isopropanol gave similar (5 per cent) yield of the polysaccharide. The dried material easily redispersed in water and retained good viscosity. The dialysed and lyophilized sample had 5-6 per cent moisture and 93 per cent carbohydrate. This preparation was used for studying the properties.

Fig. 1a shows the increase in viscosity with concentration. At 0.5-0.6 per cent levels, guar gum dispersions had nearly twice the viscosity as compared to linseed polysaccharide. Fig. 1b shows that viscosity was maximum around pH 5-7 and the decrease was greater on the acidic side of pH. Viscosity decreased considerably at higher temperatures (Fig. 1c) and greater decrease occured at higher concentrations. After heat treatment and cooling, viscosity of dispersions was close to that of unheated sample (Fig. 1d) indicating a reversibility of thermal decrease in viscosity. These changes are similar to what was reported for a few other gums and mucilages of plant origin[14].

Effect of salts: Fig. 2 shows that chlorides of sodium and potassium decreased the viscosity by 10 per cent, calcium by 20 per cent and ferric salts by 50 per cent. Potassium iodide decreased the viscosity by 40 per cent. Carbonates of calcium, magnesium increased the viscosity by 10-12 per cent, around 0.1-0.2M levels and 15-20 per cent at 0.5M levels Above 0 5M level, aggregation of the polysaccharide occurred and hence viscosity could not be determined. Sulphates of sodium, potassium, calcium, and magnesium caused about 10 per cent decrease, but that of iron (ferrous sulphate) caused about 50 per cent decrease. Phosphates of sodium and potassium reduced the viscosity by 20-

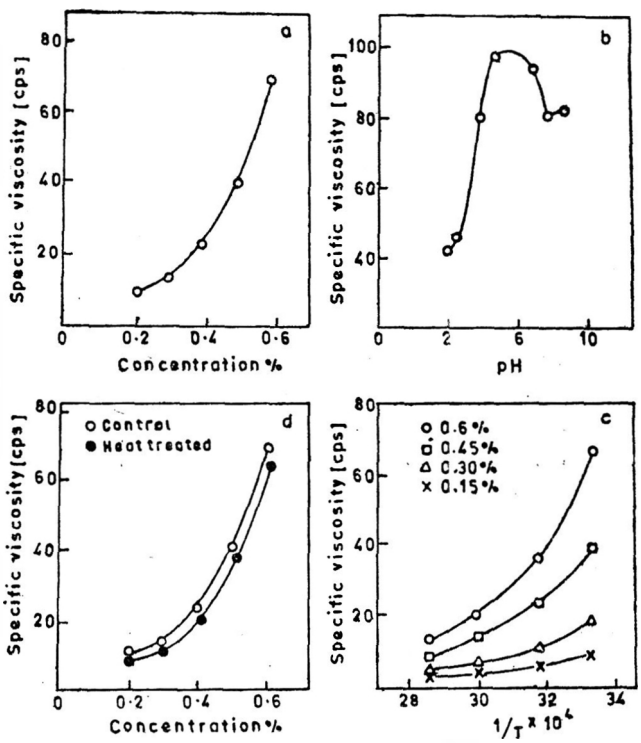


Fig. 1. Relationship between viscosity and (a) concentration (b) pH (c) temperature (d) heat treatment of linseed mucilage.

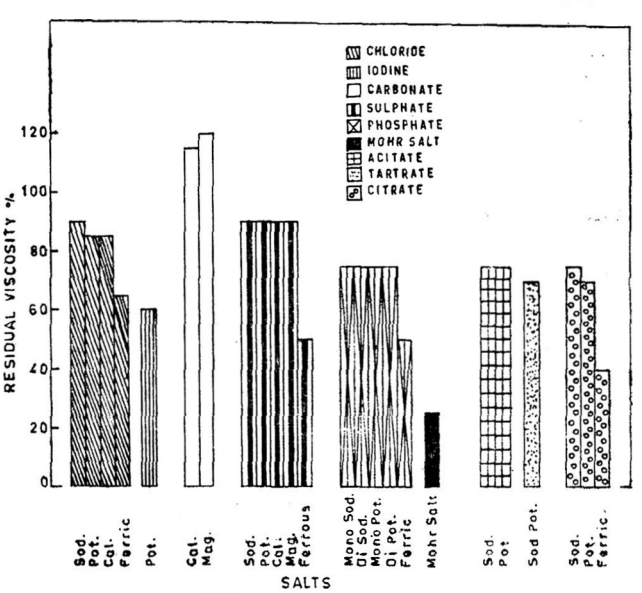


Fig. 2. The effect of salts on viscosity of linseed mucilage.

TABLE 1. THE EFFECT OF LINSEED MUCILAGE AND GUAR GUM ON THE FOAM FORMED BY PEANUT AND SESAME FLOURS

Component wt (mg)				Foam vol* (ml)			
Linseed mucilage	Guar gum	Peanut flour	Sesame flour	After acidification	After 10 min of acidification	At 95°C	After cooling
0		30		2	2	5	0.5
0			30	3	2	6	0.5
5		30		6	5	15	1.0
10		30		6	4	16	1.0
15		30		5	3	15	4.0
20		30		4	2	12	3.0
5			30	6	5	16	1.0
10			30	5	4	18	1.0
15			30	5	4	14	4.0
20			30	5	4	16	4.0
	5	30		7	6	10	1.0
	10	30		6	5	10	1.0
	15	30		4	4	12	3.5
	20	30		3	3	14	3.0
	5		30	5	4	17	1.0
	10		30	5	4	18	1.0
	15		30	4	3	18	5.0
	20		30	4	3	16	4.0

* The volume before acidification was 1.75 ml in all cases.

25 per cent and ferric phosphate by 50 per cent. Ferrous ammonium sulphate caused a maximum decrease of 75 per cent.

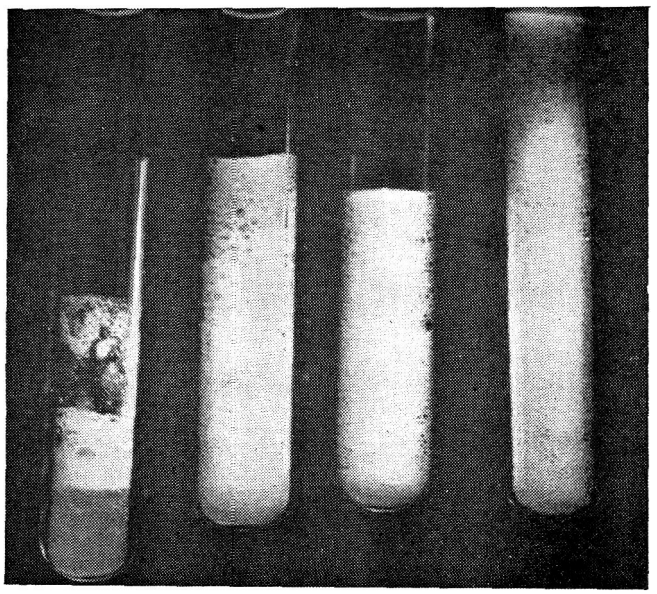
Among the organic salts, sodium acetate decreased the viscosity by 25-30 per cent, sodium potassium tartrate by 20-35 per cent, sodium citrate and potassium citrate by 25-30 per cent, ferric citrate reduced the viscosity by 50 per cent.

Foam stabilization: It is seen from Table 1 that peanut and sesame proteins by themselves in the presence of a source of gas such as CO_2 showed a tendency to form the foam with smaller increase in foam volume after heating. In the presence of lower concentrations of the polysaccharide, the foam column showed considerable expansion but was unstable to heat. At slightly higher concentrations of the polysaccharide, the foam columns showed a decrease in volume due to the compactness of the foam but were stable against thermal disruption. This behaviour could be explained as follows. The addition of small amounts of the polysaccharide imparted some viscosity to the aqueous dispersions and exerted a beneficial effect on the surface active protein, but the foam formed was unstable to heat. At slightly higher concentrations of the polysaccharide, viscosity of aqueous dispersion increased significantly which partly minimised the free expansion of the foam columns, but made them more compact resulting in a decrease in foam volume but increased the thermal stability of the foam columns. Using linseed or guar gum dispersions for stabilization, the trend of foam volumes was similar with both peanut and sesame proteins.

Fig. 3 shows the protective action of the mucilage on the surface active proteins. At higher concentrations (1.5 per cent and above) of gum, the foam columns were stabilized even at high temperature. This behaviour is similar to that observed with black gram polysaccharide, the functional role of which in imparting a soft and spongy texture to leavened foods has been reported.[13]

In our studies, linseed mucilage has been prepared avoiding thermal or mechanical treatment of the seeds (as this results in the release of other constituents which could not be easily separated) in order to obtain a polysaccharide sample with significantly unaltered functional properties.

PEANUT



0 0.5 1.0 1.5

SESAME

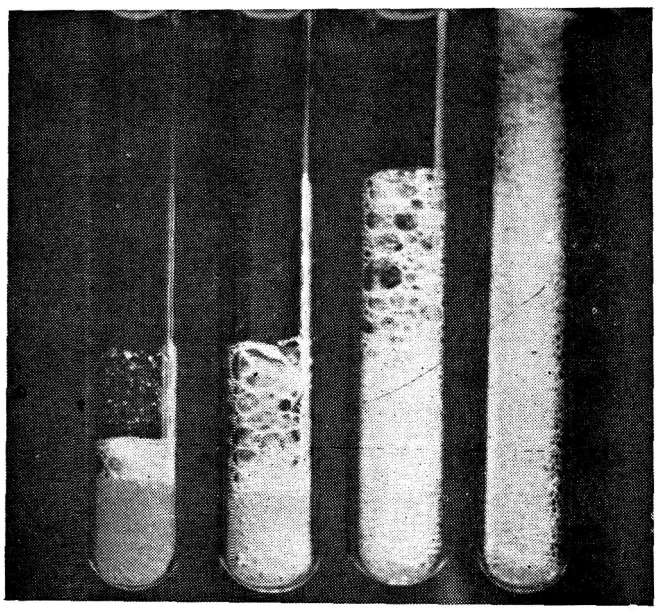


Fig. 3. Stabilization of foam formed by peanut and sesame flours by linseed polysaccharide at 0, 0.5, 1.0, and 1.5% levels against thermal disruption.

Acknowledgement

The encouragement and suggestions given during the course of this work by Dr. M. R. Raghavendra Rao are gratefully acknowledged.

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Quality of "Marsh" Grapefruit Following Cold Treatment as a Method of Disinfestation Against the Caribbean Fruit Fly[1]

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Received 5 *December* 1985; *revised* 13 *August* 1986

Fruits of 'Marsh' grapefruit (*Citrus paradisi* Macf.) were stored at 1.1°C (34°F) for 17 days as a quarantine treatment for disinfesting the Carribbean fruit fly as per regulation in the Plant Protection and Quarantine Treatment manual. Time required for cooling was different when fruits were exposed to 15.5°C (60°F) for 1 week before cold treatment compared with fruit not so exposed. Fruit conditioned at 15.5°C (60°F) for 1 week before cold treatment developed 7.3% slight chilling injury and 6.2% decay. Without conditioning, slight chilling injury was 30.3%; decay 15.0%. External fruit colour was not affected by the treatments. Weight loss was less than 3.5% throughout the storage period. Fruit quality was evaluated during subsequent storage at 20.1°C (70°F) for 3 weeks. Flavour was found to be acceptable with cold treatment fruit irrespective of pre-treatment conditioning. Ascorbic acid content at ambient temperature (21.1°C) increased. Cartons containing fruit stored at 1.1°C absorbed more moisture when transferred to 21.1°C than those stored at 12.5°C (55°F) or 21.1°C.

Florida exports approximately 7 million cartons of grapefruit to Japan annually. Currently, Florida grapefruit (*Citrus paradisi Macf*) must be fumigated with ethylene dibromide (EDB) to eliminate the possible infestations by Caribbean fruit fly (*Anastrepha suspensa* Loew).[1] However in 1980, the Environmental Protection Agency proposed a "phase out" of the use of EDB in quarantine fumigation of citrus and tropical fruits and vegetables[2]. Cold treatment and vapour heat quarantine treatments mentioned in the Plant Protection and Quarantine treatment manual are considered safe[3]. In cold treatment fruits are held at 0 to 1.6°C (32 to 35°F) for 11 to 17 days once the pulp of fruit attains this temperature. Hatton and Cubbedge[3] reported that continuous storage of early midseason and late 'Marsh' and 'Ruby Red' grapefuit at 1°C for 28 days resulted in considerable chilling injury. However, exposing the fruit for 7 days at 10°C 16°C, or 21°C before storage at 1°C significantly reduced chilling injury.

In the present investigation, the effects of cold treatment on the quality of fruits and the absorption of water by boxes have been studied with and without pre-treatment conditioning.

Materials and Methods

Commercial 'Marsh' grapefruit of size 32 prepared for market were obtained from a Lake Wales, Florida packing house. Thirty two fruits were filled in each box which subsequently were randomized in four treatments with six replications.

The treatments were as follows:

1. Stored at 21.1°C designated as "A"
2. Stored at 12.5°C designated as "B"
3. Pre-treatment conditioning at 15.5°C for one week before storing at 1.1°C for 17 days followed by storage at 21.1°C for 2 weeks designated as "C".
4. Stored at 1.1°C for 17 days followed by storage for 3 weeks at 21.1°C designated as "D".

The relative humidity within the storage room was 85-95 per cent.

A PD 2064 Exterline Augus data logger was used in recording internal fruit pulp temperature. Thermocouple sensors were inserted through the equatorial plane of the fruit and sealed with wax tape. Five sensors were used to study the thermal properties of fruit in a box and one was used to monitor the air temperature. The positions of the monitored fruit in the box were as

1 Florida Agricultural Experiment Station Journal Series No. 2.

2 Small Industries Service Institute, Madras, India.

per the specifications in the Manual on Plant Protection and Quarantine Programme[3].

The cooling rate of fruit was recorded until the internal pulp attained the desirable temperature of 1.1°C for the purpose of disinfestation and held there for 17 days. The warming rate of fruit following cold treatment was also monitored after completion of the cold treatment in separate fruits kept for this purpose.

Upon completion of the cold treatment and during subsequent storage, the fruits were examined for chilling injury, colour, weight loss and decay. Internal quality was measured in the juice by determining degree Brix, acidity, ascorbic acid and flavour.

Ten fruits from each replication were numbered and used for colour and weight loss determinations. Skin colour change was measured with a Hunterlab D25 colour difference meter and the a/b ratio values were reported. Decay caused by *Penicillium* green mold, stem end rot, sour rot and anthracnose were evaluated at weekly intervals by counting number of spoiled fruits per box and noting cumulative decay. Juice was extracted from 18 fruits (complete sample of 3 fruits from each replication) using a small laboratory hand reamer and analysed for degree Brix, acidity, ascorbic acid and flavour.

Ascorbic acid content was determined by the method suggested by the Association of Vitamin Chemists[5], degree Brix with an Abbe refractometer; acidity by titration with standard NaOH using phenolphthalein indicator; and flavour by an experienced 13 to 15-member taste panel. Evaluations were based on a 9-point Hedonic scale. Flavour data were subjected to analysis of variance. Moisture estimation was made on composite samples of cut pieces taken from the top, bottom and sides of the boxes in each treatment by drying the sample at 100°C (212°F) for 48 hr.

Results and Discussion

The desired pulp temperature (1.1°C) in the fruit was reached in 15.6-1.1-21.1°C treatment (C) and 1.1-21.1°C fruits (D) after 28 and 48 hr respectively (Fig. 1). Warming rate of both these treatments upon the completion of cold treatment was found to be the same. Cooling rate of fruits exposed to cold treatment is important in determining the holding period for fruit. The trend observed in the cooling rate conforms to that observed for other horticultural produce[6]. The longer period for cooling in the 1.1-21.1°C (D) fruit may pose a problem on commercial scale due to the build up of respiratory and field heat which eventually will require more holding time for fruit at low temperature. Therefore, it is advisable to reduce the fruit temperature in the fruit by forced air cooling before commencement

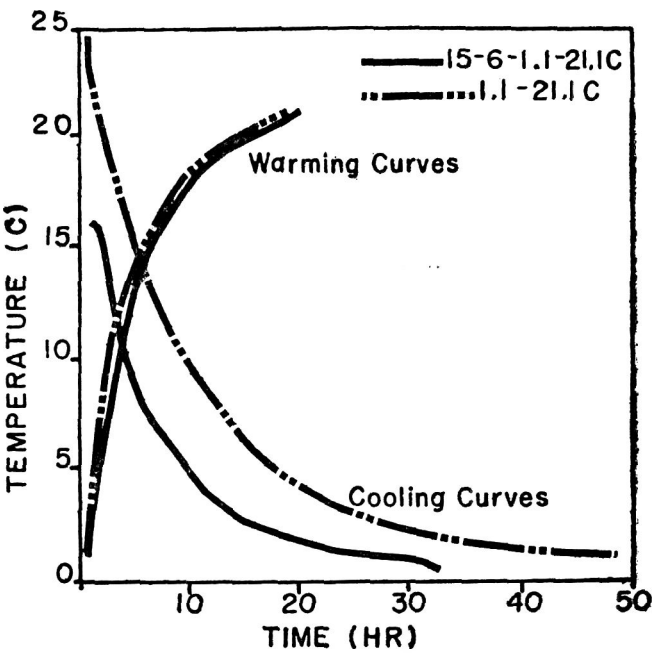

Fig. 1. Cooling and warming rates of preconditioned (14.6 – 1.1 – 21.1°C) and non-conditioned (1.1 – 12.1°C) 'Marsh' grapefruit. Preconditioning involved storage at 15.6 for 7 days prior to exposure to 1.1°C. Warming rates were measured at 21.1°C.

of cold treatment or pre-conditioning at 15.5°C for 1 week as suggested by Hatton and Cubbedge[4] to reduce chilling injury. Our studies (Table 1) also confirmed the findings of Hatton and Cubbedge that the pre-treatment conditioning of fruit produced only 7.29 per cent chilling injury against 30 per cent in case of non-preconditioned fruit.

Decay increased over a period of storage in all treatments (Table 1). The conditioning of fruit at 15.5°C

TABLE 1. PERCENTAGE DECAY DURING STORAGE AT DIFFERENT TEMPERATURES

Storage period (days)	Storage at 21.1°C (A)	12.5°C (B)	15.5-1.1-21.1°C (C)	1.1-21.1°C (D)
7	3.12a$_Z$	0.52b	3.64a	—
14	5.72a	1.04b	—	—
21	7.81a	1.56b	—	—
28	9.37a	1.56b	—	3.64a
35	12.50a	3.12b	5.20b	8.85a
42	13.02a	5.20b	6.25b	15.10a

"Z" Mean separation within each storage time by Fisher's least significant difference test p=0.05.

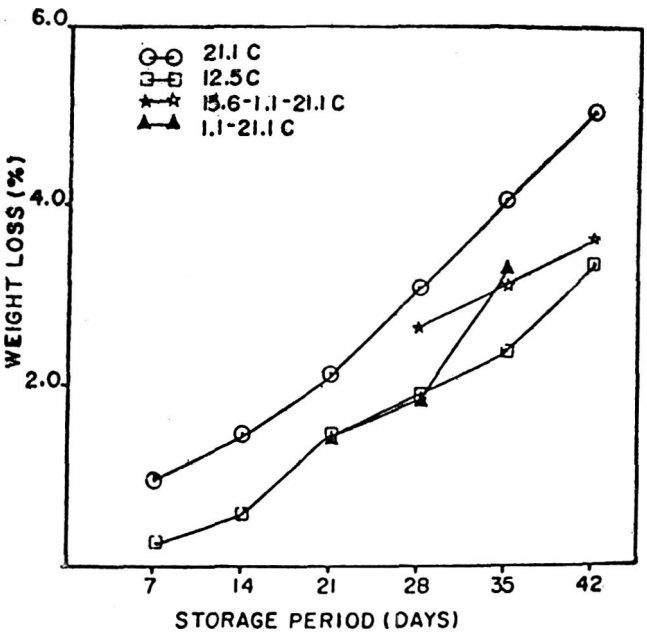

Fig. 2. Weight loss (%) of 'Marsh' grapefruit during storage of different treatments. 1) 21.1°C; 2) 12.5°C; 3) 15.6 - 1.1 - 21.1°C; 4) 1.1 - 21.1°C.

for 1 week before cold treatment reduced decay when compared with 1.1-21.1°C fruit. Pre-treatment conditioning of fruit before cold treatment seems to be advisable considering the lower percentage of decay which was comparable with the fruits stored throughout at 12.5°C. Increased decay in non-conditioned fruits may have been caused by chilling injury; this condition may have pre-disposed the fruit to invasions by mold.

Irrespective of treatments, cumulative weight loss increased over a period of storage (Fig. 2) and was at a maximum in fruit stored at ambient temperature followed by fruits that had received cold treatment and fruits stored at 12.5°C.

As expected, loss in weight did not have any effect on quality of juice in terms of degree Brix, per cent acidity, Brix/acid ratio and ascorbic acid, as most of the water was lost from the peel rather than the pulp[7].

Irrespective of treatment, there was a slight increase in skin colour values after storage for 42 days (Table 2). Cold treatment did not inhibit or impair the colour and the colour values in the 15.6-1.1-21.1°C and 1.1-21.1°C fruit were comparable to fruits stored at 12.5°C.

Regardless of treatment, changes in degree Brix and acidity during the storage period were minimal (Table 3). The values observed for 15.6-1.1-21.1°C fruit were comparable with the 1.1-21.1°C fruit. Retention of ascorbic acid is often used as an indicator of overall nutrient retention and there is a belief that other nutrients being more stable, are less affected during storage than ascorbic acid.

As noted, fruit stored at 21.1°C temperature in the present study had a stable level of ascorbic acid (Table 3). The ascorbic acid levels in other treatments were reduced slightly during storage.

During storage, flavour of juice remained acceptable in all treatments except in fruits stored at 21.1°C

TABLE 2. CHANGES IN PEEL COLOUR (a/b RATIO) DURING STORAGE AT DIFFERENT TEMPERATURES

Storage period (days)	Storage at 21.1°C (A)	12.5°C (B)	15.6–1.1–21.1°C (C)	1.1–21.1°C (D)
0	0.078a[Z]	0.071a	0.045a	0.058a
7	0.049a	0.028a	0.040a	—
14	0.036a	0.023a	—	—
21	0.053a	0.061a	0.076a	—
28	0.088a	0.065a	0.046cb	0.041bc
35	0.11a	0.088b	0.091b	0.096ab
42	0.11a	0.11a	0.12a	0.12a

Z Mean separation within each storage time by Fisher's least significant difference test, P=0.05.

TABLE 3. CHANGES IN CHEMICAL COMPOSITION OF JUICE DURING STORAGE

Storage period (days)	Storage at 21.1°C °Brix	Acid %	AA[Y]	12.5°C °Brix	Acid %	AA[Y]	15.6-1.1-21.1°C °Brix	Acid %	AA[Y]	1.1-21.1°C °Brix	Acid	AA[Y]
28	8.2	0.60	36	8.1	0.70	37				8.3	0.66	34
35	8.2	0.63	38	8.3	0.64	33	8.2	0.65	35	8.3	0.61	33
42	8.5	0.61	35	8.6	0.58	34	8.1	0.53	30	8.2	0.54	34

Initial : Brix—8.50 Acid %=0.70 Brix/Acid=12.14 AA[Y]=38 mg/100 ml Ascorbic Acid

TABLE 4. MEAN HEDONIC FLAVOUR[1] DURING STORAG AT DIFFERENT TEMPERATURES

Storage period (days)	Storage at 21.1°C	12.5°C	15.6-1.1-21.1°C	21.1°C
28	4.10[c,b]	6.40	—	6.20
35	3.80[c]	6.40	5.86	5.80
42	4.07[d,e]	6.07	6.21	5.57

1. Where 9—like extremely, 5=neither like nor dislike
1—dislike extremely.

[a]Sig. diff at 1% level from treatment D
[b]Sig. diff at 0.1% level from treatment B
[c]Sig. diff at 0.1% level from treatments B, C and D
[d]Sig. diff at 1% level from treatments B and C
[e]Sig. diff at 5% level from treatment D

(Table 4). The deleterious change in flavour of fruits stored at ambient temperature might be ascribed to sprouting of seeds.

During storage, cartons subjected to cold treatment had absorbed moisture at a maximum level (13.55 and 12.86 per cent) followed by cartons at 21.1°C (11.22 per cent) and 12.5°C (10.31 per cent). The high moisture content of these cartons was due to the condensation of moisture on fruit when they were transferred to 21.1°C. When a 2-tier stacking system was used during storage, the top portion of the bottom tier of cartons and the bottom portion of the upper tier system became damp. This was due to the percolation of condensed water from the surface of fruit in boxes from upper layers. The lower moisture in cartons used for 1.1-21.1°C fruit may be due to 3 weeks storage at 21.1°C after cold treatment as compared with 2 weeks storage for cartons used for 15.6-1.1-21.1°C fruit. This long storage period (3 weeks) at 21.1°C may have helped in evaporating some moisture from the boxes. Therefore, rate of moisture absorption by cartons under these two treatments may be considered similar.

In order to avoid the sweating of boxes, fruit must be conditioned to ambient temperature or strong cartons must be employed for packing fruit Condensation of moisture on the surface of fruit is also not desirable if fruit is to be fumigated with hydrogen cyanide (HCN) as required in Japan since the solubility of HCN will be increased and may cause injury to the fruit peel. This phenomenon is only prevalent in regions of high humidity such as Florida. Arid or dry regions may not be faced with this situation.

Acknowledgement

The senior author expresses his gratitude to the Government of India (Ministry of Industry and Ministry of Home Affairs) and to the Florida Department of Citrus for financial support during this study period.

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Metabolism of Organic Acids in Modified Atmosphere Stored Ripening Mangoes *(Mangifera indica)*

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Received 2 *April* 1986; *revised* 10 *September* 1986

^{14}C succinate, aspartate, citrate, acetate and malate were used to study the ripening behaviour of mango fruits kept under self built CO_2 atmosphere. Among the organic acids studied, citrate metabolism was totally affected as 98% of the label remained in the organic acid fraction and only less than 2% was incorporated into CO_2 as against 36% of the ^{14}C citrate incorporation into CO_2 in normally ripened fruits. The metabolism of succinate and malate was affected in fruits kept under modified atmosphere (MA) of 7 days. It, however, picked up when the fruits were transferred to ambient conditions for ripening. ^{14}C aspartate distribution into various fractions showed some deviation from the control fruits especially at the ripe stage. Acetate utilization was not disturbed in the experimental fruits as could be seen by its distribution pattern in CO_2, amino acids, organic acids, and sugars. In general, except for citrate utilization, the metabolism of fruits stored for 5 days in MA did not deviate much from the control fruits unlike the fruits stored for 7 and 13 days under MA.

Fruits after harvest undergo a series of biochemical and physiological changes during ripening and storage[1]. Various methods like irradiation, controlled or modified atmosphere (CA or MA) and low temperature storage or a combination of both are employed to extend the ripening and the storage life[2].

There are reports indicating accumulation of succinic, malic, maleic and some amino acids in certain fruits stored under CA with various CO_2 concentrations[3,4]. Low O_2 and high CO_2 levels retarded starch, pectin and chlorophyll degradation in tomatoes, apples and peaches respectively[5-7]. Inhibitory effect of CO_2 on some mitochondrial enzymes has been noted in a few fruits[8,9]. In mangoes treated for storage extension, retardation of chlorophyll disappearance, changes in sugar levels and total soluble solids (TSS) have been recorded[10,11].

Research on MA stored fruits is so far restricted to determinations of carbohydrate, sugar, acid levels, etc. No biochemical studies have been reported using tracer compounds. It is important to understand the mechanism underlying extended ripening by comparing the biochemical behaviour of normally ripened fruits and MA stored fruits. Work was, therefore, undertaken to focus attention on organic acid metabolism in MA stored ripening mangoes using labelled organic acids.

Materials and Methods

Mature mangoes (*Mangifera indica*) harvested fresh were washed, dipped in Benlate (1000 ppm for 5 min), dried and were allowed to ripen in ventilated crates at room temperature (26±2°C). Experimental fruits were kept in the same type of crates (40 fruits/crate) but covered air tight with a polythene film (150 gauge) to create a modified atmosphere with endogenously generated CO_2. Provision was made for taking out gas samples from the atmosphere in the crate. Gas was analysed every day to know the rate of CO_2 built in the crates by the fruits. CO_2 and O_2 were analysed by GLC on a column of porapak with injector and detector temperature of 40°C and N_2 flow rate of 25 ml/min. After a specified period of exposure of the fruits to the MA(5, 7 and 13 days) the polythene films were removed and the fruits were stored in the same crate for subsequent ripening.

Labelled compounds were suitably diluted in 0.4M mannitol to give 1.8 μci of radioactivity/0.05 ml. The radioactive material thus prepared was injected into the fruit with a syringe (0.05 ml) by vacuum infiltration. The amount of label entering into each fruit was calculated by subtracting the counts of syringe wash from the total counts taken initially in the syringe for injecting into the fruit. Thus, the total amount of activity injected in each fruit was accounted for[12].

The fruits injected with labelled compounds were transferred to desiccators individually fitted with an inlet for air. The $^{14}CO_2$ respired was trapped for $7\frac{1}{2}$ hr in 3N NaOH by applying suction[13]. After collecting $^{14}CO_2$ for $7\frac{1}{2}$ hr, the fruits were removed from the desiccators. The pulp was homogenized and 25 g of pulp was

fixed in alcohol. Amino acids, organic acids and sugars were extracted thrice in 90, 70 and 60 per cent simmering alcohol for 60,30 and 20 min respectively. The alcohol extracts were pooled, concentrated to 10 ml and centrifuged. The clear supernatant was separated into amino acid, organic acid and sugar fractions by ion exchange column chromatography. Each fraction was suitably concentrated and 0.5 ml was taken in 10 ml of scintillation medium and counted in a liquid scintillation counter. The $^{14}CO_2$ fraction collected in NaOH was precipitated with $BaCl_2$ as Ba $^{14}CO_3$ was analysed for radioactivity[12].

Results

The distribution pattern of the labelled compounds in normally ripened mango fruits and in fruits ripened after controlled storage conditions (fruits exposed for 5 days in MA) is compared in Table 1. The interconversion of ^{14}C succinate and ^{14}C malate into CO_2, amino acid and sugar fractions of mango were not very much different at raw and ripe stage in control fruits. Labelled aspartate, citrate and acetate entering into sugar fractions was more at the ripe stage than at the raw stage. The conversion of ^{14}C citrate into $^{14}CO_2$ decreased from 36 to 13 per cent in the ripe fruit. On the other hand, the label conversion from acetate to CO_2 increased from 27 to 42 per cent in the ripe stage. The distribution of label in organic acids in the experimental fruits (stored for 5 days in MA) (Table 2) did not, in general, deviate much from the control fruits except for ^{14}C citrate incorporation which was depressed quite significantly.

In fruits kept for 7 days under MA (Table 3), the conversion of citrate into CO_2, amino acid and sugar fraction was drastically curtailed and was of the order of 1,8,0.9 and 1.0 per cent as against 36.4 and 7.4 per cent in the control fruits. Even on ripening, the fruits showed the same trend of depressed citrate conversion. On the other hand, ^{14}C succinate and ^{14}C malate label distribution though altered in the experimental fruits (raw state) showed higher inter-conversion on ripening as evidenced by the per cent incorporation values. In ripe fruits, the conversion of aspartate was somewhat deperssed in the experimental fruits when compared

TABLE 1. DISTRIBUTION OF PER CENT RADIO ACTIVITY IN VARIOUS FRACTIONS OF MANGO INJECTED WITH LABELLED ACIDS (CONTROL)

Fruit type	CO_2	Amino acid	Organic acid	Sugar	Total counts (μ ci)
		1-4-^{14}C-Succinate			
Raw	45.4±3.1	2.7±0.1	27.9±0.8	24.0±2.0	0.95
Ripe	45.2±4.6	1.7±0.1	24.8±2.3	28.1±1.9	0.88
		U-^{14}C-Aspartate			
Raw	22.8±0.6	47.4±2.8	15.1±1.3	14.6±1.0	1.00
Ripe	25.8±2.5	35.0±2.0	15.3±1.3	23.0±1.7	0.87
		1-5-^{14}C-Citrate			
Raw	36.0±1.0	4.1±0.4	52.5±2.8	7.4±1.0	0.85
Ripe	13.1±1.1	5.9±0.6	65.2±5.4	15.2±0.5	0.70
		U-^{14}C-Acetate			
Raw	27.7±1.1	5.1±0.8	47.9±3.5	19.3±1.5	0.72
Ripe	42.1±3.5	7.8±0.8	11.3±1.5	38.6±3.0	0.50
		U-^{14}C-Malate			
Raw	27.6±2.0	4.0±0.8	43.6±3.8	24.7±1.6	1.15
Ripe	22.8±3.2	5.1±0.6	51.1±3.6	20.9±2.2	0.98

*The fruits in this group did not ripen normally after removal from MA storage.

The values are averages of 6 fruits.

Raw—Control fruits were taken 1 day after harvest. In case of experimental, the fruits were taken immediately after removal from MA.

Ripe—The fruits taken after subsequent ripening at ambient temperature.

TABLE 2. PER CENT RADIO ACTIVITY IN FRUITS REMOVED AFTER FIVE DAYS EXPOSURE TO MODIFIED ATMOSPHERE

Fruit type	CO_2	Amino acid	Organic acid	Sugar	Total counts (μ ci)
		1-4-^{14}C-Succinate			
Raw	42.0±2.4	1.6±0.1	35.4±1.9	20.0±1.2	1.00
Ripe	44.5±4.6	2.1±0.1	27.0±1.4	26.4±1.8	0.86
		U-^{14}C-Aspartate			
Raw	25.0±1.7	51.1±3.2	10.1±0.9	13.9±0.8	0.90
Ripe	21.5±3.0	35.3±1.9	9.2±0.8	24.0±3.1	0.86
		1-5-^{14}C-Citrate			
Raw	10.2±0.9	1.9±0.2	84.5±2.4	4.5±0.5	0.81
Ripe	11.4±1.1	2.4±0.2	83.0±3.6	3.2±0.2	0.63
		U-^{41}C-Acetate			
Raw	22.0±1.2	8.3±0.8	46.1±3.4	23.7±3.8	0.59
Ripe	43.5±5.2	9.0±1.0	11.0±1.3	37.4±1.6	0.41
		U-^{14}C-Malate			
Raw	16.1±1.0	2.9±0.1	72.0±4.0	9.1±0.7	1.04
Ripe	26.1±2.3	5.1±0.2	50.3±2.0	19.1±1.4	0.91

*Foot Note as in Table 1.

TABLE 3. PER CENT RADIO ACTIVITY IN FRUITS REMOVED AFTER SEVEN DAYS OF EXPOSURE TO MODIFIED ATMOSPHERE

Fruit type	CO_2	Amino acid	Organic acid	Sugar	Total counts (μ ci)
		1-4-^{14}C-Succinate			
Raw	37.7±3.5	0.7±0.1	48.9±2.7	12.7±1.2	0.97
Ripe	45.7±1.8	3.0±0.2	32.7±2.5	18.5±1.9	0.90
		U-^{14}C-Aspartate			
Raw	26.4±2.2	53.9±1.8	11.8±1.3	7.9±1.0	0.85
Ripe	16.4±1.5	73.2±2.9	4.6±0.9	5.7±0.9	0.84
		1-5-^{14}C-Citrate			
Raw	9.9±0.1	1.0±0.1	95.5±4.8	1.9±0.1	0.88
Ripe	2.2±0.1	1.2±0.2	95.1±4.6	1.5±0.1	0.70
		U-^{14}C-Acetate			
Raw	13.8±2.0	12.6±1.6	52.3±3.6	21.2±2.1	0.60
Ripe	36.2±2.0	15.8±1.0	16.3±2.2	31.4±1.6	0.58
		U-^{14}C-Malate			
Raw	4.5±0.8	1.1±0.1	90.2±3.5	4.2±0.7	1.00
Ripe	26.8±2.0	2.5±0.1	54.9±3.1	15.7±2.0	1.21

*Foot Note as in Table 1.

TABLE 4. PER CENT RADIO ACTIVITY IN RAW MANGO FRUITS REMOVED AFTER 13 DAYS OF EXPOSURE TO MODIFIED ATMOSPHERE*

CO_2	Amino acid	Organic acid	Sugar	Total counts (μ ci)
		1-4-^{14}C-Succinate		
30.4±1.0	2.8±0.5	54.2±0.8	12.6±1.1	1.40
		U-^{14}C-Aspartate		
6.7±1.0	79.4±5.0	6.9±0.8	7.1±0.8	1.22
		1-5-^{14}-CCitrate		
0.5±0.1	1.0±0.1	97.5±3.6	1.0±0.1	1.81
		U-^{14}C-Acetate		
10.8±1.1	9.0±1.6	16.3±1.7	63.4±3.4	1.10
		U-^{14}C-Malate		
17.3±1.5	3.1±0.5	72.8±2.9	6.9±1.0	1.40

Foot note as in Table 1.

to controls. 14C acetate label distribution was not affected in the experimental fruits at both raw and ripe stages.

The label distribution in fruits (raw) stored for 13 days in MA (Table 4) was different from that in fruits (raw) stored for 7 days (Table 3). It rather resembled the distribution pattern of ripe fruits (Table 2). It must be mentioned here that these fruits were already soft and resembled ripe fruits more than the raw fruits in taste and colour at the time of their removal from the closed atmosphere. There was no clear out demarkation between raw and ripe stages and the fruits exhibited irregular, non-uniform ripening when they were exposed to MA for 13 days. Here, the fruits showed an even more suppressed distribution pattern than the fruits ripened after 7 days exposure to MA. Unlike the other labelled compounds used in the present study, the ^{14}C acetate conversion to sugar fraction in 13 days stored fruits (63 per cent) was about two times higher than control ripe fruits (38 per cent) as experimental ripe fruits stored for 7 days in MA (31 per cent).

The trend of increase in CO_2 levels and decrease in O_2 levels in the endogenously generated atmosphere around the fruits is given in Fig. 1. The CO_2 and O_2 levels were 4.4 and 9 per cent respectively in the 5 day stored fruits. The levels changed to 7.6 and 5 per cent at 7 day storage. The fruits stored for 14 days built more than 16 per cent CO_2 and had less than 2 per cent O_2.

The fruits exposed for 5 days to MA ripened normally and visual observations revealed that ripening was extended by 4 to 5 days.

Discussion

The results indicate a somewhat undisturbed metabolism of acetate and a completely altered metabolism of citrate in mangoes kept for 7 days in MA atmosphere (7 per cent CO_2 and 4 per cent O_2). Succinate and malate utilization though disturbed in the fruits which were removed freshly from the MA, showed recovered metabolism on subsequent ripening. It is also clear that fruits which had built 4-5 per cent CO_2 and 8-9 per cent O_2 showed almost normal metabolism with respect

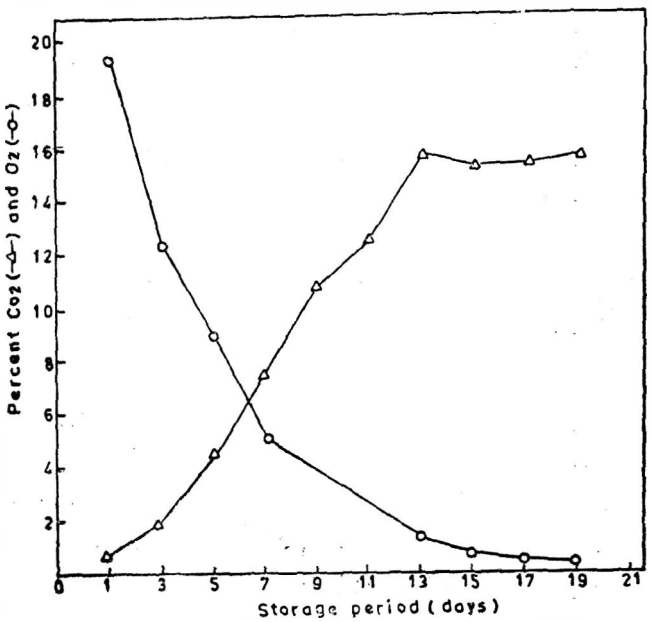

Fig. 1. Endogenous levels of CO_2 and O_2 built within mango crates kept sealed in polyethylene bags.

to acetate, succinate, malate and aspartate and hindered metabolism with respect to citrate. But much higher CO_2 levels (7 per cent and above) and lower O_2 levels (less than 5 per cent) caused a marked deviation in the metabolism of tnese organic acids when compared to control fruits.

Valmayor[14] obtained a significant extension of ripening of mangoes when stored at 5 per cent CO_2 level at 10°C.

Lakshminarayan and Subramanyam[10] observed fermentative decarboxylation at higher CO_2 concentration which was thought to be toxic to mango fruit. High CO_2 levels offset tricarboxylic acid metabolism in some plants[15]. Inhibition of some enzymes like succinate oxidase and succinic dehydrogenase by CO_2 and inefficient cytochrome oxidase system under low O_2 levels have been reported[9]. CO_2 has been stated to be a competitive inhibitor of C_2H_4, a ripening hormone. Further, it is thought that O_2 is required for the conversion of l-amino cyclo propane l-carboxylic acid a final step in C_2H_4 biosynthesis from methionine[16].

Accumulation of organic acids in MA stored fruits[3,4] is compatible with poor utilization of the labelled acids found in our experiment. It is worth looking into the mitochondrial enzyme levels in these fruits and the inhibitory effect of CO_2 on different enzyme systems as citrate utilization was specifically affected even when the fruits were kept for a minimum period (5 days with 4 per cent CO_2 and 9 per cent C_2 levels) indicating some deviation in the normal TCA cycle.

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Biochemical Changes Associated with Ripening of Cheddar Cheese from Buffalo Milk: The Lipid Composition of Buffalo Milk and Zero-Day Curd

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Received 28 *May* 1986; *revised* 28 *August* 1986

Buffalo milk was curdled by presalting method using a starter culture 'Y' (*S. lactis*+*S. cremoris*+*S. lactis* subsp. *diacetylactis*). Analysis of total lipids of buffalo milk and zero-day cheese revealed more than 98% neutral lipids and less than 1% phospholipids. Neutral lipids consisted chiefly of triglycerides with smaller amounts of di- and monoglycerides. The major component phospholipids were phosphatidyl choline, phosphatidyl ethanolamine and sphingomyelin with small amounts of phosphatidyl serine and phosphatidyl inositol. Palmitic acid was the major saturated fatty acid in total and neutral lipids and free fatty acids of milk cheese; the unsaturated fatty acids detected in the total and neutral lipids of milk and cheese were 14:1, 16:1, 18:1, 18:2, 18:3. In free fatty acids, the only unsaturated fatty acid present was oleic acid. Free fatty acids amounted to 0.95 μeq/ml in buffalo milk while in zero-day cheese, it was 33.6 μg/g . Lipase activity in buffalo milk was found to be 1.25 μeq FFA ml milk^{-1} hr^{-1} and it was inactivated at pasteurization tempertature.

Attempts to prepare Cheddar cheese from buffalo milk[1–4] have not yielded a product comparable to that from cow milk. Buffalo milk cheese has a hard and dry body with a short and crumbly texture and high fat content. Further, it has a slow ripening capacity and flat flavour. In India, 60 per cent of the total milk produced is from buffalo milk and in many other countries, buffalo is an important dairy animal. This prompted us to investigate the ripening of buffalo milk Cheddar cheese in greater detail.

The present study deals with the manufacture of Cheddar cheese and compositional studies of buffalo milk and zero-day cheese. Emphasis is on the lipid composition since they have a pronounced effect on the rheological as well as flavour properties.

Materials and Methods

Cow butter oil was purchased from Indian Dairy Corporation. Fatty acid methyl esters, tripalmitin, sodium metaperiodate and silicic acid (100-200 mesh) were of Sigma, U.S.A. N-methyl urea was of Fluka AG, Switzerland and BF_3 methanol reagent, Silica gel G and chromotropic acid were from E. Merck, W. Germany. Hydrazine sulphate, lithium lactate, sodium molybdate, cholesterol were purchased from Loba Chemicals and gum acacia from S.D. Fine Chemicals, Bombay. Calf rennet powder was procured from the Christan Hanson's Laboratory, Denmark. Salt was of commercial grade. Buffalo milk (Murrah) and starter cultures named 'Y' (*Streptococcus lactis*+*S. cremoris*+*S. lactis* subsp. *diacetylactis*) were obtained from the National Dairy Research Institute Karnal. All other chemicals and solvents were of analytical reagent grade. The solvents were freshly distilled or purified before use.

Preparation of cheese: Cheddar cheese from 90 kg of buffalo milk was prepared by presalting method as described in Table 1. Cheddar cheese prepared from cow's milk with no presalting acted as control in all studies. The cheese blocks were paraffin waxed and stored at 8±1°C for further studies.

Extraction and separation of lipids from milk and zero-day cheese: The lipids were extracted from 10 g of milk using 20 volumes of chloroform: methanol (2:1, v/v) at room temperature under nitrogen for 12 hr with occasional shaking. The solvent was filtered on a Buchner funnel and the residue was extracted twice more with 5 vol of the same solvent for 6 and 3 hr. The three extracts were combined and then washed with 0.2 volumes of 0.37 per cent KCl[5]. The lower organic layer containing the lipid was removed and the upper

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NDRI Publication No. 86-60.

TABLE 1. DETAILS OF PREPARATION OF CHEDDAR CHEESE FROM BUFFALO MILK BY PRESALTING METHOD

Particulars	Av. value of 3 trials
Quantity of milk (kg)	90
C/F ratio	0.7
Pasturization temp/time (°C/min)	63/30
Acidity of milk (% lactic acid, LA)	0.16
Addition of salt at 45°C (g)	900
Setting temp. (°C)	28
Starter addition (%)	1.5
Quantity of rennet (g)	2.25
Setting time (min.)	55
Whey acidity at cutting (% LA)	0.12
Maximum cooking temp. (°C)	38.5
Total cooking time (min)	45
Whey acidity at draining (% LA)	0.145
Cheddaring time (min)	180
Milling acidity (% LA)	0.45
Amount of green cheese (kg)	13.8
Rate of salt addition (%)	2
Mellowing time (min)	15
Zero-day cheese yield/100 kg milk (kg)	13.2
Ripening conditions, Temp. (°C)	8±1
R. H. (%)	80–90

water phase washed twice with 0.1 volume of the pure solvent lower phase of the Folch system. The lipid extract was dried over anhydrous Na_2SO_4. The solvent was evaporated and the total lipids estimated gravimetrically. For zero-day cheese, 10 g of milk curd was ground with 25 ml of chloroform: methanol in a pestle and mortar and the slurry obtained was used for the extraction of lipids.

Total lipids were fractionated into neutral and phospholipids by silicic acid column chromatography[6]. The solvents were evaporated and the weight of each class of lipid was determined. Separation of lipid classes into component lipids was achieved by thin-layer chromatography (TLC) using 0.5 mm thick layer of silica gel G on glass plates activated at 110°C for 1 hr. The lipids of milk and zero-day cheese were freed of cholesterol by treatment with 1 per cent digitonin solution at 60-70°C for 10 min. The precipitate was removed by filtration and the filtrate was evaporated to recover cholesterol free fat. Neutral lipids were separated into different glyceride fractions using the solvent mixture of hexane, ethyl ether and acetic acid (70:30:1, by vol) for development. Phospholipids were separated by the two dimensional technique of Rouser, *et al.*[7]

Identification and characterization of spots: Visualization of different spots was carried out by spraying the plates with 40 per cent sulphuric acid and charring at 180°C for 30 min. The mobilities of unknown spots were compared with those of authentic samples run under identical conditions. The different glyceride fractions were located with iodine vapour. The areas were circumscribed and scraped into a centrifuge vial and assayed by the method of Van-Handel and Zilversmith[8]. Phospholipids were quantified through estimation of their phosphorus content by Ahovcova and Odavic[9] method and multiplying by 25. Cholesterol content of milk and zero-day cheese was determined by the method of Abell *et al*[10]. Free fatty acids in milk were estimated according to Deeth, Fitz-Gerald and Wood[11] and those from zero-day cheese as follows:

Grated cheese packed into centrifuge tubes were kept in a water bath at 50-55°C for 1 hr. The tubes were centrifuged at 1,500×g for 5 min and fat was decanted. Two grams of cheese fat was dissolved in 25 ml n-hexane and titrated against 0.01 N KOH in methanol. The results are expressed as μg of free fatty acids (FFA) as decanoic acid/g cheese.

Analysis of methyl esters: Methyl esters of the total and neutral lipids were prepared by the method of deMan[12] and those of FFA according to Chapman[13]. They were separated on a 1 mm×4 mm glass column packed with 20 per cent DEGS on Diatomite C-AN (100-200 mesh) in a Pyeunicham series 304 dual column gas chromatograph equipped with a FID, temperature programmer and CDPI Integrator under the following conditions:

Initial temp.	=	70°C
Initial time	=	2.5 min
Rate of increase in temp. (I)	=	20°C/min
Upper temp. (I)	=	155°C
Rate of increase in temp. (II)	=	20°C/min
Upper temp. (II)	=	185°C
Upper time (II)	=	6 min
Rate of increase in temp. (III)	=	20°C/min
Upper temp. (III)	=	198°C
Upper time (III)	=	10 min

The fatty acid methyl esters were eluted with nitrogen at a flow rate of 30 ml/min. Peaks were identified by comparing the retention time of the component fatty acids with those of known standards.

Lipase activity in milk and zero-day cheese: Lipase activity in milk and zero day cheese extract prepared by the method of Umemoto and Sato[14] was determined according to Deeth and Fitz-Gerald[15]. The results are expressed as μeq FFA/g protein/hr.

Analytical methods: pH of the cheese samples was determined as suggested by O'Keeffe *et al*[16]. after

making a paste of 10 g cheese with 10 ml distilled water. The moisture content of cheese was determined according to a standard method[17]. The titrable acidity and salt were quantified according to AOAC[18] and for lactic acid, the method of Harper and Randolph[19] was used. Fat in milk and cheese samples was quantified by the Gerber method[20] and casein was determined by formol titration[21]. Protein was estimated by micro-Kjeldahl method of Maneffee and Overman[22].

Results and Discussion

Starter bacteria: Though considerable work has been done on the starter bacteria to be used for the manufacture of Cheddar cheese from cow's milk, similar studies for making Cheddar cheese from buffalo milk are few. A systematic study was carried out in which different combinations of commercial starter cultures (CH4, 48, 70, 72, 253) and pure single strain cultures (*S. lactis*, *S. cremoris*, *S. lactis* subsp. *diacetylactis*) were employed to make Cheddar cheese from buffalo milk. Seventy batches of Cheddar cheese were prepared by Czulak method[3] and presalting method separately (Table 1). The latter method proved to be good and starter culture named 'Y' gave good cheese.

Composition of buffalo milk zero-day cheese: In hard cheeses, like Cheddar, Hargrove and Alfred[23] have reported typical analysis (in per cent) figures of moisture 37, fat 32, protein 22 and salt 1.6 for the ripening process. Buffalo cheese prepared by presalting method in the present study showed 39.5 per cent moisture on zero-day which advantage was retained throughout the ripening period of 8-9 months[24]. Analysis of buffalo milk cheese showed (in per cent) protein 23.25, fat 29.50, salt 1.90, titrable acidity (per cent lactic acid) 0.58, lactic acid 0.28 and pH 5.25. The corresponding figures for cow's milk cheese were 22.50, 32.0, 1.75, 0.66, 0.28 and 5.26 per cent respectively.

Lipid composition: On weight basis, the total lipid content of buffalo milk worked out to be 6.88 per cent which is 1.8 times that found in cow's milk and is in agreement with the results published earlier[25]. Buffalo and cow's milks and zero-day cheeses had very similar distribution of component neutral lipids. The total triglycerides, (tri-, di-and mono-) constituted about 98 per cent with triglycerides present as the major component neutral lipid. Total cholesterol and free fatty acid concentrations were slightly lower than in cow's milk and a similar trend was observed in the milk curds of the two species (Table 2).

TLC analysis of phospholipids of buffalo and cow milks indicated the presence of phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl serine, phosphatidyl inositol and sphingomyelin and their

TABLE 2. LIPID CONSTITUENTS OF MILK AND ZERO-DAY CHEDDAR CHEESE

	Milk (g/100g)		0-day cheese (g/100g)	
	Buffalo	Cow	Buffalo	Cow
Total lipids	6.88	3.80	29.50	32.00
	% of total lipids			
Neutral lipids	98.45	98.20	98.40	98.12
Phospholipids	0.62	0.58	0.62	0.58
	% of neutral lipids			
Triglycerides	93.91	93.85	93.64	93.56
Diglycerides	3.70	3.40	4.00	3.72
Monoglycerides	0.49	0.60	0.42	0.52
Cholesterol (Total)	0.30	0.39	0.30	0.38
Free fatty acids (μeq/ml milk; μg decanoate/g cheese)	0.95	1.02	33.57	52.29
	% of phospholipids			
Phosphatidyl ethanolamine	29.80	31.00	29.50	30.30
Phosphatidyl choline	28.80	33.50	28.40	33.40
Phosphatidyl serine	4.20	3.60	4.20	3.80
Phosphatidyl inositol	3.80	4.00	3.85	4.00
Sphingomyelin	29.80	24.00	29.70	23.70

TABLE 3. FATTY ACID (PER CENT OF TOTAL) COMPOSITION OF ZERO-DAY CHEDDAR CHEESE TOTAL LIPIDS*

Fatty acid	Buffalo milk	Buffalo milk 0-day cheese	Cow milk	Cow milk cheese
4:0	5.4	5.8	4.1	4.2
6:0	2.0	2.1	2.4	3.0
8:0	0.9	2.9	1.4	1.1
10:0	1.6	0.7	1.8	1.9
12:0	2.3	2.1	2.5	2.4
14:0	12.1	11.7	12.3	12.4
14:1	1.2	1.8	1.5	1.3
15:0	0.9	0.8	0.8	0.6
16:0	37.2	34.6	35.0	33.9
16:1	2.5	1.1	3.4	2.2
18:0	11.1	10.2	10.7	11.1
18:1	21.1	24.1	22.3	24.0
18:2	1.0	1.3	1.4	1.1
18:3	0.7	0.8	0.4	8.8

*Values are average of 3 batches of Cheddar cheese analysed in duplicate.

quantitative distribution is presented in Table 2. Similar data for component phospholipids (Table 2) were also obtained for zero-day cheeses which would indicate that phospholipids were retained in the curd along with the glycerides.

Fatty acid composition: Analysis of methyl esters of fatty acids of total and neutral lipids revealed similar distribution in milks of buffalo and cow and their corresponding zero-day cheeses (Tables 3 and 4). The principal acids present were 4:0, 14:0, 16:0, 18:0, 18:1 in both total fat and neutral fat. Rama Murthy and Narayanan[26] have, however, reported that the fatty acids 4:0, 16:0 and 18:0 were distinctly higher and the fatty acids 6:0, 8:0, 10:0 12:0 and 14:0 were significantly lower in buffalo than in cow's milk total lipids.

Analysis of free fatty acids revealed 4:0 and 18:1 to occur at higher concentrations in buffalo milk, while 6:0 and 14:0 were in larger proportions in cow milk. The fatty acid composition of zero-day cheese was observed to be identical to that of milk in both species (Table 5).

Lipase activity of milk (E.C. 3.1.1.3.): Cow milk showed a slightly higher lipase activity (1.85 μeq FFA/ml milk/hr). Milk lipase is normally inactivated at pasteurization temperatures[27]. Pasteurization at 63°C for 30 min inactivated the lipase activities in the milks of both the species.

The foregoing studies reveal that the curdling of buffalo milk by presalting method using starter culture 'Y' gave good acid production and adequate moisture retention. Glyceride fraction constitutes the major lipid of buffalo milk which was retained in the curd as well. Phospholipids constituting less than 1 per cent of the total lipids of buffalo milk and curd and the milk lipase are likely to have no role during ripening.

TABLE 4. FATTY ACID (PER CENT OF TOTAL) COMPOSITION OF MILK AND ZERO-DAYCHEDDAR CHFESE NEUTRAL LIPIDS*

Fatty acid	Buffalo milk	Buffalo milk 0-day cheese	Cow milk	Cow milk 0-day cheese
4:0	8.3	8.0	7.6	6.5
6:0	3.3	3.2	3.4	3.3
8:0	2.0	2.2	1.8	2.0
10:0	2.7	1.9	3.4	3.3
12:0	2.5	2.9	2.4	3.0
14:0	13.4	11.3	15.0	13.3
14:1	1.5	2.0	1.3	1.2
15:0	1.4	1.0	0.9	0.8
16:0	34.4	35.8	32.4	33.4
16:1	2.0	1.1	1.8	1.2
18:0	7.9	8.4	7.5	7.7
18:1	18.5	20.5	20.2	22.5
18:2	1.6	1.3	1.3	1.1
18:3	0.5	0.6	0.3	0.8

*Values are average of 3 batches of Cheddar cheese analysed in duplicate.

TABLE 5. FREE FATTY ACID (PER CENT OF TOTAL) COMPOSITION OF MILK AND ZERO-DAY CHEDDAR CHEESE*

Fatty acid	Buffalo milk	Buffalo milk 0-day cheese	Cow milk	Cow milk 0-day cheese
4:0	15.1	11.5	10.8	10.4
6:0	4.6	3.3	11.5	12.1
8:0	3.3	2.6	3.6	3.7
10:0	4.5	3.4	4.6	3.6
12:0	4.5	4.3	4.4	3.9
14:0	3.8	10.6	10.6	13.9
16:0	37.3	38.8	35.7	32.1
18:0	8.7	7.3	6.3	5.5
18:1	18.1	18.3	12.6	12.9

*Values are average of 3 batches of Cheddar cheese analysed in duplicate

Acknowledgement

The award of Senior Research Fellowship to one of us (A.V.) under the United Nations Development Programme is gratefully acknowledged.

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ERRATA

Vol. 24 No. 2, Page 61, Paper entitled "Physical Properties of Unsweetened Soymilk". by R. R. Lal, S. C. B. Siripurapu and B. P. N. Singh.
In Fig. 1. X-axis numbers **should be 0, 10, 20, 30 ....100** instead of 1.0, 2.0. 3.0 ....10.0

Biochemical Changes Associated with Ripening of Cheddar Cheese from Buffalo Milk: Ripening Changes at 8°C

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Received 28 *May* 1986; *revised* 28 *August* 1986

Buffalo milk Cheddar cheese was ripened at 8±1°C. The changes observed in moisture content, titrable acidity, pH and lactic acid formation were comparable to those in cow milk Cheddar cheese prepared and ripened under identical conditions. Per cent decrease in total glycerides and triglycerides in buffalo milk cheese was 1.02 and 4.11 compared to 1.66 and 4.36 for cow milk cheese. Soluble nitrogen (mg tyrosine/g cheese) was lower for buffalo milk cheese than cow milk cheese. Similarly, the active -SH group at 6 months of ripening was 20 μg cysteine-HCl/g cheese for buffalo milk cheese and 32 μg for cow milk cheese. Buffalo milk cheese lipase and proteinase had lower levels of activity than their counterparts in cow milk cheese. At 9 months of ripening, 206.6 μg free fatty acids (FFA)/g were released in buffalo milk cheese compared to 280.7 μg FFA/g in cow milk cheese. Maximum proteinase activity in cow milk cheese (1.5 hide powder azure units/g protein) developed in 6 months was 1.5 times that in buffalo milk cheese. Typical Cheddar flavour in cow's milk cheese was evident by 6 months of ripening while buffalo milk cheese had developed mild flavour at this time and desired flavour at 7 months. Body and texture, were however, not satisfactory.

In cheese making, the porteins, lipids and carbohydrates present in milk are broken down to simpler substances during ripening. The agents responsible for ripening are the native milk enzymes, rennet and other adventitious organisms like starter bacteria. It is obvious that the individual biological entities (proteins, lipids, carbohydrates) or their metabolites in the total cheese system may not operate in isolation; they would interact with one another. The appearance and disappearance of metabolites of the three major macromolecules involving definite biochemical pathways may be subject to regulatory control[1]. Such changes may eventually lead to a condition in the total cheese system which may be described biochemically as "steady state". The breakdown of lipids, proteins and carbohydrates during the ripening of buffalo milk cheese at 8±1°C are described and for comparison similar studies in cheese made from cow milk are reported in this paper.

Materials and Methods

Hide powder azure (HPA) was obtained from Sigma, U.S.A. L-cysteine hydrochloride and L-tyrosine were obtained from Loba Chemicals, Bombay. The source of all other chemicals was the same as described in the earlier paper[2].

Preparation of cheese: Buffalo milk Cheddar cheese was prepared by presaiting method using starter culture 'Y' as described earlier[2]. Cheddar cheese prepared from cow's milk using the same culture but no presalting served as control. The cheese blocks were paraffin waxed and stored at 8±1°C and samples were taken for chemical analysis and organoleptic evaluation at one month intervals.

Lipid analysis and analytical methods: The methods for the estimation of glycerides (tri, di, mono), free fatty acids, their conversion to methyl esters and analysis by GLC, pH, moisture content, titrable acidity and lactic acid content have been described earlier[2].

Enzyme assays: Lipase activity was determined according to the method of Deeth and Fitz-Gerald[3] using cheese extract (2 ml) prepared according to Umemoto and Sato[4]. The released fatty acids were extracted and titrated by the method of Deeth, Fitz-Gerald and Wood[5]. The results are expressed as μeq free fatty acids (FFA)g protein/hr.

Proteinase activity was measured by using hide powder azure (HPA) as the substrate[6]. One unit of proteinase activity was defined as the amount of enzyme which would release dye-labelled soluble peptides from HPA in 1 hr to give optical density of 0.25 at 595 nm. The results are expressed as HPA units/g protein.

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NDRI Publication No. 86-61.

Estimation of soluble nitrogen constituents and active -SH groups in cheese: TCA soluble fraction was obtained by the method of Dulley[7]. An aliquot of TCA soluble fraction was taken and tyrosine content was measured by the method of Lowry *et al*[8] and expressed as mg tyrosine/g cheese. The acetate buffer (pH 4.6)[9] extract was prepared from 2 g cheese blended with 25 ml of buffer at 45°C for 1 min on Sorvall Omnimixer setting 7. This was then centrifuged at 4°C to remove the fat and supernatant was filtered through Whatman filter paper; an aliquot was taken for determining tyrosine by the method of Lowry *et al*[8].

Active -SH groups were determined by a modification of the DTNB (5, 5'-dithiobis 2-nitrobenzoic acid) procedure for heated skim milk[10]. Cheese (1 g) was made into a slurry with 8.9 ml 2 per cent sodium citrate and 0.1 ml of DTNB solution (39.60 mg in 10 ml 0.1M KPO_4 buffer, pH 7.0) was added. The mixture was stirred and after 20 min 3 g of ammonium sulphate was added to precipitate the proteins. The mixture was filtered through Whatman No. 42 filter paper and optical density of the filtrate was determined at 412 nm. The concentration of active -SH groups was determined from a standard curve prepared with cysteine-HCl. Results are expressed as μg cysteine-HCl/g cheese.

Flavour assessment: After 3 months of ripening of the cheese, flavour, body and texture evaluation were carried out at one month intervals by an expert panel of five judges. The samples taken from salted and unsalted cheese of buffalo milk and cow milk were judged for defects in 3 different batches. Ideal score for flavour, body and texture were 45 (normal range 35 to 42) and 30 (normal range 26 to 29.6).

Results and Discussion

Moisture, pH, titrable acidity and lactic acid changes: High moisture content in the curd is essential for the promotion of good and rapid ripening. Presalting method for cheese preparation from buffalo milk[2] gave 39.5 per cent moisture content in the curd which decreased to 36.7 per cent after 9 months of ripening (Table 1). The comparable figures for cow milk cheese were 38 per cent at zero-day and 36 per cent at 9 months.

Titrable acidity for a typical Cheddar cheese sample has been observed to increase from 0.7 per cent at day 1 to 1.0-1.25 per cent in 12 months[11]. The titrable acidity values for 9-month period (Table 1) are lower for buffalo milk cheese when compared with cow milk cheese. This could be due to lower production of fatty acids and amino acids as a consequence of lower lipase and proteinase activities respectively of buffalo milk cheese preparation. Titrable acidity, however, is not a true index of effective acidity. A pH value ranging between 5.40 and 5.50 at the time of milling of the curd is suitable for ripening[11]. For buffalo milk Cheddar cheese, pH ranged between 5.25 and 5.40 and for cow milk cheese it was between 5.26 and 5.52 over the 9-month ripening period (Table 1). The coagulating activity of rennet varies with the pH of the milk[11]. At lower pH values, the solubility of calcium salts increases which accelerates the coagulation of casein. Addition of salts in the presalting method used in the present study allowed the exchange of Ca^{2+} with NA^{+} ions. Lactic acid is essential for the preparation of cheese in many ways, viz. ripening, flavour development and good keeping quality. No differences were found in lactic acid formation between the ageing buffalo and cow milk cheeses.

TABLE 1. PROFILE OF MOISTURE, PH, TITRABLE ACIDITY AND LACTIC ACID DURING RIPENING OF CHEDDAR CHEESE*

Ripening period (months)	Buffalo milk cheese				Cow milk cheese			
	Moisture	pH	Titrable acidity (% LA‡)	Lactic acid (%)	Moisture	pH	Titrable acidity (% LA)	Lactic acid (%)
0	39.5	5.25	0.58	0.28	38.0	5.26	0.66	0.28
1	39.4	5.19	0.66	0.36	37.9	5.15	0.72	0.35
3	38.5	5.20	0.76	0.48	37.4	5.20	0.84	0.50
5	38.2	5.35	0.90	0.58	37.1	5.30	0.95	0.56
7	37.3	5.20	1.10	0.65	36.4	5.28	1.20	0.65
9	36.7	5.48	1.20	0.72	36.0	5.52	1.36	0.75

*Values are average of 3 batches of Cheddar cheese analysed in duplicate.
‡LA: lactic acid.

Lipid changes: In buffalo milk cheese, the decrease in total glycerides was 1.02 per cent in 9 months while the corresponding decrease for cow's milk Cheddar cheese was 1.66 per cent (Table 2). An evaluation of individual glycerides showed slight increase for monoglycerides; for diglycerides: 2.05 per cent for buffalo and 2.65 per cent for cow milk cheese and for triglycerides decrease was 4.11 per cent for buffalo and 4.36 per cent for cow milk cheeses. The formation/accumulation of partial glycerides in cheese as ripening advances was noticed by de Man[12] and Rama Murthy[13]. In the latter study on buffalo milk cheese ripening, a gradual increase in the mono- and diglyceride content was seen but the amount of triglycerides hydrolysed was not estimated. Except the monoglycerides for which a greater hydrolysis was observed in buffalo than in cow milk cheese, the hydrolysis of glycerides in two cheeses are not markedly different (Table 2). The cow milk cheese had developed desired flavour while the buffalo milk cheese was flat with no flavour which may be related to the specificity of the lipase activity during ripening and the difference in the glyceride structures between buffalo and cow's milk fats.

Lipase activity and the formation of free fatty acids: Buffalo milk cheese had a lower lipase activity than the cow milk cheese (Fig. 1). The cow's milk cheese had the highest lipase activity at 4 months of ripening. In contrast, buffalo milk cheese lipase showed downward trend at 4 months but by 5 months it increased again. After 5 months, a continuous decline was noticed in both buffalo and cow milk cheese lipases. Lipase activity has been detected in lactic acid bacteria and adventitious bacteria isolated from Cheddar cheese[14]. Psychrotrophic bacteria that possess heat stable lipases are also potential contributors to free fatty acids production during cheese ripening[15]. The free fatty acids produced

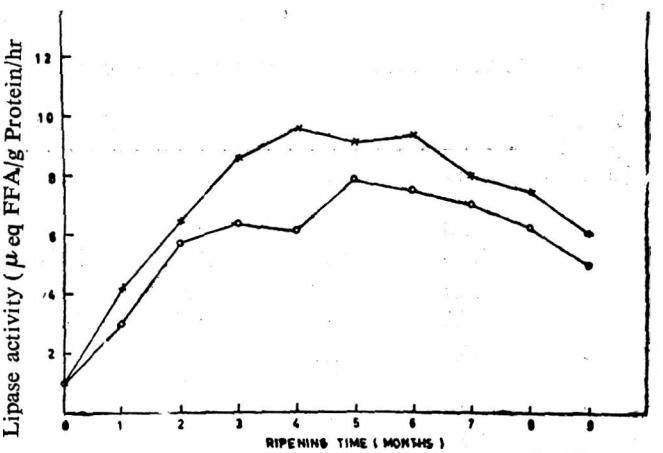

Fig. 1. Lipase activity during ripening of Cheddar cheese: Buffalo (O———O), Cow (X———X).

between 0 and 9 months were more for cow milk cheese than for buffalo milk cheese (Table 3). Stadhouders and Veringa[16] have reported that the lipases elaborated by lactic streptococci prefer di-and mono-glycerides as the substrates. The triglycerides were feebly hydrolysed in both buffalo and cow milk cheese preparations and the likely reason could be the lactic streptococci used as starter culture in this study. Both the buffalo and cow milk cheeses had fatty acids with chain length 4 to 18 carbons; the proportions of each fatty acid varied with ripening. These proportions were also different in the two species.

Protein changes: The role of amino acids and peptides released by proteinases in hard and semi-hard cheeses is not clearly understood but these may act by directly contributing to flavour[17]. The estimation of soluble nitrogen and/or free tyrosine has been used as an indicator of the degree of maturity of ripening cheese[18]. In two studies on Cheddar cheese, [19], [20] a

TABLE 2. CHANGES IN PARTIAL GLYCERIDES OF NEUTRAL LIPIDS DURING RIPENING OF CHEDDAR CHEESE* FROM BUFFALO AND COW MILK

Ripening period (months)	Monoglycerides (%)		Diglycerides (%)		Triglycerides (%)	
	B. milk cheese	Cow milk cheese	B. milk cheese	Cow milk cheese	B. milk cheese	Cow milk cheese
0	0.42	0.52	4.00	3.70	93.64	93.56
1	0.78	0.45	4.70	4.95	92.42	92.25
3	0.85	0.57	4.88	6.20	91.89	90.65
5	1.05	0.54	5.80	6.37	90.55	90.15
7	1.06	0.65	5.90	6.45	90.22	89.50
9	1.05	0.60	6.05	6.35	89.95	89.20

*Values are average of 3 batches of Cheddar cheese analysed in duplicate.

TABLE 3. CHANGES IN FREE FATTY ACID COMPOSITION OF BUFFALO MILK CHEDDAR CHEESE DURING RIPENING*

Ripening period (months)	Fatty acid as % of total fatty acids								
	4:0	6:0	8:0	10:0	12:0	14:0	16:0	18:0	18:1
0	11.5 (10.4)	3.3 (12.1)	2.6 (3.7)	3.4 (3.6)	4.3 (5.9)	10.6 (13.9)	38.8 (32.1)	7.3 (5.5)	18.3 (12.9)
1	12.4 (25.2)	1.3 (4.4)	1.5 (3.7)	3.4 (4.7)	4.8 (5.1)	13.8 (10.8)	37.9 (27.8)	7.8 (5.4)	17.0 (13.0)
3	13.0 (24.0)	3.3 (4.0)	2.6 (1.3)	3.2 (3.7)	4.5 (3.5)	14.8 (10.5)	36.0 (33.8)	6.3 (5.9)	16.5 (13.6)
5	16.9 (13.1)	4.1 (15.0)	2.2 (2.1)	3.0 (4.7)	4.0 (6.4)	14.5 (11.0)	34.8 (27.9)	6.0 (6.3)	14.5 (13.1)
7	15.2 (7.6)	2.6 (2.8)	2.7 (3.3)	3.5 (4.7)	4.1 (6.0)	14.6 (11.5)	36.0 (36.7)	6.4 (8.6)	15.1 (15.9)
9	17.7 (15.1)	1.7 (1.4)	3.1 (3.1)	3.2 (3.7)	4.8 (7.9)	13.6 (11.7)	36.2 (36.6)	5.4 (5.9)	14.0 (14.5)

*Values are average of 3 batches of Cheddar cheese analysed in duplicate.
Data in parantheses are for cow milk Cheddar cheese.

highly significant relation was observed between flavour and the phosphotungstic acid (PTA) soluble N and trichloroacetic acid (TCA) soluble tyrosine levels. In the present study, the TCA soluble nitrogen increased from 0.65 to 3.36 mg tyrosine/g cow milk cheese while for buffalo milk cheese it was 0.55-3.25 mg tyrosine/g cheese. Similar trend was observed in soluble nitrogen extracted with acetate buffer (Fig. 2). These results are in agreement with the proteinase activity profiles of the two cheeses. A higher rate of proteolysis was observed in cow milk cheese throughout the 9-month ageing period in comparison with buffalo milk cheese (Fig. 3). Differences in the protein composition of buffalo and cow milk[21] have been reported but this may not be the only reason for the low development of proteinase activity.

Active -SH groups and interaction with free fatty acids: Volatile sulphur compounds were detected in ripening cheese and were considered to be important in the development of cheese flavour. Buffalo milk cheese had initial active -SH group (2.5 μg cysteine-HCl/g cheese at 1 month) which increased to a maximum of 20 μg over 6 months ageing period. In contrast, cow milk cheese had a higher level of 16 μg cysteine-HCl/g cheese at 1 month which increased to the maximum value of 36 μg at 7 months of ripening. Ripening beyond these periods decreased the -SH concentrations in both cheeses (Table 4). The ratios of free fatty acids to active -SH groups were related to the flavour development of the cheeses. Though it has been reported that

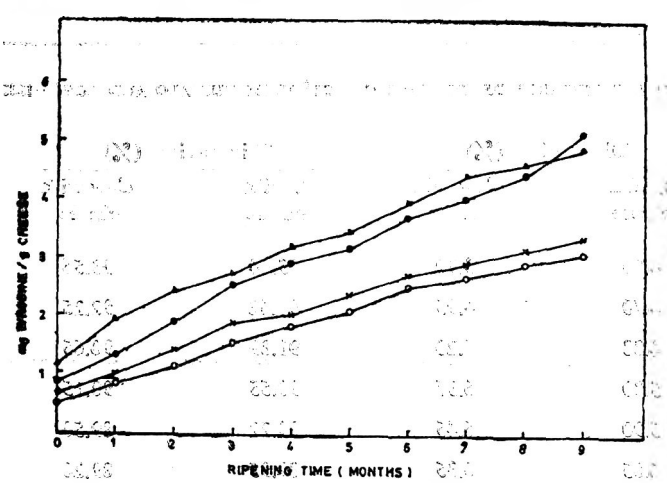

Fig. 2. Soluble nitrogen during ripening of Cheddar cheese. TCA: Buffalo (O——O), Cow (X——X). Acetate buffer (pH 4.6): Buffalo (●——●), Cow (▲——▲).

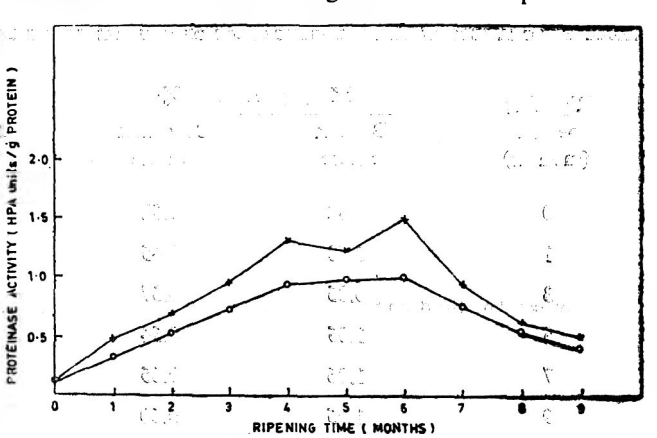

Fig. 3. Proteinase activity during ripening of Cheddar cheese: Buffalo (O——O), Cow (X——X).

TABLE 4. FREE FATTY ACIDS, ACTIVE -SH GROUPS AND FLAVOUR IN CHEDDAR CHEESE*

Ripening period (months)	Buffalo milk cheese			Cow milk cheese		
	Total FFA (μg/g cheese)	Total -SH (μg cysteine-HCl/g cheese)	Total FFA/ -SH groups	Total FFA (μg/g cheese)	Total -SH (μg cysteine-HCl/g cheese)	Total FFA/ -SH groups
0	33.57	—	—	52.29	—	—
1	56.81	2.5	22.72	74.30	16.0	4.64
2	68.16	5.0	13.63	85.31	18.0	4.74
3	90.39	10.0	9.04	99.07	25.0	3.96
4	113.63	14.0	8.11	126.59	23.5	5.38
5	138.44	17.2	8.05	165.12	31.2	5.29
6	150.00	20.0	7.50	187.13	32.0	5.84
7	175.61	16.5	10.64	231.17	36.0	6.42
8	189.19	18.5	10.22	253.18	32.5	7.79
9	206.60	14.5	14.25	280.70	29.0	9.68

*Values are average of 3 batches of Cheddar cheese analysed in duplicate.

the flavour of cheese is related more to the ratios of free fatty acids to hydrogen sulphide concentrations than to any other compound or a number of compounds[22,23], there does not seem to be any definite pattern between these ratios and flavour development in the present study.

Sensory evaluation (data not presented) revealed that in cow milk Cheddar cheese, typical "Cheddar flavour" was evident by six months of ripening while the buffalo milk cheese had mild flavour. Desirable but not typical flavour was detected in buffalo milk cheese only after 7 to 8 months of ripening. However, body and texture were not satisfactory.

Acknowledgement

The award of Senior Research Fellowship to one of us (A. V.) under the United Nations Development Programme is gratefully acknowledged.

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Effect of Blending Goat and Buffalo Milk on Shelf-life of Ghee

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Received 18 *March* 1986; *revised* 4 *September* 1986

This study was undertaken to determine the shelf-life of ghee prepared from goat, buffalo and mixed milk in the ratio 3:1 and 1:1 (goat: buffalo milk). At 30°C, shelf-life of goat ghee was almost at par with that of buffalo ghee being about 4 months by *desi* method and 10 months by creamery butter (CB) and direct cream (DC) methods. The final flavour quality was the best in case of DC method. No goaty flavour was observed. The samples were also analysed for free fatty acids, peroxide value and carbonyl content during storage.

Goat ghee (dehydrated butter oil) is reported to show a typical flavour and greasy texture[1]. Its flavour is relatively less appealing and it has higher acidity as compared to buffalo ghee. Since no information is available regarding keeping quality of goat ghee, a systematic study was undertaken to determine the shelf-life of ghee made from goat milk, buffalo milk and their blends.

Materials and Methods

Goat and buffalo milk were obtained from the cattle yard of the Institute. Goat milk was mixed with buffalo milk in 3:1 and 1:1 (V/V) ratios. Ghee was prepared from pure goat and buffalo milk and two types of mixed milk by three different methods viz. *desi*, creamery butter (CB) and direct cream (DC) methods at a clarification temperature of 125, 120 and 115°C, respectively. Twenty litres of milk were taken for preparation of ghee by *desi* method and 80 litres by CB and DC methods.

Ghee samples were filled in 125 ml glass stoppered wide mouth bottles and stored in an incubator at 30±1°C for 6 months those prepared by *desi* method and 10 months those made by CB and DC methods. These samples were analysed at two-month intervals for organoleptic and chemical changes. Two trials were conducted for each type of ghee.

Sensory evaluation of ghee was done by a panel of six trained judges by the composite scoring method of ISI[2] with slight modification as suggested by Rajorhia *et al*[3].

Free fatty acids (FFA) and peroxide value were determined by ISI procedure[4], with following modifications for the latter: 10 g of the sample was taken instead of 1 g. 3g of KI was taken instead of 1 g and 0.01 N $Na_2S_2O_3$ was used instead of 0.002N $Na_2S_2O_3$. Peroxide value was calculated as: $\frac{V \times 5}{W}$ (ml of 0.002N $Na_2S_2O_3$ per g). Carbonyl content was determined by the modified Swartz method[5].

Results and Discussion

Flavour: Data in Table 1 show that the flavour score of fresh ghee made from goat milk, buffalo milk and their blends by *desi* method ranged from 48.7 to 49.0, exhibiting no significant variation. All were ranked as 'good' ghee. No typical flavour defect was observed with goat ghee. However, buffalo ghee and its blends tended to be slightly superior. The same trend was

TABLE 1. EFFECT OF STORAGE ON FLAVOUR SCORE OF BLENDED GHEE

Type of ghee	Flavour score* during indicated storage period (months)					
	0	2	4	6	8	10
Desi Method						
Goat	48.7	46.8	42.4	42.4	—	—
Goat: Buffalo (3:1)	48.8	46.8	42.9	41.5	—	—
Goat: Buffalo (1:1)	48.9	46.9	43.3	41.7	—	—
Buffalo	49.0	47.3	44.3	42.2	—	—
Creamery Butter Method						
Goat	49.7	49.0	46.5	44.9	42.4	42.1
Goat: Buffalo (3:1)	50.5	49.1	47.0	45.3	42.9	42.3
Goat: Buffalo (1:1)	52.0	49.2	47.5	45.7	43.3	42.5
Buffalo	53.0	50.8	49.0	47.3	44.7	42.7
Direct Cream Method						
Goat	50.6	48.9	47.0	46.0	44.0	43.1
Goat: Buffalo (3:1)	51.8	50.6	48.5	47.0	45.2	44.2
Goat: Buffalo (1:1)	53.8	52.2	49.8	48.0	46.0	44.7
Buffalo	54.8	53.8	51.3	50.0	48.0	46.1

*Maximum score 60.

observed with fresh ghee made by CB and DC methods. Though the score of ghee made by CB and DC methods was slightly superior in increasing order, the differences between goat and buffalo ghee became more conspicuous. All the samples were considered to be 'good' except the last two made from buffalo milk or its blends with goat milk (1:1) which were rated as 'excellent'.

All the samples deteriorated during storage, the deterioration being fastest in *desi* method prepared ghee followed by CB and DC methods. The ghee samples made by *desi* method were 'fair' after 4 months of storage but were rated as 'poor' after 6 months. On the other hand, ghee made by CB and DC methods were 'fair' up to 10 months. These observations are in accordance with those of Rangappa and Achaya[6] who also reported lower flavour stability of *desi* ghee as compared with direct cream and creamery butter ghee. In general, buffalo ghee and its blends tended to have slightly better shelf-life than goat ghee, regardless of methods of manufacture. The relative slower rate of deterioration observed in case of goat ghee could be due to its relative higher phospholipid content[7,8].

Body and texture: The results in Table 2 show the effect of different kinds of milk on body and texture of ghee. The score of fresh ghee made by *desi* method ranged from 20.2 to 20.7 without any significant effect of type of milk, although buffalo milk and its blends tended to be slightly superior. The same trend was observed with ghee made by CB and DC methods. The improving effect of buffalo milk was more conspicuous with CB and DC methods[9]. The body and texture of all fresh samples were categorized as 'good' regardless of methods of manufacture. Though the granule size of goat ghee was relatively smaller as compared to buffalo ghee, none of the samples was termed as 'greasy'.

Though all the samples at the end of storage were rated as 'fair', there was some deterioration in body and texture during storage. Deterioration in body and texture could be attributed to the fact that some of the granules are dissolved by the solvent action of the liquid portion[9].

Colour: The colour score of fresh ghee made by *desi* method ranged from 8.4 to 8.6 without any significant effect of type of milk. However, buffalo ghee tended to be slightly superior. There was slight bleaching of colour during storage although all the samples were fairly acceptable. The ghee made by CB and DC methods showed similar trend (Table 3). Lalitha and Dastur[10] also reported decline in colour of buffalo ghee during 4 months storage at 42°C.

Chemical changes: In order to correlate the sensory evaluation data with the more objective chemical changes, ghee samples were analysed for free fatty

TABLE 2. EFFECT OF STORAGE ON BODY AND TEXTURE OF BLENDED GHEE

Type of ghee	Body and texture score* during indicated storage period (months)					
	0	2	4	6	8	10
Desi Method						
Goat	20.2	20.0	19.5	19.2	—	—
Goat: Buffalo (3:1)	20.3	20.0	19.5	19.4	—	—
Goat: Buffalo (1:1)	20.6	20.1	19.4	19.4	—	—
Buffalo	20.7	20.3	19.6	19.4	—	—
Creamery Butter Method						
Goat	21.4	21.4	21.3	20.6	20.4	19.9
Goat: Buffalo (3:1)	22.0	21.8	21.6	21.4	20.8	19.8
Goat: Buffalo (1:1)	22.5	22.4	22.2	21.9	21.0	19.7
Buffalo	23.5	23.0	22.7	22.5	21.8	20.1
Direct Cream Method						
Goat	19.3	19.3	18.6	18.4	18.3	18.0
Goat: Buffalo (31:1)	20.0	19.8	19.6	19.3	19.0	18.6
Goat: Buffalo (1:1)	21.0	20.5	20.0	19.6	19.0	18.8
Buffalo	22.5	22.0	21.5	20.4	20.0	19.8

*Maximum score 25.

TABLE 3. EFFECT OF STORAGE ON COLOUR SCORE OF BLENDED GHEE

Type of ghee	Colour score* during indicated storage period (months)					
	0	2	4	6	8	10
Desi Method						
Goat	8.4	8.4	8.2	8.1	—	—
Goat: Buffalo (3:1)	8.5	8.5	8.3	8.2	—	—
Goat: Buffalo (1:1)	8.6	8.5	8.3	8.3	—	—
Buffalo	8.6	8.6	8.4	8.4	—	—
Creamery Butter Method						
Goat	8.2	8.2	8.1	8.0	8.0	7.8
Goat: Buffalo (3:1)	8.3	8.3	8.2	8.1	8.1	7.9
Goat: Buffalo (1:1)	8.5	8.4	8.3	8.2	8.2	8.0
Buffalo	8.7	8.6	8.5	8.4	8.3	8.0
Direct Cream Method						
Goat	8.6	8.6	8.5	8.4	8.3	8.1
Goat: Buffalo (3:1)	8.6	8.6	8.5	8.5	8.3	8.2
Goat: Buffalo (1:1)	8.7	8.6	8.6	8.5	8.4	8.3
Buffalo	8.9	8.8	8.7	8.6	8.5	8.4

*Maximum score 10.

TABLE 4. EFFECT OF STORAGE ON ACIDITY OF BLENDED GHEE

Type of ghee	Free fatty acids (%) during indicated storage period (months)					
	0	2	4	6	8	10
Desi Method						
Goat	0.38	0.60	0.84	0.91	—	—
Goat: Buffalo (3:1)	0.33	0.54	0.76	0.81	—	—
Goat: Buffalo (1:1)	0.25	0.45	0.66	0.72	—	—
Buffalo	0.23	0.35	0.49	0.56	—	—
Creamery Butter Method						
Goat	0.19	0.20	0.22	0.26	0.27	0.35
Goat: Buffalo (3:1)	0.18	0.19	0.21	0.25	0.27	0.34
Goat: Buffalow (1:1)	0.16	0.18	0.21	0.23	0.26	0.34
Buffalo	0.14	0.15	0.16	0.18	0.27	0.34
Direct Cream Method						
Goat	0.19	0.22	0.30	0.37	0.42	0.57
Goat: Buffalo (3:1)	0.18	0.21	0.28	0.35	0.41	0.50
Goat: Buffalo (1:1)	0.17	0.20	0.27	0.34	0.41	0.48
Buffalo	0.16	0.19	0.25	0.28	0.36	0.41

TABLE 5. EFFECT OF STORAGE ON PEROXIDE VALUE OF BLENDED GHEE

Type of ghee	Peroxide value (ml of 0.002 N $Na_2S_2O_3$/g) during indicated period of storage (months)					
	0	2	4	6	8	10
Desi Method						
Goat	0.62	0.74	0.92	1.01	—	—
Goat: Buffalo (3:1)	0.64	0.78	0.95	1.05	—	—
Goat: Buffalo (1:1)	0.65	0.82	1.00	1.10	—	—
Buffalo	0.68	0.88	1.09	1.28	—	—
Creamy Butter Method						
Goat	0.66	0.71	0.81	0.98	1.07	1.16
Goat: Buffalo (3:1)	0.66	0.73	0.83	0.98	1.09	1.17
Goat: Buffalo (1:1)	0.67	0.75	0.84	0.98	1.10	1.18
Buffalo	0.68	0.77	0.87	0.98	1.16	1.24
Direct Cream Method						
Goat	0.74	0.82	1.04	1.36	1.60	1.82
Goat: Buffalo (3:1)	0.78	0.86	1.12	1.40	1.65	2.15
Goat: Buffalo (1:1)	0.83	0.96	1.20	1.46	1.73	2.20
Buffalo	0.93	1.12	1.35	1.62	1.85	2.56

acids, peroxide value and carbonyl content during storage.

Free fatty acids (FFA): The results in Table 4 show the effect of type of milk on FFA content of ghee during storage. The acidity level of fresh ghee made by *desi* method ranged from 0.23 to 0.38 per cent, the highest being in goat ghee and the lowest in buffalo ghee. Reducing the proportion of goat milk in the blend resulted in a corresponding decrease in FFA level. Acidity increased during storage regardless of methods of manufacture. The final acidity after 6 months of storage ranged from 0.56 to 0.91 per cent: the highest and the lowest being in goat ghee and buffalo ghee, respectively. The development of acidity during storage was inversely related to flavour quality.

The FFA content of fresh ghee made by CB method ranged from 0.14 to 0.19 per cent, again highest and lowest being in goat and buffalo ghee, respectively. These values were considerably lower than those of ghee made by *desi* method. The FFA content increased during storage reaching final values of 0.34 to 0.35 per cent after 10 months of storage. Similar trend was noted in case of ghee made by DC method. The increase in acidity during storage was maximum in *desi* ghee followed by ghee prepared by DC and CB methods.

Peroxide value: The peroxide value of ghee made by *desi* method ranged from 0.62 to 0.68, the lowest being in goat ghee and the highest in buffalo ghee (Table 5). Increasing the proportion of goat milk had a corresponding depressing effect on PV. The PV increased during storage, the final values ranging from 1.01 to 1.28 after 6 months.

The PV of fresh ghee made by CB method ranged from 0.66 to 0.68, the highest being in buffalo ghee. The PV increased during storage, reaching the final value of 1.16-1.24. The PV values of fresh ghee made by DC method were significantly higher than those made by the other two methods, the range being from 0.74 to 0.93. These values increased during storage, reaching final level of 1.82 to 2.56.

It may be noted that rate of peroxide development was less in goat ghee than in buffalo ghee, irrespective of methods of manufacture. This may be assigned to higher level of phospholipids in goat ghee (0.049 per cent) than in buffalo ghee (0.021 per cent)[7,8]. Phospholipids suppress peroxide development.

Carbonyls: The results in Table 6 show that the carbonyl content of fresh ghee ranged from 3.81 to 5.39 micromoles per g, the lowest and highest being in goat and buffalo ghee, respectively. The increasing proportion of goat milk had corresponding depressing effect on carbonyl content. The content of carbonyl increased during storage, reaching final value of 8.52 to 8.81 micromoles/g. The depressing effect of goat milk was maintained during storage.

In case of creamery butter ghee, the level of carbonyls

TABLE 6. EFFECT OF STORAGE ON CARBONYL CONTENT OF BLENDED GHEE

Type of ghee	Carbonyls (μ M/g fat) during indicated period of storage (months)					
	0	2	4	6	8	10
			Desi Method			
Goat	3.81	4.26	5.83	8.52	—	—
Goat: Buffalo (3:1)	4.54	4.96	6.35	8.58	—	—
Goat: Buffalo (1:1)	5.09	5.62	6.71	8.63	—	—
Buffalo	5.39	5.95	6.97	8.81	—	—
			Creamy Butter Method			
Goat	3.98	4.70	5.63	6.58	7.08	7.61
Goat: Buffalo (3:1)	4.35	4.76	5.67	6.67	7.29	7.62
Goat: Buffalo (1:1)	4.45	4.88	5.70	6.78	7.45	7.63
Buffalo	4.85	4.98	5.90	6.93	7.73	7.93
			Direct Cream Method			
Goat	3.54	3.79	3.90	4.10	4.40	4.60
Goat: Buffalo (3:1)	3.65	3.82	3.99	4.18	4.59	4.86
Goat: Buffalo (1:1)	3.75	3.86	4.10	4.25	4.75	5.08
Buffalo	3.99	4.15	4.20	4.50	4.90	5.40

in fresh as well as stored ghee was similar to that of *desi* ghee. Here also, goat ghee had relatively lower carbonyl content than corresponding buffalo ghee. In DC ghee, the initial as well as final carbonyl content at the end of storage was relatively lower than that of *desi* and CB ghee. However, with respect to the type of milk, the trend was the same i.e. goat ghee showed relatively lower carbonyls than buffalo ghee. Gaba and Jain[11] reported similar trend in carbonyl content of buffalo *desi* ghee during storage for 100 and 200 days at 37°C.

It may be noted that rate of increase in carbonyl content of goat ghee was faster than that of buffalo ghee, both prepared by *desi* and CB methods, whereas reverse was the case in DC method. Also, rate of increase of carbonyl content of all types of ghee prepared by *desi* method was comparatively faster than that of ghee prepared by CB and DC methods.

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Studies on the Brewing of Lager Beer from Nigerian Sorghum

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Received 11 *February* 1986; *revised* 8 *August* 1986

Lager beer was brewed from sorghum malt using the three stage decoction method and 30% sucrose as adjunct. Physical and chemical properties of the beer as well as organoleptic tests showed that it was very similar to barley beer. It had the clear sparkling straw-coloured, hop-taste, characteristic of lager beer and was different from the sour, cloudy indigenous beers of Africa, which are produced by lactic acid bacteria and yeast fermentation. The sorghum lager beer had a shelf-life of at least fourteen months.

Lager beer, by definition is a beer brewed from barley malt and stored for a period of time for clarification and maturation[1]. In West African countries, barley is imported from Europe and this involves expenditure of scarce foreign exchange. The problem has, in recent times, become acute and these studies were, therefore, conducted to examine the grain of *Sorghum bicolor*, which grows well in tropical countries, as a substitute for barley in beer brewing.

To our knowledge, no scientific study of the produc-

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tion and the properties of lager beer from sorghum exists. Skinner[2] reported the production of a "tropical lager acceptable to tasters" from sorghum without giving analytical data on the product.

Materials and Methods

Sorghum malt: A local red Nigerian variety (LRV)[3] of *Sorghum bicolor* was malted for 4 days at 28°C. The methods described below were arrived at after preliminary experiments reported by Aniche[4].

Mashing: A three-stage decoction mashing method was used as described below: 3.86 kg of coarse-milled (No. 30 sieve) sorghum malt was mixed in 12.87 litres of brewing water at 40°C for 30 min. The temperature was raised to 50°C and allowed to fest for 15 min. About one third of the mash was withdrawn and transferred into a weighed aluminium pot which served as the boiling vessel. The mash was boiled until the malt particles were gelatinized and added back to the mashing vessel. This raised the temperature to about 60°C which was maintained for 60 min. One third of the entire mash was withdrawn once more and boiled. This was added back to the main mash and this raised the temperature to 65°C, at which temperature the heating was maintained for another 60 min. For the third time, one third of the entire mash was withdrawn and boiled and then added back to the mash. The temperature was raised to 60°C and was maintained for 20 min. After this, the temperature of the mash was raised to 75°C and mashing was terminated after 30 min at this temperature.

Wort boiling, hopping and filtration: The quantity of hops added is dependent on the specific gravity of the wort. Twelve litres of wort of specific gravity 1.050 was obtained. According to the American Society of Brewing Chemists' (ASBC) recommendations[5], 25.2 g of ground hops (0.65 per cent of total weight of malt) were added. The hops were added in two stages of equal amounts, namely, at the beginning and at the end of the 2-hr period of the boiling of the wort. About mid-way in the boiling period, 30 per cent sucrose of the original weight of malt (1.58 kg) was added. After boiling, the hopped wort was allowed to cool and then filtered first through a 120-mesh sieve and finally through a cotton gauze. The residue was flushed with water at 80°C until the washings were clear.

Fermentation of wort: One ml of yeast suspension containing 2.25 mg of *Saccharomyces uvarum* (kindly supplied by Premier Breweries, Onitsha) was used for pitching 12 litres of wort[7], having been grown in glucose yeast extract broth[8].

Wort pH and temperature: The wort was adjusted to pH 5.5 using I M citric acid. The temperature of wort at the time of pitching of the yeast was 11°C. A temperature of 8°C was maintained after the first day by using brine to cool the fermenting wort.

Lagering: The fermentation lasted for five days. By this time, the foam head formed in the primary fermentation in the jar had collapsed. The green beer was lagered for two weeks at 6°C in a glass jar, thereafter the temperature of the beer was decreased to 4°C and the beer was left at this temperature for another two weeks in a refrigerator.

Carbonation and bottling of beer: The lagered beer was carbonated with carbon dioxide with a specially designed carbonator for one hr. The beer was then passed through a filter disc and bottled. Each bottle of beer was crown-corked immediately.

Pasteurization of beer: The filled and crowned bottles were placed in a water bath at 60°C for 30 min. The bottles took about 30 min to attain the pasteurizing temperature, remained in the water bath for 30 min and took about 30 min to cool down.

Analysis of unfermented wort: The wort was analysed for extract, total acidity, pH, colour, and reducing sugars[4-7, 9].

Analysis of beer: To prepare the beer for analysis it was brought to 20°C. The beer was decarbonated by transferring the sample to a larger Erlenmeyer flask and by shaking, gently at first and then vigorously, until no more gas escaped. Foam was removed by filtering through dry Whatman No. 1 filter paper. After decarbonation, the beer was attemperated to 20°C. Specific gravity, original apparent and real extracts, alcohol content, apparent and real degree of attenuation, pH, total acidity, protein content and reducing sugar were determined. Iodine reaction, and the foam head retention capacity of the beer were determined according to the Institute of Brewing Methods[7].

Tasting: Twenty tasters evaluated the beer. Twelve of them drank beer occasionally whereas eight of them drank at least a bottle of beer a day. The sorghum beer and a commercial local beer were both chilled to 12°C and were served in opaque glasses to keep the identity of the beer secret to the taster. Each of the tasters was asked to score the two types of beer on a scale of 5.

Results and Discussion

To our knowledge so far, no work has compared lager beer produced from sorghum with that produced from barley. Indeed, the only report of a lager beer from sorghum was that of Skinner[2] which merely described beer produced from Botswana sorghum as being acceptable to tasters. No detailed analysis was done.

The data in Table 1 show that the wort from sorghum malt did not differ much from that of barley malt.

The progress of the fermentation as measured by various parameters including specific gravity, pH, temperature, apparent and real extracts, alcohol content by weight, real and apparent degress of attenuation and yeast concentration (Table 2) followed similar properties reported for barley fermentation[11]. Chemical composition of sorghum beer is given in Table 3.

Statistical analysis of tasting scores in Table 4 shows that there was no difference between the barley beer and sorghum beer at 95 per cent confidence level. Thus, lager beers produced from barley malt and sorghum malt are similar.

Extensive work has been done by Novellie,[13-15] on the highly popular South African sorghum beer "Kaffir beer". This beer, essentially an indigenous product of the Bantu people of South Africa, is, however, very different from lager beer in that a lactic fermentation occurs. Indeed, in kaffir beer brewing, the mash is held initially at 48-50°C for 16-24 hr to encourage the growth of thermophilic lactic acid bacteria occurring naturally on the grain. Although this beer has about the same amount of alcohol as lager beer (3.2 per cent by weight) it is sour due to the high lactic acid content (2.13 mg/l) and cloudy because of the high amount of total solids of about 4.9 per cent[4,13,16].

Other beers prepared from sorghum on smaller scales throughout Africa include "burukutu", "pito", or "otika" from Nigeria[3] 'Mayek' and 'Mosa' in Kenya and Malawi, respectively and 'Merisa', Bouza' and 'Pombe' in Sudan, Ethiopia and parts of East Africa[4,16]. These are all different from lager and are closer to kaffir beer. In all these beers, a lactic fermentation

TABLE 1. PROPERTIES OF SORGHUM WORT

	Sorghum wort	Barley* wort
Specific gravity (20°C/20°C)	1.04758	1.04768
Apparent extract (°Plato)	11.64	11.8
Total acidity (as lactic acid %)	0.12	0.11
Iodine reaction	Faint yellowish tinge	
pH	5.5	5.2
Colour at 430 nm (°SRM)	6.2	6.2
Reducing sugars‡	3.89	—
Protein (%)	2.30	—

*American Society of Brewing Chemists, 1958.
‡As anhydrous maltose (%) without sucrose addition.

TABLE 3. PROPERTIES OF SORGHUM BEER

Colour at 430nm (°SRM)	3.3 (3.9)
Specific gravity (20°C/20°C)	1.01608 (1.01410)
Apparent extract (%)	3.102 (3.60)
Real extract (%)	5.8 (5.45)
Extract of original wort (°Plato)	13.14
Real degree of fermentation (%)	55.5
Apparent degree of fermentation (%)	76.4
Total acidity (%)	0.44 (0.13)
Alcohol by wt (%)	3.09 (3.74)*
Protein (%)	0.34 (0.302)*
Iodine reaction	yellowish colour
pH	4.3 (4.5)

Figures in parentheses are from American Society of Brewing Chemists, 1958, *Journal of the American Society of Brewing Chemists, 1979, 37, 129.

TABLE 2. CHANGES IN VARIOUS PARAMETERS OF SORGHUM WORT DURING FERMENTATION

Days of fermentation	Sp. gr.	pH	Apparent extract (%)	Real extract (OP)	Alcohol (% by wt)	Original gravity (OP)	Extract of original wort (OP)	Degree of attenuation (%) Real	Apparent	Yeast concn (mg/ml DM)
1	1.046	5.0	11.35	11.41	1.009	33.71	13.25	14.54	14.98	2.8
2	1.026	4.8	6.27	7.65	2.39	29.58	12.27	37.65	48.90	8.8
3	1.029	4.6	5.80	5.90	3.19	28.91	12.08	1.16	31.99	20.0
4	1.0162	4.4	4.28	4.68	3.35	25.83	11.48	59.27	62.72	15.4
5	1.0100	4.0	2.60	3.93	3.50	21.72	10.76	13.48	75.84	7.8

TABLE 4. ANALYSIS OF TASTE SCORES

Commercial barley beer			Sorghum beer		
Tasters (No.)	Total Marks	Marks scored/ taster	Tasters (No.)	Total marks	Marks scored/ taster
2	6	3	2	4	2
6	24	4	6	24	4
12	60	5	12	60	5

occurs and are usually sour in taste. These sorghum beers are not hopped and are all cloudy because of their high solids content. In contrast, the lager beer produced from sorghum and described in these experiments was clear, sparkling and was close to barley beer in colour, pH, taste and other properties. There was also no deterioration in the taste of the sorghum beer after storage for 14 months at room temperature. The various parameters of fermenting sorghum wort described in this study may be used in monitoring the fermentation of lager beer procured from sorghum.

In conclusion, sorghum is inherently a cereal suitable for producing the indigenous African beer. It is the technique of production that produces opaque beers. Sorghum can also be used to produce the sparkling, clear lager beer.

Acknowledgement

One of us (GNA) acknowledges with gratitude the encouragement given by the Director and staff of the Projects Development Institute (PRODA), Enugu.

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EFFECT OF SOME OILS, FATS AND BILE SALTS ON GROWTH AND LIPASE PRODUCTION BY *STREPTOMYCES* SP. L_4

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Received 15 *July* 1985; *revised* 28 *July* 1986

Growth and lipase production by *Streptomyces* sp. L_4 were studied in the presence of some oils, fats and bile salts. Triolein, castor oil, rapeseed oil, cholesterol, lard, sodium taurocholate, linseed oil, Tween-80, sodium cholate, olive oil and coconut oil increased the production of lipase in the broth.

Lipases are now being used to improve the quality of foods as also to impart desired flavour and texture to cheese and similar foods[1]. During a soil screening programme in our laboratory, an exocellular lipase producing *Streptomyces* sp. designated as L_4 was isolated[2]. Production of lipases by fungi has been shown to be stimulated by a number of glycerides of fatty acids[3]. In this paper, effect of some oils and fats as also some bile salts on the growth and lipase production of *Streptomyces* sp. L_4 are reported.

Lipolytic activity of *Streptomyces* sp. L_4 was at first assayed by cup-assay using Tween-80 agar medium[4]. Modified liquid medium[5], containing (g/l): peptone, 7.5; soluble starch, 10.0; NaCl, 3.0 and Mg SO_4. $7H_2O$, 1.0 was used for cultivating the organism. Different compounds (Table 1) were added to the medium at concentration of 0.1 per cent (w/v). All chemicals and natural vegetable oils were obtained from commercial sources. The conical flasks containing 50 ml broth were inoculated with one ml spore suspension of L_4 (9×10^8 spores/ml) and incubated for 4 days at 28°C.

A shake flask fermentation was conducted employing the *Streptomyces* sp. following the above described procedure and to the culture broth, ammonium sulphate was added to a concentration of 60 per cent saturation, for the precipitation of the crude enzyme. The optimum concentration of ammonium sulphate for precipitation of the enzyme was determined by trial and error. The precipitate was collected after centrifugation (12,000g for 20 min) and was then dissolved in deionised water and dialysed against deionised water in cold (4°C) for 2 days. The dialysed product was concentrated to 1/10 of its volume using polyethylene glycol.[6] DEAE cellulose column was then employed for further purification using sodium acetate buffer (0.02M) and sodium chloride solution (0-0.4M) followed by a mixture of sodium chloride solution (1M) and sodium hydroxide solution. A single peak of lipase was eluted with acetate buffer.

Fifty per cent pure olive oil emulsion using gum acacia and sodium benzoate as stabiliser was used as substrate. Seven grams of gum acacia and 0.2g of sodium benzoate were dissolved in 100 ml water. Equal volumes of mixture of this solution and olive oil were homogenised and thus olive oil emulsion was prepared[7]. The lipolytic activity was measured by titrimetric method[2]. The nitrogen content of the culture filtrate and enzyme solutions at different steps of purification was determined by micro-Kjeldahl method and from these values amounts of protein were calculated.

The lipase produced by *Streptomyces* sp. L_4 was found to be exocellular in nature and no lipolytic activity was noted in the mycelial mat. Table 1 shows different purification steps of the lipase. Table 2 indicates the effect of lipids on lipase production in the culture broth by the *Streptomyces* L_4. It is evident from Table 2 that all the compounds tested increased the lipase activity of the broth. Among them triolein, rapeseed oil, cholesterol and lard increased the lipase production by 46, 42, 40 and 36 per cent respectively. Olive oil, coconut oil and Tween-80 were effective in stimulating growth of the *Streptomyces*.

In case of *Achromobacter lipolyticum*, Khan *et al.*[8] reported that the addition of 1 per cent olive oil, corn oil or milk fat to the growth medium and slow stirring stimulated the lipase production 3 fold. The addition

TABLE 1. PURIFICATION OF LIPASE FROM STREPTOMYCES SP. L_4

Steps	Activity (units/ml)*	Protein (mg/ml)	Specific activity	Yield (%)
Culture broth	1250	416.6	3.0	100
Ammonium sulphate precipitation (60% saturation)	760	122.0	6.2	58.6
Dialysis	360	14.5	24.8	28.8
Column chromatography on DEAE cellulose	250	1.24	201.6	10.2

*1 unit = μ moles of fatty acids/ml/hr.

TABLE 2. INFLUENCE OF ADDITIONS TO THE CULTURE MEDIUM ON LIPASE PRODUCTION

Addition to the medium	Total lipase activity of the extract* (units**/ml)+	Dry wt. of mycelium (mg/50ml)++
Control***	1000	60
Castor oil	1450	75
Olive oil	1200	83
Coconut oil	1050	80
Rapeseed oil	1420	82
Lard	1360	69
Linseed oil	1280	72
Triolein	1460	74
Tween-80	1280	81
Cholesterol	1400	79
Na-taurocholate	1340	67
Na-cholate	1260	70

* 5 ml extract made from 50 ml broth after 4 days.
** 1 unit=1 μ mole of fatty acids/ml/hr.
*** Medium for control contained g/l peptone=7.5, soluble starch 10.0, NaCl 3.0, $MgSO_4$, $7H_2O$ 1.0.
+ Standard deviation $\pm$ 10.
++ Standard deviation $\pm$ 5.

of fats and oils to the basal medium at a concentration of 0.2 per cent increased the production of extracellular lipase in *Torulopsis ernobii*[9]; highest yield was obtained with olive oil. Ota *et al*[10]. working on *Candida paralipolytica* stated that castor oil and triolein were the best stimulators for lipase production. Ota *et al.*[11] found that there was a difference between *C. paralipolytica* and *C. cylindracea* in sterol requirement. They concluded that perhaps the induction of lipase production was carried out in a novel manner since most of the inducers were insoluble in water.

The data presented in this paper as well as the literature cited above indicate that addition of lipids to the growth medium usually increases the yield of enzyme in culture broth. The reason for this stimulation seems to be a genetic inducer effect.

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PHYSICO-CHEMICAL TESTS—A BASIS FOR SELECTING THE SIZE OF WHEAT FLOUR

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Received 29 *June* 1986; *revised* 20 *August* 1986

Wheat flour was obtained from the ground hammer mill product by manual sieving using different ASTM standard sieves. These flours were tested for different physico-chemical characteristics such as alpha-amylase, gluten washing, sedimentation value and chapati baking tests. Chapatis were baked at pan temperatures of 220 and 280°C with different proportions of salt and water. These tests showed that wheat flour of particle size of 1000 microns and below was satisfactory for baking chapatis with desirable sensory characteristics.

The modern technique of producing wheat flour pays more attention towards getting flours with more white and finer particle sizes which needs sophisticated, complex and expensive machinery. Therefore, the resultant product becomes expensive. Although fine flour is

required for many food products, but that finer particles are not good for digestibility.

Chapati, commonly used in the Indian sub-continent and some parts of Middle-East is mainly made of coarse type of flour, especially whole wheat flour. Very limited studies were reported in this area. The objective of this study was to decide upon the maximum coarse size of flour for baking chapati based on physico-chemical tests.

Wheat of 12 per cent moisture content was milled in a hammer mill (Model-PRESIDENT made in Denmark, Rotor diameter-270 mm and width-48 mm. Rotor speed-3075 rpm, bottom sieve opening-1.5 mm, bottom sieve location-lower 180° electric motor driven-1.865 KW) in the laboratory and the flour was manually sieved by a single sieve. Eight different types of flour were chosen by sieving them using ASTM standard sieve sizes 14, 16, 18, 25, 30, 35, 45 and 50 mesh (1410, 1190, 1000, 710, 590, 500, 350 and 297 micron respectively). Each type of flour contained the maximum size corresponding to the sieve size used and all the lower size fractions. The flours were tested in the laboratory by sensory evaluation for the purpose of selecting maximum size acceptable for baking chapati.

Although the western standards for white bread are very different from chapatis (about 75 per cent extraction, particle size less than 140 micron[1], use of leavening with yeast or chemicals and an oven temperature around 225°C applied from all sides), it was interesting to compare the data observed by subjecting these coarse flours to some of the simpler tests that are employed to control the quality of flours as measured by (*i*) water absorption capacity at low temperatures (40°C), pentosans and (*ii*) starch quality as measured by the viscosity of a heated (about 100°C) suspension of flour in water.

Chapati baking tests: Two 50 g flour samples from each of the eight different types were chosen for preparing chapatis. One per cent salt was added to each of the samples. A series of chapati baking tests were performed by adding 60, 70 and 80 per cent water by weight of flour. Two chapatis were prepared from each kind of flour by using two frying pans at two different temperatures of 220 and 280 °C.

Salt, water and flour were thoroughly mixed and were kept for about one hr as leavening period followed by stretching the dough by hand. Chapatis were prepared by stretching the dough ball by hand and were baked on the frying pan. Later, they were tasted by a group of 3 judges in the laboratory (Table 1). The judges made their comment according to their choice of chapati. The results presented in Table 1 are the maximum scores.

Alpha-amylase test: Starch stability during the baking process depends on the amount of alpha-

TABLE 1. LABORATORY TESTS FOR SELECTION OF WHEAT FLOUR FOR CHAPATI MAKING

Test criteria	Water added (%)	Particle size of flour (< μ)							
		1410	1190	1000	710	590	500	350	297
Gluten washing tests (moisture content % (d.b.)*		153	157	157	200	225	240	240	300
Sedimentation tests*		13.5	13.0	12.7	11.8	11.5	11.7	11.6	11.7
Falling number test (sec.)*		242	251	268	272	280	283	290	303
Workability	60	Easy							
	70	Easy							
	80	Acceptable							
Appearance	60	Too coarse		Good					
	70	Pale with brown spots		Even, good, Light brownish					
	80	Not good		Good					
Taste	60	Acceptable		Very Good					
	70	Acceptable		Very Good					
	80	Acceptable		Good		Very Good			
Texture	60	Stiff		Good		Very Good			
	70	Stiff		Good		Very Good			
	80	Too coarse		Good		Very Good			

*Average of three replications.

amylase present in the flour. The activity of alpha-amylase was measured by the Hagberg Falling Number Test[1]. Alpha-amylase test was carried out by standard AACC method[4] using Falling Number Apparatus (Falling Number AB, Type-GC No. 7, Stockholm, Sweden).

Gluten washing test: The wet gluten content of the flour samples was determined by Glutomat (Perten Method)[1], using a 2 per cent NaCl solution as washing medium. Moisture content of wet gluten was determined by the hot air oven method to arrive at the per cent dry gluten contents.

Sedimentation test: The sedimentation value, devised by Zeleny,[1], is essentially a simplified water retention caapcity test in the presence of lactic acid; it was determined by the standard AACC[1] Procedure.

Falling number test indicates a slow but consistant decline in starch quality with particle size. The suspension of coarse particles was thinner than that of the fine particles and therefore, the viscosity was lower which resulted in a lower falling number (Table 1).

The results of gluten washing test indicated significant effect of particle size on water absorption capacity (Table 1). Muller[2,3] reported that the sedimentation value is related to the granularity of the flour, and that sedimentation is an agglomeration of the coarse particles rather than swollen protein. The results indicated gradual decrease in sedimentation value with smaller particle size of flour. The results of gluten washing test indicated significant effect of particle size on water absorption capacity.

Sensory evaluation showed that good chapatis can be made from flours upto 1000 micron particle size. Also, the gluten washing test presented a guideline for the optimal percentage of water to be used. Use of 70 per cent water absorption (on flour basis) was found suitable for chapati making (Table 1).

The authors acknowledge the co-operation received from the Dept. of Technology of Plant Food Products. The Royal Veterinary and Agricultural University, Copenhagen, during the experiment and are thankful to Danish International Development Agency for financial assistance.

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FATTY ACID COMPOSITION OF SOME EUROPEAN EDIBLE VEGETABLE SEED LIPIDS. PART II

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Received 16 *May* 1986; *revised* 12 *August* 1986

Fatty acid composition of the seed lipids of *Brassica rapa*, *Sinapis alba* (*Brassicaceae*), *Spinaca oleracea* and *Beta vulgaris* (*Chenopodiaceae*) were determined by capillary-gas-chromatography for the first time in a comprehensive manner. Neutral lipid content ranged from 4.0 to 10.0%. Neutral lipids of *B. rapa* and *S. alba* contained 46.1 and 15.5% erucic acid and 10 and 8.2% eicosenoic acid; Oleic, linoleic and linolenic acid contents in these two species were 13.6, 24.7, 13.5, 20.6 and 8.6, 20.7% respectively. *S. oleraceae* and *B. vulgaris* seed lipids contained 10.4 and 15.7% palmitic, 23.5 and 31.3% oleic and 60.9 and 43.2% linoleic acid respectively. Other long-chain fatty acids (20:2, 20:3, 22:0, 22:2, 22:3, 24:0 and 24:1) have been found in small amounts (0-4.3%).

As part of a continuing programme, hexane extracted lipids from four oilseeds namely *B. rapa* var. *esculenta*, *S. alba*, *S. oleracea* and *B. vulgaris* have been examined for the fatty acid composition of their oils by a capillary-column gas chromatograph (GC). Except mustard, other oilseeds do not contribute much to the edible oil supply. However, they are frequently eaten as part of the flora along with their vegetative edible organs, either raw or cooked. They are grown even in kitchen gardens, and add to the daily nutrition. As oilseeds are the major source of fatty acids, it was thought desirable to screen the fatty acids carried by them to the human system.

Farming grade vegetable seeds and mustard paste were purchased from the local market (Munster City, FRG). All solvents used were from Merck. Fatty acid compositions of methyl esters derived from hexane extracted lipids were determined using a gas-chromatograph (Perkoin Elmer, Model F22) equipped with FID and fused silica capillary column (50 feet) coated with OV 351. Nitrogen was used as carrier gas (2 ml/min) with split ratio 1:60, and hydrogen for the flame (20 ml/min). Analysis was carried out by temperature programming (155 to 190°C/min rate). A computerised integrator was used for percentage calculation. Lipids from the seeds were extracted using hexane as per the

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TABLE 1. FATTY ACID COMPOSITION OF EUROPEAN EDIBLE-VEGETABLE SEED LIPIDS

Fatty acid*	*B. rapa*	*S. alba*	*S. oleracea*	*B. vulgaris*
12:0	—	0.2	0.1	—
14:0	0.1	0.4	0.2	0.1
16:0	2.2	5.1	10.4	15.7
16:1	0.2	0.6	0.3	0.5
18:0	0.9	1.4	0.6	1.3
18:1	13.6	24.7	23.5	31.3
18:2	13.5	20.6	60.9	43.2
18:3	8.6	20.7	2.0	0.5
20:0	0.8	0.6	0.2	0.9
20:1, n–9	8.7	7.2	0.8	0.8
20:1, n–7	1.3	1.0	—	—
20:2	0.7	0.5	—	—
20:3	—	0.1	0.2	—
22:0	0.9	0.3	0.3	0.5
22:1	46.1	15.5	0.3	0.3
22:2	0.7	0.2	—	—
22:3	0.1	0.1	—	—
24:0	0.3	0.1	0.2	0.5
24:1	1.3	0.7	—	4.3

*Carbon number: no. of double bonds, position of double bond from methyl terminal.

conventional soxhlet procedure; for mustard paste (Speisensenf), a separating funnel was used. Purified lipids were saponified. The unsaponifiable matter was removed[1] and the liberated fatty acids were transformed into methyl esters using boron-trifluoride[2]. Purity of esters was checked by TLC and impure esters were purified by loading on to a glass column (10 × 1.5 cm) packed with silica gel in toluene[3]; esters were eluted with 100 ml toluene and again checked for purity as before.

Lipid contents of the seeds of *B. rapa* (rubesteel, stielmuss), *S. alba* (mustard paste, Speisensenf), *S. oleracea* (spinach, Spinat) and *B. vulgaris* (Foliage beet, Mangold) were found to be 40.0, 6.6, 4.0 and 5.6 per cent respectively. There is no mention about the fatty acid composition of seed lipids of *B. rapa* var. *esculenta* and *B. vulgaris*. However, major fatty acids are known for *S. alba*[4] and *S. oleracea*[5]. The lipids of *B. rapa* and *S. alba* (Table 1) contained erucic acid upto 46.1 and 15.5 per cent respectively. It is inferred that the mustard seeds used for the preparation of mustard paste contained lower level of erucic acid, The percentage (wt.) of oleic, linoleic and linolenic acids in *B. rapa* and *S. alba* were 13.6 and 24.7; 13.5 and 20.6; and 8.6 and 20.7, respectively. *S. oleracea* and *B. vulgaris* contained 10.4 and 15.7 per cent palmitic, 23.5 and 31.3 per cent oleic, and 60.9 and 43.2 per cent linoleic acids respecpectively. Unusual amount of C24:1 (4.3 per cent) was found in *B. vulgaris*. While eicosenoic acid was present appreciably in *B. rapa* and *S. alba*, some previously unreported long-chain fatty acids, 22:0 and 24:0, were found in all the four species but 20:2 20:3 22:2, and 22:3 were present in some of the species in very small amounts.

Nasirullah thanks the Federal Ministry of Nutrition, Agriculture and Forestry, FRG for financial assistance.

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IMPROVEMENT IN COLOUR OF DEEP FAT FRIED POTATO CHIPS FROM COLD STORED POTATOES BY LACTIC ACID FERMENTATION (*LACTOBACILLUS PLANTARUM*)

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Received 30 *May* 1986; *revised* 2 *September* 1986

Fermentation conditions were standardized to obtain golden yellow to white coloured deep fat fried potato chips/ wafers from cold stored potatoes with high sugar content. Potato slices, 1.2 mm thickness, were fermented with pure culture of *Lactobacillus plantarum* (10^8 CFU/ml) in 2.5% pasteurized brine at 32–35°C. The optimum ratio of brine to potato slices was lower than 1:1 for fermenting the slices more effectively in 8–12 hr. Changes in moisture, pH, acidity, reducing and total sugars were recorded periodically. A fall in pH (6.65 to 3.96), reducing sugar (10.03 to 4.29% on moisture free basis) and an increase in lactic acid content (0.02 to 0.18%) were noticed after 12 hr fermentation.

The most important problem in the potato chips industry is to maintain the desirable colour of the

finished product throughout the storage period. Some of the factors that contribute to the colour of potato chips are the varieties and growing conditions which affect sugar content of potatoes, methods of storing or handling in order to maintain low sugar level and several other methods of treating potatoes or the slices in hot water and various chemical solutions[1-3] to deplete the sugars. Potatoes with high specific gravity or high total solids are preferred for processing. However, the potato varieties which accumulate high levels of reducing sugars during storage are not considered fit for making chips as high sugar content causes browning or discolouration of the end product when deep fat fried and subsequently stored at ambient temperature.

Lactic acid fermentation is considered to be one of the important processes in the food industry as it imparts a characteristic flavour. Bacteria which produce predominantly lactic acid from available sugars, are responsible for the fermentation. Little is known about the use of lactic acid fermentation of potato slices for reducing the total sugar contents and to produce potato chips/wafers of desirable colour on frying. However, potato solids have been subjected to fermentation by using a yeast strain.[4] The present study covers lactic acid fermentation of potato slices made from high sugar, cold-stored potatoes to obtain light golden yellow coloured chips upon frying. This low energy process of making potato chips with desirable sensory quality attributes may have an edge over the chemical methods as well as over the process of fermentation using yeast strain where cost and energy involvement is more.

A pure culture of *Lactobacillus plantarum* procured from Pathology Division, National Chemical Laboratory, Pune, was maintained in an enriched medium comprising (in 100 ml distilled water) glucose 0.5 g, lactose 0.5 g, liver extract 600 mg, sodium acetate 600 mg, yeast extract 500 mg, KH_2PO_4 0.05 g, K_2HPO_4 0.05 g, $MgSO_4.7H_2O$ 0.02g, NaCl 1 mg, $MnSO_4.4H_2O$ 1 mg, $FeSO_4.7H_2O$ 1 mg. pH was adjusted to 7.6. The stock culture of *L. plantarum* was activated by 2-3 successive transfers of 24 hr intervals in fresh broth[5]. Inoculum (1.0 ml/kg of potato slices) containing 10^8 colony forming units (CFU)/ml was dispensed to potato slices dipped in 2.5 per cent pasteurized brine in 8 lb glass jars and incubated for 12 hr at 32-35°C. The ratio of potato slices to brine was kept at 1:1.

TABLE 1. CHANGES IN CHEMICAL CHARACTERISTICS OF POTATO SLICES DURING FERMENTATION AND THEIR QUALITY CHARACTERISTICS* UPON DEEP FAT FRYING

Treatment	Incubation period+	Moisture	Sugars (moisture free basis)			Steeping solution	% lactic acid
			Reducing	Non-reducing	Total		
	(hr)	(%)	(%)	(%)	(%)	pH	
Steeped in tap water (control)	0	78.4	10.03	7.36	17.79	6.53	0.01
	4	85.4	8.20	6.49	15·03	6.40	0.01
	8	85.9	7.80	5.61	13.71	5.85	0.04
	12	86.2	7.26	5.23	12.77	4.80	0.08
Fermented naturally in 2.5%	0	78.4	10.03	7.36	17.79	6.65	0.01
pasteurised brine (T_1)	4	75.8	8.22	6.70	15.27	6.30	0.01
	8	75.4	6.95	5.98	12.93	5.56	0.05
	12	75.8	6.86	4.51	11.32	4.65	0.15
Fermented in 2.5% pasteurised	0	78.4	10.03	7.36	17.79	6.65	0.02
brine using *plantarum* (T_2)	4	75.2	7.81	6.41	14.56	5.68	0.15
	8	74.8	6.31	5.71	12.02	4.26	0.10
	12	75.3	4.29	4.50	9.32	3.96	0.18

*Quality characteristics of potato chips:

Control —dark brown coloured potato chips upon deep fat frying—undesirable product with respect to sensory quality attributes.

T_1 —dark to light brown coloured potato chips upon deep fat frying with charred taste—undesirable product.

T_2 —0–8 hr: Dark to light brown coloured chips upon deep fat frying—undesirable product.

12 hr: Golden yellow to white coloured good potato chips upon deep fat frying. Satisfactory product with respect of colour flavour/aroma, texture and general acceptability.

+ Incubation at 32–35°C.

The cold-stored potatoes (commercial varieties i.e. 'Kufri Chandramukhi' and 'Numbery' in trade) with high level of reducing sugars (2.17 on fresh weight basis or even higher) were washed, peeled and sliced to a uniform thickness (approximately 1.2 mm) using a pineapple slicer. Slices were washed, with water and divided into three lots. One lot was steeped in tap water, (control), the second in 2.5 per cent pasteurized brine (T_1) and the third was inoculated with a pure culture of *L. plantarum* in 2.5 per cent pasteurized brine (T_2). The samples were examined at intervals of 4 hr for changes in moisture, pH, acidity and sugars (reducing and total) using standard AOAC methods[6].

Samples of potato slices (100 g) were washed thoroughly and deep fat fried in refined groundnut oil (750 g) by lowering the slices into oil at 160°C and frying for 3-4 min at 110-130°C. Deep fat fried potato chips/wafers were evaluated for colour, flavour/aroma, texture and overall acceptability by a panel of judges comprising scientific workers.

Data presented in Table 1 reveal that the moisture content of slices derived from cold-stored potatoes varied from 78.4 to 79.0 per cent. Potato slices steeped in tap water had a moisture content of 7.9 per cent while those kept in 2.5 per cent brine for fermentation with or without pure culture of *Lactobacillus*, lost 2.6-3.0 per cent moisture during 12 hr incubation. A gradual fall in pH and sugars, and an increase in per cent acidity as lactic acid were observed during the course of fermentaton. Potato slices steeped in 2.5 per cent pasteurised brine (1:1) along with *Lactobacillus* inoculum had attained a lactic acid level of 0.18 per cent in 12 hr incubation. The reducing sugar content of these slices got reduced from 2.17 per cent (10.03 per cent on moisture free basis (MFB) to 1.06 per cent (4.29 per cent on MFB) when compared with slices steeped in tap water (from 10.03 to 7.26 per cent) and those subjected to natural fermentation (10.03 to 6.86 per cent).

Fermentation period of 12 hr could further be minimised to 8-8.5 hr by increasing the *Lactobacillus* inoculum containing 10^8 CFU/ml to the tune of 2-3 ml/kg of the material to be fermented and cutting down the ratio of brine to potato slices (0.5:1) without affecting the overall acceptability of the end product.

The potato slices inoculated with *L. plantarum* gave satisfactory end product upon deep fat frying with respect to colour, flavour/aroma, texture and overall acceptability. The potato slices subjected to natural fermentation in tap water and those in 2.5 per cent brine yielded dark brown and light brown coloured potato chips respectively with charred taste on deep fat frying. This darkening and charring of potato chips is caused by non-enzymatic browning[7]. The data given in Table 1 indicate that when the reducing sugar level in the slices is reduced to 4.29 per cent, the intensity of the browning is decreased giving a satisfactory product upon frying.

The rise in lactic acid content in potato slices due to fermentation does not appreciably affect the organoleptic quality of the chips upon deep fat frying as most of the acids are lost during washing of the slices after fermentation. Moreover, a small amount of acid and salt left in the slices will add to the taste of the chips.

The authors are grateful to Dr. B.L. Amla, Director of the Institute and Shri K. K. Mookerji, Area Co-ordinator, CFTRI Regional Centres, Mysore for their keen interest in the investigations.

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CHEMICAL COMPOSITION OF DIFFERENT VARIETIES OF APRICOTS AND THEIR KERNELS GROWN IN LADAKH REGION

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Received 24 *April* 1985; *revised* 29 *July* 1986

The chemical composition of the kernels from eleven varieties of apricots grown in Ladakh region of India has been investigated. The oil content of the kernels, ranged between 27 and 67% and it is composed of three fatty acids, viz., oleic (51-80%), linoleic (10-46%) and palmitic acid (3-11%). Kernels contain 20 to 45% proteins.

Apricot (*Prunus armeniaca* L.) is one of the important temperate fruits grown in India. It mostly grows in

mid-Himalayas at elevations of 1000-2500 m above mean sea level (MSL). The fruit also grows extensively in Ladakh region of Jammu & Kashmir State which is a perpetually dry area characterised as the high altitude desert. The growing areas are located at altitudes above 2500 m MSL and fruit matures far late i.e. mid July to mid September. The area besides being far off from consuming centres, is also poorly equipped with communication channels. The only avenue for disposal is to dry the fruits in the open sun. Better varieties are dried along with stones for marketing, whereas the inferior and wild varieties are utilized for oil extraction from the kernels. According to rough estimates about 2500 quintals of dry apricots are marketed from Ladakh annually. The oil is traditionally extracted from mildly roasted kernels using a stone mortar, the yield being 30-35 per cent. Other details of the habit, habitat and chemical composition of the fruits have already been reported[1,2].

Authentic fruit varieties were collected from Government Fruit Plant Nursery, Saspol (District Leh) by harvesting 100 fruits in each case at eating-ripe stage. Pits and kernels were removed manually and data recorded on the fresh fruit yield. The kernels were brought to the laboratory for analysis.

All the samples were analysed in duplicate and their mean values reported. Moisture, protein, sugars, crude fibre and total ash contents were analysed by AOAC methods[3]. The kernels were crushed coarsely and extracted in a soxhlet extractor using n-hexane (69-71 °C) as solvent. Total of thirty siphons in about 6 hr were found to be adequate for each sample. The oils obtained on complete desolventisation of extracts under reduced pressure were analysed[4] for their physico-chemical characteristics and component fatty acids.

Methyl esters for each sample were obtained by treating their mixed fatty acids with excess of methyl alcohol in the presence of 1.5 per cent concentrated sulphuric acid. The esters were analysed on a Perkin Elmer 881 Dual Column Gas Chromatograph equipped with a flame ionization detector, 2m x 4.8 mm S S column packed with 15 per cent Reoplex-400 on Chromosorb W (40-60 mesh) at 190°C isothermal.

The pits constituted 7.3 to 19.0 per cent (w/w) of the fresh fruit. The kernels formed 21.9 to 38.0 per cent of the pits with the single exception of 'Koban' variety which had only 13.5 per cent kernel content by weight

TABLE 1. PHYSICAL CHARACTERISTICS OF APRICOT PITS

Variety	Pit* (% w/w)	Kernel* (% w/w)	Kernel in pin (% w/w)	Av kernel wt (g)
Halman	9.4	2.35	25.0	0.3 2
Rakchay-karpo	9.2	2.96	32.2	0.52
Tokpopa	14.5	4.65	32.1	0.52
Rogan	19.0	7.22	38.0	0.43
Koban (bitter)	10.5	1.42	13.5	0.39
Khochuli (bitter)	11.0	3.60	32.7	0.34
Shakarpara	8.5	1.86	21.9	0.25
Nari	9.8	2.89	29.5	0.26
Australian	7.3	2.02	27.7	0.50
Sufaidi	9.1	2.59	28.5	0.40
Normoo	9.4	2.23	23.8	0.32

Mean of duplicates * In fresh fruit.

TABLE 2. CHEMICAL COMPOSITION OF LADAKH APRICOT KERNELS

Variety	Oil (%)	Protein (%)	Moisture (% w/w)	Sugars (%)	Fibre (%)	Ash (%)
Halman	37.6	34.5	4.6	11.6	3.8	2.7
Rakchay-karpo	44.0	31.9	4.1	12.9	2.6	2.5
Tokpopa	41.1	42.3	4.1	5.2	4.8	2.4
Rogan	36.4	39.3	4.8	8.1	4.9	2.2
Koban	27.7	45.3	4.9	4.3	6.6	3.2
Khochuli	41.4	37.3	3.9	4.1	6.1	3.1
Shakarpara	45.2	41.3	4.4	5.4	2.3	2.1
Nari	51.6	26.1	4.2	10.8	3.7	2.3
Australian	48.0	30.9	4.8	5.0	3.9	2.2
Sufaidi	66.7	20.3	3.7	5.2	2.2	1.9
Normoo	36.5	38.8	5.9	7.8	7.2	3.9

Mean of duplicates.

TABLE 3. PHYSICO-CHEMICAL CHARACTERISTICS AND COMPONENT FATTY ACIDS OF APRICOT KERNEL OILS

Variety	Colour	nD^{37}	Unsap. matter (%)	Sap. Eq.	Iodine value	% free fatty acids	Component fatty acids (% by wt of total) 16:0	18:1	18:2
Halman	Pale Yellow	1.4679	0.80	294.5	85.0	0.41	10.68	79.80	9.62
Rakchay-karpo	Yellow	1.4672	0.34	295.4	102.0	2.70	4.30	71.80	23.90
Tokpopa	Yellow	1.4679	0.86	291.7	80.8	2.90	4.55	75.53	19.92
Rogan	Yellow	1.4675	0.89	290.8	102.0	0.34	4.39	64.91	30.70
Koban	Redish	1.4692	0.51	298.5	111.0	1.20	7.19	56.18	36.63
Khuchuli	Yellow	1.4679	0.57	293.0	104.0	0.81	3.77	67.98	28.25
Shakarpara	Yellow	1.4685	0.78	290.0	86.8	0.56	5.12	67.89	26.99
Nari	Pale Yellow	1.4688	0.45	301.0	85.0	0.62	5.30	63.63	31.07
Australian	Yellow	1.4677	0.36	293.0	105.0	1.10	6.43	83.33	10.24
Sufaidi	Pale Yellow	1.4689	0.81	304.3	123.0	0.47	3.15	50.95	45.90
Normoo	Yellow	1.4673	0.28	296.8	88.9	1.10	5.86	66.43	27.71

Means of duplicates.

(Table 1). From Table 2, it may be observed that 'Sufaidi' variety had the highest oil content (66.7 per cent) and 'Koban' the lowest (27.7 per cent). Sugars varied from 5.2 to 12.9 per cent except in case of bitter kernels of 'Koban' and 'Khochuli' varieties which had low values. The protein content in the kernels ranged from 20.27 to 45.25 per cent. These characteristics of Ladakh apricot kernels are nearly the same as in other apricot varieties grown in mid Himalayan ranges[5,6].

The physico-chemical characteristics and fatty acid composition of the oils are given in Table 3. No unusual fatty acids like the ones possessing epoxy, cyclopropene, hydroxy or other oxygenated groups were detected spectroscopically (UV, IR and NMR)[7]. HBr titration[8] and chromatographic techniques[9] also indicated the absence of these fatty acids.

The fatty acid composition of oils in all apricot varieties is well in agreement with the general composition of the *Prunus* sp. (Rosaceae)[10,11] by virtue of not only being rich in oleic acid but also containing appreciable amounts of linoleic acid.

The authors are thankful to Central Instrumentation Section, Regional Research Laboratory, Jammu for GLC and spectral analyses.

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QUANTITATIVE VARIATIONS IN SOME METABOLITES IN THE DIFFERENT PARTS OF TWO VARIETIES OF PEAR FRUITS

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Received 30 *April* 1986; *revised* 1 *September* 1986

Quantitative variations in total soluble solids, reducing and total sugars, acidity, vitamin C and total phenolics in five different regions viz. upper, middle and lower regions of the receptacle, the stem and calyx ends of two cultivars of pear fruit-('LeConte' and 'Patharnakh') were followed. In both the varieties, the total soluble solids were higher in the calyx end of the fruit. They were more in the upper part of the receptacle. Acidity was maximum in the lower receptacle and increased progressively towards the upper receptacle while total phenolics were significantly higher in the stem end and least in the lower receptacle regions of the fruit.

Pear cultivation has assumed greater importance in Northern India only in recent years. Not much information is available on the biochemical status of the fruit. The concentration of total soluble solids has been reported to increase from stem end to the calyx end of the fruit in Japanese pear[1]. The present investigation was undertaken to know the quantitative variations in some metabolites in different parts of the pear fruit cultivars 'Patharnakh' and 'LeConte'. The fruits were obtained from 13-year old trees of Cv. 'Patharnakh' (*Pyrus pyrifolia* (Burm) Nakai) and Cv. 'LeConte' (*Pyrus pyrifolia x Pyrus communis*) maintained under uniform cultural practices at the Fruit Research Station, Bahadurgarh (Patiala). In each cultivar, ten fruits were taken and peeled off. Segments of the flesh from stem end, calyx end, upper receptacle, middle receptacle and lower receptacle were taken for analysis. Total soluble solids were estimated with the help of a hand refractometer and values were corrected to 20°C. Total acidity was estimated by titrating a known volume of the juice against N/10 NaOH using phenolphthalein as indicator and expressed in terms of malic acid. Vitamin C, sugars and total phenolics were determined by using the standard techniques[2].

Data in Table 1 show that total soluble solids in 'Patharnakh' pear differ significantly in different fruit segments. The highest total soluble solids were recorded in calyx end and upper receptacle and the minimum in the lower receptacle. In 'LeConte' pear (Table 1) the total soluble solids were more at the calyx end than at the stem end. Kajiura *et al*[1]. also reported

TABLE 1. COMPOSITION OF DIFFERENT PARTS OF FRUIT CULTIVAR 'PATHARNAKH' AND 'LECONTE'

Fruit part	Total soluble solids (%)	Acidity %	°Brix-acid ratio	Vitamin C (mg/100g)	Sugars		Total phenolics (mg/100g)
					Reducing (%)	Total (%)	
			Patharnakh				
Stem end	10.13	0.29	35.04	1.91	5.01	6.43	50
Upper receptacle	10.40	0.20	51.35	1.75	5.51	6.91	36
Middle receptacle	10.00	0.28	35.94	2.54	5.02	6.51	22
Lower receptacle	9.27	0.32	29.35	1.81	4.31	5.81	18
Calyx end	10.40	0.28	37.29	1.72	5.03	6.52	35
C.D. at 5%	0.296	0.041	6.21	0.092	0.131	N.S.	7.475
			Leconte				
Stem end	12.40	0.25	50.58	2.00	6.03	8.33	36
Upper receptacle	13.00	0.17	76.91	2.15	7.04	8.53	22
Middle receptacle	12.20	0.22	55.74	2.49	5.81	7.87	15
Lower receptacle	12.00	0.24	50.00	2.09	5.25	7.14	12
Calyx end	13.93	0.27	52.33	1.80	6.15	8.33	23
C.D. at 5%	0.23	0.029	9.20	0.109	0.237	0.167	4.834

that total soluble solids increased from stem end to calyx end in Japanese pear.

There was not much difference in the acid content of stem end and calyx end in 'Patharnakh' pear, but a progressive increase in its level was observed from upper receptacle to lower receptacle (Table 1). In 'LeConte' pear, the per cent acidity was maximum at the calyx end and the lowest in the upper receptacle. Like in 'Patharnakh', the acid content in 'LeConte' increased from upper receptacle to lower receptacle.

Data in Tables 1 reveal that the upper receptacle has highest °brix-acid ratio in both the cultivars. The trend of decreasing °brix-acid ratio from upper receptacle to lower receptacle was almost the same in both cultivars. 'LeConte' had higher °brix-acid ratio than the 'Patharnakh'.

The vitamin C content was the highest (2.54 mg/100g) in the middle receptacle in 'Patharnakh' as well as in 'LeConte' pear (2.49 mg/100g). The calyx end of both the cultivars contained the lowest vitamin C. Similarly, Krivencov *et al*[3]. reported more vitamin C at the centre in the jujube fruit than near the skin and more in the upper part than in the stylar end.

In 'Patharnakh' pear, the levels of both reducing and total sugars were maximum in the upper receptacle. The levels of total sugars were the same in the stem end and the calyx end; however, it decreased progressively towards the lower receptacle. In 'LeConte' pear, the stem end and the calyx end also maintained higher levels of sugars (Table 1). The highest levels of reducing and total sugars in both the cultivars were found in the upper receptacle and their levels decreased towards the cente of the fruit.

The total phenolics were significantly higher in the stem end portion of the fruit in both the cultivars (Table 1). The total phenolics decreased from the upper receptacle towards the lower receptacle where the minimum phenolics were recorded.

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LACTOSE CONTENT—A FACTOR TO DISTINGUISH *CHANNA* AND *KHOA* BASED SWEETS

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Received 12 *March* 1986; *revised* 18 *August* 1986

Lactose content can be used to distinguish *Channa* and *Khoa* based milk sweets. True lactose was estimated in milk sweets such as *Rasagolla*, *Rasbhari*, *Apple*, *Rajbhog*, *Burfi*, *Pedha*, *Anarkali*, *Champakali* and sandwich by the colorimetric method. True lactose was not present in *Channa* based sweets, whereas *Khoa* based sweets contained 5.1 to 11.9% lactose and of those milk sweets prepared from a mixture of *Channa* and *Khoa* contained 2.6 to 5.0% lactose.

Many successful attempts have been made to retain its wholesomeness of milk for a long period. Concentration of milk, coagulation and addition of sugars, flour, flavouring and colouring matter have led to the preparation of many traditional milk products. Milk powder, infant foods, *khoa*, *pedha*, *burfi*, *kheer*, *rasmalai*, cream, ice-cream, casein, *rasagolla*, *champakali* and *anarkali* are milk products available in the market[1]. *Khoa* is prepared by heating and concentrating the milk, whereas *channa* is prepared by coagulation of hot milk with citric or lactic acids or by adding cleansed sour whey[2]. Although *channa* and *khoa* are derived from milk, sweets prepared from them have different tastes, consumer acceptability and market value. Instances have been noticed where *khoa* has been adulterated with *channa*. So far, no method has been devised to check such adulteration and also sufficient information is not available regarding their composition. Since sucrose is considered specific to sweets and lactose to milk sweets, a study was undertaken to estimate true lactose and sucrose in *channa* and *khoa* based sweets.

Thirty samples of *channa* based, *khoa* based and *channa-khoa* mixed sweets were procured from the local market. Each sample was blended thoroughly and used for analysis. Lactose as original reducing sugar (ORS) and sucrose were estimated by the Lane Eynon method[3].

Estimation of lactose by colorimetric method: True lactose was estimated by the colorimetric procedure as described by Nickerson[4].

(*a*) *Zinc acetate-phosphotungstic acid* (ZAPT): Twenty five grams of zinc acetate and 12.5 g of phosphotungstic acid in water; 20 ml of glacial acetic acid is added and diluted to 100 ml.

(*b*) *Glycine-NaOH buffer:* 150 ml of glycine solu-

tion containing 2.4768 g glycine and 1.9359 g NaCl were mixed with 850 ml of 0.385 N NaOH to give a pH of 12.8.

(*c*) *Methylamine solution:* 5.0 g of methylamine-HCl was dissolved in distilled water and diluted to 100 ml and stored in a refrigerator.

(*d*) *Sodium sulphite solution:* 1 per cent (W/V) sodium sulphite was dissolved in water and diluted to 100 ml, prepared daily.

(1) *Stock solution:* 2.6315 g of lactose monohydrate (USP grade) was dissolved in 200 ml of 0.1 per cent (W/V) benzoic acid stored in a refrigerator.

(2) *Working solution*: 10, 15, 20, 25 and 30 ml stock solution was diluted to 250 ml separately to get 0.5 to 1.5 mg of lactose per ml.

Preparation of the sample: (*a*) To 8 g of the sample, 1 ml of ZAPT was added, diluted to 10 ml, filtered after 10 min with Whatman No. 1 filter paper.

(*b*) To 0.5 ml of filtrate, 0.5 ml of 1N NaOH was added and diluted to 10 ml and filtered with Whatman No. 1 filter paper.

(*c*) 5 ml of filtrate is diluted to 10 ml.

Procedure: (*a*) 5 ml each of working solution unknown i.e., (*c*) and water were pipetted into 25 ml test tubes, 5 ml of glycine NaOH buffer, 0.5 ml of methylamine solution and 0.5 ml of sodium sulphite solution were added and mixed thoroughly. Tubes were heated in a thermostatically controlled water bath at 65°C for 25 min and cooled immediately in an ice-water bath for 2 min to stop the reaction. Absorbance was read against blank at 540 nm in a Spectronic-20 spectrophotometer. A standard curve was drawn by plotting absorbance against concentration of lactose solution.

Polarimetric[5], enzymatic[6] and titrimetric[7] (Chloramine T) methods are available for the estimation of lactose, but they have drawbacks like large concentrations of lactose required, non-availability of enzyme and interference from other reducing substances, respectively. Therefore, it was thought desirable to have a more sensitive method for its estimation. Lactose reacts with methylamine in hot alkaline solution to form a red complex which absorbs at λ max 540 nm. This principle is applied in the present study to estimate true lactose.

True lactose, lactose as original reducing sugars and sucrose contents of 4 varieties of *channa* based, 2 varieties of *khoa* based and 3 varieties of *channa* and *khoa* mixed sweet preparations are given in Table 1. Each sweet preparation was taken in triplicate for estimation and average values are reported. It can be seen that true lactose in *channa* based sweet preparations is absent and ranges between 5.1 and 11.9 per cent in *khoa* based sweets and 2.6 to 5.0 per cent in mixed sweet preparations. The lactose as original reducing sugar reported may be due to hydrolysis of sucrose during preparation of milk sweets. True lactose content of fresh *channa* ranges from 1 to 1.95 per cent. Lactose may undergo hydrolysis as well as lactic fermentation during preparation of *channa* based sweets resulting in negligible proportion of true lactose in the finished product. Hence, it is concluded that true lactose content can be used to distinguish *channa* and *khoa* based milk sweets.

TABBE 1. LACTOSE AND SUCROSE CONTENTS OF CHANNA, KHOA AND CHANNA-KHOA MIXED SWEETS

Sweet preparation	No. of samples	Lactose* (%)	True lactose (%)	Sucrose (%)
Channa based				
Rasagolla	3	Traces	Nil	36.3–40.0
Rasbhari	2	0.1–2.5	Nil	58.0–62.6
Apple	2	0.8–3.9	Nil	50.0–57.0
Rajbhog	1	0.3	Nil	58.3
Channa (Market)		Nil	Nil	Nil
Channa (Fresh)		Nil	1.95	Nil
Khoa based				
Burfi	5	10.0–15.3	8.1–11.9	33.9–49.3
Pedha	5	5.9–14.7	5.1–10.5	30.1–37.6
Khoa (Market)		16.0	15.8	Nil
Khoa channa mixed				
Anarkali	1	6.0	5.0	38.5
Champakali	3	2.7–3.2	2.6–3.0	42.7–47.5
Sandwich	1	2.8	2.9	68.4

*As original reducing sugar

Authors thank the Director, Central Food Technological Research Institute, Mysore for providing necessary facilities.

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FEASIBILITY OF INCORPORATION OF PARTIALLY DEODOURISED FISH MEAT IN EXTRUSION COOKED PRODUCTS

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Received 3 *February* 1986; *revised* 1 *September* 1986

Croaker (*Johnius dossumuri*) meat was partially deodourised by boiling the dressed fish in 0.3% orthophosphoric acid followed by passing through a screw press to separate the meat. The meat, having a moisture content of 50%, was mixed with flour and was subjected to extrusion cooking. The product had no fishy odour suggesting the feasibility of using partially deodourised meat from miscellaneous fishes in extrusion cooked products.

A sizable portion of annual marine landings in India comprises miscellaneous fishes having poor marketability. Although, these fishes have high nutritional content, they are commercially under-utilised due to poor taste, small size, bony nature and unattractive appearance. The scope of using meat from these fish varieties in various processed products has been pointed out[1]. Attempts have been made to develop several secondary products incorporating meat from these fishes. Some of the processes include preparation of minced fish muscle blocks[2], fish protein concentrates[3], protein hydrolysates[4] and cereal based formulations[5-7]. A process has been worked out in our laboratory for preparation of partially deodourised meat from these fishes[4]. Extrusion cooking technology offers scope for development of a variety of texturised products[7,8]. Only a few attempts have been reported for development of extrusion-cooked products using fish meat[9,10]. Incorporation of whole fish meat, however, could give products having objectionable fishy flavour. The present communication reports on the feasibility of using partially deodourised meat from a typical low-priced trash fish, croaker, as a protein supplement in extrusion cooked products.

Croaker (*Johnius dossumuri*) obtained from a Bombay market was used in the experiments. The fish was beheaded, cut open, eviscerated and washed in potable tap water. The fish was then passed through a laboratory model deboning machine and the meat was collected. The meat was partially deodourised according to the method described earlier[4]. One-kg lots of the meat were boiled for 15 min in 3 l of water containing 0.3 per cent orthophosphoric acid. After cooking, the slurry was passed through cheese cloth and the meat was collected. The cheese cloth containing the meat was pressed between layers of absorbant paper by a screw press to remove excess of water which contained odour-bearing compounds. The process was repeated till no more water was released from the meat. The cooking of the meat at low pH followed by removal of excess of water significantly deodourised the meat. The meat press cake had moisture and lipid contents of about 50 and 2.5 per cent respectively.

The deodourised meat was used for preparation of cereal based extrusion cooked products. The press cake (150 g) was mixed with 800 g refined wheat flour (*maida*), 20 g salt, 5 ml refined oil and 25 ml water. The mixture was passed through a laboratory model extrusion cooker[7] at a barrel temperature of about 150°C. The prepared product was crisp, light coloured having negligible fish odour and had good rehydration properties. The product did not have any significant fish odour even after storage for a period of two years. Fig. 2 shows the flow sheet of the process.

Applicability of fish protein concentrate as a protein supplement in cereal based products has been pointed out. However, the process of making fish protein concentrate is comparatively expensive and time consuming, Incorporation of fish meat in extrusion cooked products offers an inexpensive way for its utilisation. Maga and Reddy[10] observed that the type of fish used is important in determining the quality of extrusion cooked product. The lipid content of the fish used could impart fishy odour to the extruded product as in the case of product prepared from Atlantic cod[9]. The present process for isolation of partially deodourised fish meat is much simpler and suggests scope for incorporation of meat from miscellaneous fish as a protein

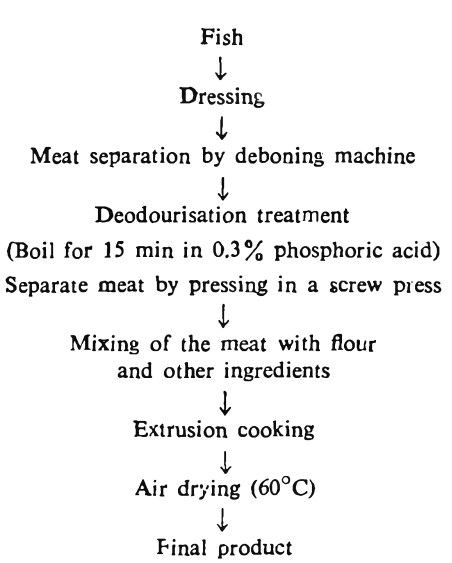

Fig. 1. Flow diagram for extrusion cooking of deodourised fish meat.

supplement in cereal based extrusion cooked products having almost no fishy flavour.

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STUDIES ON HIGH PROTEIN BREAK-FAST FOOD BASED ON CALCIUM GROUNDNUT PROTEINATE

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Received 29 *September* 1984; *revised* 2 *December* 1986

Based on calcium groundnut proteinate and wheat flour, a process has been standardised for the preparation of breakfast food with desired texture, crunchiness and non-gruel forming property on rehydration. Protein efficiency ratio of the untoasted food was 2.26 which decreased to 1.4 on toasting in an electrical oven. The PER data correlated well with lower values of available lysine and *in vitro* digestibility for toasted products.

Commonly available breakfast cereal products which enjoy a favourable consumer market are often not nutritious[1,2]. From the nutritional point of view, a breakfast food based on a proper admixture of cereal, and oilseed protein provides the necessary calories and protein with a fairly well balanced amino acid composition. Taking advantage of the texture forming ability of the calcium derivative of groundnut protein, a process has been developed for the preparation of high protein breakfast food.

Calcium groundnut proteinate was prepared from edible groundnut flour by extraction at alkaline pH and subsequent precipitation of proteins with calcium chloride at neutral pH[3]. Whole wheat flour as a cereal base and additives such as sugar and salt were used.

The process consists of: (*a*) blending of wet groundnut protein (60 per cent moisture) and wheat flour for the preparation of dough, (*b*) autoclaving at 1 kg/cm^2 pressure for 20 min, (*c*) tempering to a moisture content of 30 per cent, (*d*) disintegrating the heated dough into small pieces by passing through Gansons multimill (*e*) flaking in a Malmo flaker and partial drying in a Kilburn truck dryer at 60°C for 30 min to a moisture content of 15 per cent and (*f*) toasting for 15 min in an electrical roaster at 140-150°C to get a typical cereal like flavour and colour. Wheat flakes were also processed similarly for comparison.

For the measurement of physical characteristics, the flakes were reduced to a particle size of 2.4 mm. Physical characteristics like water absorption capacity[4], (ml/g), bulk density (g/ml), textural integrity[4] (weight of washed wet solids retained on a 16 mesh sieve per 100 g dry material), apparent visocosity of the reconstituted material (1 part of material dispersed in 6 parts of water at 60°C and cooled) were studied. The nutritive value was assessed by protein efficiency ratio (PER) assay[5]. *In vitro* digestibility was determined by the pepsin-pancreatin digestion method of Saunders[6]. Available lysine content was estimated by Carpenter's method[7].

Apart from the nutritional attributes, characteristics like shape, size, crunchiness and non-sogginess are important for breakfast food. Breakfast flakes prepared from either groundnut cake or sodium groundnut proteinate on rehydration tend to become pasty. Further, it is essential to extrude the dough through expensive equipment like macaroni press or extrusion cookers. These drawbacks can be overcome by the use of calcium groundnut proteinate in the formulation.

Some of the physical characteristics of the high protein breakfast food as compared to the product based on wheat alone indicated that there are some changes as a result of addition of protein (Table 1). Water absorption capacity (WAC) of protein food granules was low as compared to wheat granules which

TABLE 1. CHARACTERISTICS OF HIGH PROTEIN BREAKFAST FOOD

Characteristics	Untoasted granules[a]		Toasted granules[a]	
	Wheat	Protein food	Wheat	Protein food
Water absorption capacity (ml/g)	4.0	3.1	3.9	2.7
Bulk density (g/ml)	1.76	1.48	1.94	1.59
Texture integrity[b](%)	190	246	260	150
Viscosity[c] (Cp)	4160	1030	3440	460

[a]Particle size: 2.4 mm (8 mesh)

[b]Wt of washed wet solids retained on the sieve per 100 g dry material.

[c]Reconstituted powder measured by Model LVT Brookfield viscometer at spindle speed 60 rpm.

TABLE 2. NUTRITIVE CHARACTERISTICS OF BREAKFAST FOOD

Diet	Available lysine (g/16g N)	Av. protein intake (g)	Gain in wt. (g)	PER*	*In vitro* digestibility (%)
A. Toasted breakfast food	2.20	11.94	16.25	1.39	67
B. Untoasted breakfast food	5.10	18.10	40.88	2.26	71
C. Casein (control)	7.86	21.15	74.75	3.56	83

*Determined at 10% level of protein in diet: Duration of experiment 4 weeks: 8 male rats per group with an initial wt of 38.88 g.

indicates the possibility of higher crunchiness of the former on rehydration. Bulk density of toasted protein food granules was a little lower than that of wheat granules. This may be due to swelling and the porous texture forming ability of calcium groundnut proteinate on heating. There was considerable decrease in gruel viscosity of high protein granules due to toasting indicating that the rehydrated product is not gluey or pasty. Textural integrity of untoasted protein food was high compared to wheat granules. This is due to the chewy texture forming ability of calcium groundnut proteinate on thermal processing. However, toasted wheat granules had high textural integrity compared to toasted protein granules. This may be attributed to hardness or grittiness resulting on toasting of wheat granules and is not a desirable characteristic.

As toasting of the product produces a pleasant aroma (removal of raw taste), the material was subjected to toasting in an electrical roaster at a temperature of 140°C. However, on toasting, the PER decreased from 2.26 to 1.4 (Table 2). The low PER was due to reduction in available lysine content. *In vitro* digestibility was also reduced on toasting. The PER values correlated well with those of available lysine and *in vitro* digestibility. The adverse effect of heat on nutritive value (PER negative) of breakfast flakes has earlier been reported[2,3]. Instantaneous toasting by exposure as is industrially carried out in the production of corn flakes may improve the nutritive value. The present limited studies have shown that it is possible to prepare a high protein breakfast food (protein content 25 per cent) based on calcium groundnut proteinate and wheat flour with desired textural and nutritional characteristics. On reconstitution in hot water, and with sugar the protein food can be consumed even without the addition of milk.

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BOOK REVIEWS

Commercial Fruit Processing: Edited by J. G. Wood Roof and B. S. Luh, AVI Publishing Company Inc., Westport, Connecticut, 1986; Second edition; pp.678; Price: $ 78.00.

This book is the second edition updated in all respects with the developments in fruit processing. The chapters on Canning of fruits (Ch. 6) freezing of fruits (Ch. 7) and Brining Cherries and other fruits (Ch. 9) have been considerably revised and updated. The chapters on harvesting, handling and holding of fruits (Ch. 2), dehydration of fruits (Ch. 8) flavour and colour of fruits as affected by processing (Ch. 11) plant sanitation and waste disposal (Ch. 15) and fruit consumption trends and prospects (Ch. 16) have been completely rewritten to do full justice by including the latest developments that took place from the time the first edition appeared.

Besides, a lot of new information on the developments that took place has been added in the preparation of the second edition. The new material added includes expanded discussion of the history of fruit processing, extended list of fruits and fruit products, list of speciality fruit products, contract packing, breeding fruit for processing, list of molds commonly associated with fresh fruits, list of antimetabolites produced by molds, developments in cryogenic rail car, freeze dried fruits, pitting dates, table of opening and closing dates for canning fruits, aseptic processing of fruit puree, fruit irradiation, fruit leather, processing of banana, mango and kiwi fruit, US Food and Drug Administration regulations, list of products covered by Food and Drug standards of identity quality and fill, list of products covered by USDA Grade standards for fresh and processing fruits, changes in regulations for U. S. wine labels and storage of raisins.

The lucid presentation, extensive coverage with brief summing up of the status of knowledge with a detailed bibliography at the end of each chapter makes reading interesting.

This comprehensive book on commercial fruit processing with the latest information added, will certainly meet the need of every student, scientist and technologist working in this area, besides being very useful to the commercial fruit processor, the world over. This book is recommended for libraries of research institutes, universities and the R&D wings of the fruit processing concerns.

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

Membrane Separations in Biotechnology: Edited by W. Courtney McGregar; Marcel Dekker, Inc, 270, Madison Avenue, New York 10016; 1986; pp: 408; Price: $65 (US and Canada), $78 (All other countries).

Biotechnology is currently the most exciting field in the realm of applied science and entrepreneurship. Although the commercial exploitation of biotechnology has been rather limited so far, its vast potential to contribute towards human welfare is well recognised. Many an important breakthrough is expected in the near future in medicine, agriculture and industry.

The key to modern biotechnology is recombinant DNA, and there is a spate of literature on recombinant DNA pertaining to both basic and applied biology. The volume under review 'Membrane Separation in Biotechnology" deals, refreshingly, not with the main stream subject of biotechnology but rather with a related and yet a very essential topic, viz. the application of membranes for separation of biologicals, a topic that could immediately be recognised as relating to a segment of the downstream processing. It should be reckoned that the burgeoning business of biotechnology has to rely on supportive industries such as the one that provides for the separation process. The volume being reviewed is the first in the series of Bioprocess Technology. Its 13 articles written by eminent workers in the field from both academia and industry deal largely with down to earth and practical aspects of membrane application in biotechnology with just the adequate level of theoretical considerations.

The basic principle underlying membrane usage is their sieving effect: and with near precise control on pore size that becomes possible, biologicals can be separated on the basis of molecular size. With imaginative and skillful innovation almost anything can be separated in a bioprocess. The various articles featured in the volume are replete with illustrative examples where significant successes have been achieved; be it a simple harvest of cells from fermentation broth (Chapter-3) or purification of lectin and enzyme by affinity binding (Chapter-5). Chapters 8 and 9 should be of special interest to Food Scientists and Technologists. Kosikowski's article (Chapter 9) deals with the principles and practice of membrane separation in food processing with special emphasis on milk and milk products.

The membranes needed for various purposes are commercially available and they are semi-synthetic or synthetic in nature: Polysulfone, polypropylene, nylon

and the cellulosic types. The newer types viz. metal membranes of zirconium oxide will soon enter the field, offering greater versatility in the separation of biologicals.

The volume should be very useful to workers in the field of biotechnology, more particularly to those in the industrial establishments and to those contemplating in setting up of any bioprocessing industry.

RICHARD JOSEPH
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Advances in Refrigeration and Heat Pump Technology Achieved by the Application of Micro-Electronics and the Control of Systems by Micro-electronic Devices: International Institute of Refrigeration, 177, Bouleward Malesherbes, F. 75017, Paris-France, 1984-2; pp:236; Price: not mentioned.

This book is the 57th Volume in the I.I.R. series, "Refrigeration Science and Technology", consisting of introductory papers and contributed communications which were presented at a meeting of its Commission B-2 in Dresden (GDR) in 1984. About 130 participants from 20 countries participated in the meeting contributing 50 papers on the subject. Vol. 1 covers 23 papers.

Out of 3 working sessions, various topics like (1) New developments, new solutions for components and installations created by the application of micro-electronics; (2) Comparison between conventional automated components and installations with those controlled by micro-electronics (3) Principles and methods for mathematical modelling of steady state and dynamic behaviour of refrigeration components and installations were discussed covering 21 papers, while the remaining 2 papers were presented during the preliminary session.

Data processing is a new tool which finds varied applications in designing a machine, optimising a system, managing equipment or stocks, or for adopting performance of microprocessor controlled automators to the exigencies of the refrigeration apparatus. Therefore, there is a need to assess the application of data processing in various aspects associated with the construction and use of refrigerated machineries, heat pumps and air-conditioning installations. Design and construction, experimental research (automation of labs.), technological processes (materials and energy conversion), maintainance and servicing, planning and management, mental process permitting formulation are a few fields of technology where micro-electronics may be applied.

The paper titled "Comparison between conventionally automated components and installations with those controlled by micro-electronics" gives technical and economical considerations for the use of micro-electronics.

Various controls like (*a*) Traditional regulation control, (*b*) Decentralised control, (*c*) Fully centralised control and (*d*) Combined centralised and decentralised controls were dealt giving advantages and drawbacks, technical considerations, list of input and output functions, plant considerations, comparison of investments, etc., along with illustrations.

Also, paper titled "Micro-computer module for the automation of heat pumps and refrigerating plants" discusses the following aspects:

(1) Heat pump heating station, (2) Automation structure, (3) Micro-computer module device structure and (4) Control engineering for micro-computer modules.

The book gives adequate up-to-date information on advances in refrigeration and heat pump technology. The application of micro-electronics and control systems is gaining momentum in all the installations for achieving higher efficiency with less inputs. This book will be a useful addition to any scientific and technical library.

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

Guidelines for the Study of Dietary Intakes of Chemical Contaminants: WHO offset publication No. 87 WHO, Geneva, 1985; pp: 102; Price; Sw fr 11.

This book prepared under the Joint FAO/WHO Food Contamination Monitoring Programme in collaboration with the Joint FAO/WHO food standards programmes and the relevant committees of the Codex Alimentarius Commission is very timely because of the growing importance of chemical contaminant/pesticide exposure assessments in humans. Quantitation of human contaminant exposure through diets is a vital aspect in formulating the guidelines to minimise and prevent the variety of adverse health effects induced by chemical contaminants. The joint FAO/WHO food contaminants programme initiated in 1976 is one of the major health related activities of Global Environmental

Monitoring Systems (GEMS) which was established by the United Nations Environmental Programme (UNEP). Since 1978, collaborating centres have submitted information on estimated daily dietary intakes of DDT complex, total HCH isomers, the sum of aldrin and dieldrin, the sum of heptachlor and heptachlor epoxide, PCBs, lead, cadmium and aflatoxins. These data have been evaluated to determine the potential risk to human health from such exposures. This book gives useful guidelines and describes in detail the methodologies by which estimates of dietary intake of contaminants could be determined.

Three practical and basic approaches have been suggested: (*a*) Though the total diet intake studies (market basket) provide an accurate estimate of intake of contaminants for a country, it is complex and expensive, (*b*) the selective studies of individual foodstuffs and (*c*) the duplicate portion studies represent the minimum programmes where resources and technical capabilities are limited. The above contents are dealt in nine sections, chronologically describing the objectives and scope of the guidelines (Sections 1 and 2); approaches and methods most suitable for assessing the pattern of food consumption by an individual or household or population (Section 2), and discusses the validation of methods utilised, consumption data for 'extreme' eaters, use of food consumption data to derive the intake of contaminants. Section 4 describes the mode of development of food lists, preparation of guides and shopping lists and selection of representative samples of food. Sections 5, 6 and 7 deal with total diet studies (market basket), selective studies of individual foods and duplicate portion studies respectively, encompassing the selection of sample sites, population group(s), food items, collection and transportation of samples, the validity, and precision, utilisation of data on contaminant levels in food items from surveys undertaken in other countries and advantages and disadvantages of each technique.

Analytical facilities, preparation of samples, detection and quantification limits of the analytical methods, adequacy, and confirmation of the identity of the contaminants, reporting the results and quality assurance procedures are well presented in Section 8. Techniques for converting the contaminant levels to daily dietary intakes, their conversion to intakes per kilogram body weight and the calculation of 'extreme' intakes are indicated in the last Section (9).

This book with more than 10 tables, 19 annexures and 47 references, questionnaires, diary forms and proforma used globally with data forms, official and published methods, is a very useful guide for all the toxicologists, analysts, regulatory authorities, scientific policy makers, doctors and nutritionists. Particularly to those who are interested in initiating studies of the dietary intake of contaminants at the national level is of immense value. The painful efforts taken by Dr. C.F. Jelinek, Bureau of Foods, Food and Drug Administration, Department of Health and Human Services, Washington, D. C., USA and Dr. D. G. Lindsay, Food Science Division, Ministry of Agriculture, Fisheries and Food, London, England, in the preparation of this book, as acknowledged by FAO/WHO secretariat is worth mentioning.

Dr. (Mrs) M. K. Krishnakumari
C.F.T.R.I., Mysore.

AFST(I) News

Annual General Body Meeting of the Association

The twenty second Annual General Body Meeting was held on Sunday, 1 March 1987 at the Central Food Technological Research Institute, Mysore. Dr. T. R. Sharma, President of the Association presided over the meeting.

The President highlighted the activities of the Association during the year. Sri Naginchand, Hony. Exec. Secretary presented the minutes of the previous General Body Meeting and delineated the follow up action taken on the suggestions made during the last AGBM.

This was followed by the presentation of Report by the Secretary for the year 1986. The membership of the Association, the Secretary pointed out stood at 1,685. He made an appeal to the members to renew their membership in time. The activities of the Chapters were also breifly explained by the Secretary. The contribution of Rs. 30,000 made by the Bombay Chapter was appreciated and the Secretary appealed to other Chapters who are financially sound to make contribution to Head Quarters to tide over the financial difficulties. The Secretary explained about the publication of two Journals Journal of Food Science and Technology and the Indian Food Industry, during the year.

Important decisions taken at the AGBM include (*i*) increasing the membership fee to Rs. 40 from 1987, (*ii*) the members are to be given either JFST or IFI as per their choice, and (*iii*) issue of membership cards to all the Life Members.

The President requested the Hony. Exec. Secretary to announce the names of the office bearers for the year 1987. The Secretary informed that election was held for the office of the Hony. Joint Secretary. The office bearers are as follows.

1.	*President-designate*	Sri N. A. Pandit
2.	*Vice-President*	Dr. A. M. Nanjundaswamy (H.Q)
		Dr. A. C. Kapoor (Hissar)
		Dr. P, J. Dubash (Bombay)
		Prof. B. P. N. Singh (Pantnagar)
		Dr. K. L. Sarode (Madras)
	Hony. Joint Secretary	Sri N. Keshava
	Hony. Treasurer	Dr. K. Sambaiah

The new office bearers were inducted.

The meeting ended with a vote of thanks by the Hony. Exec. Secretary.

The Awards Function

The AFST (I) Awards function was held in the afternoon of 1 March 1987 at CFTRI, Mysore. The Awardees were presented by the President of the Association. Following are the persons selected for the various awards:

Prof. V. Subrahmanyan Industrial Achievement Award was presented to Dr. D. V. Rege, Hony. Professor, UDCT, Bombay.

The Young Scientist Award was presented to Dr. I. Karunasagar, Associate Professor, Fisheries College, University of Agricultural Sciences, Mangalore.

Best Student Award was given to Sri. Y. Mallikarjuna Reddy, Fisheries College, University of Agricultural Sciences, Mangalore.

Gardner's Award was given jointly to Dr. C. Arumughan, Sri N. Gopalakrishnan, Mr. P. P. Thomas, Dr. C. S. Narayanan and Dr. A. G. Mathew of the Regional Research Laboratory, Trivandrum for their paper entitled "Refining and bleaching of indigenous palm oil at pilot plant scale" published in the Journal of Food Science and Technology, Vol. 22 No. 6, 1985, 330-333.

Dr. P. B. Rama Rao Memorial Award was given to Sri Satish Kulkarni and Dr. M. K. Ramamurthy of the Southern Regional Station of National Dairy Research Institute, Bangalore for their paper entitled "Effect of moisture and solids-not-fat on rheological characteristics of butter" published in the Journal of Food Science and Technology, Vol. 22, No. 6, 1985, 358-361.

Suman Food Consultant Travel Award and Laljee Godhoo Smark Nidhi Award were not given.

Pune Chapter

The Annual General Body Meeting was held on 20th February '87. The following office-bearers were elected for the year 1987.

President	Dr. S. H. Godbole
Vice-President	Dr. (Mrs) P. P. Kanekar
Secretary	Mrs. N. R. Joshi
Joint Secretary	Mrs. K. S. Reddy
Treasurer	Dr. (Mrs) S. Dey

Mr. B. R. Bedekar gave a talk on "Fast Foods and Indian Culture". A discussion followed.

Bangalore Chapter

The following persons have been elected as office bearers for the year 1987-88.

President	Mr. O. P. Dang
Vice-President	Dr. P. Muddappa Gowda
Hon. Secretary	Mr. B. K. Ramaiah
Hon. Treasurer	Mr. R. Gururaj Rao
Hon. Jt. Secretary	Mrs. Mariamma Tharakan

Executive Committee Members

Mr. P. J. J. Tilak
Mr. Ambadav
Mr. A. Devariya
Mr. S. V. Dravid

Delhi Chapter

The Delhi Chapter of AFST (I) organised 12 lectures on various topics on food science and technology during 1986, from eminent scientists and technologists. It also organised five one-day seminars on food industries like Soybeans, Potato, Fruit-vegetables, processed foods. The Spices Symposium which was to have taken place during February 1987 will now be held during April 10-11, 1987 at Delhi.

Madras Chapter

An International Symposium on 'Proteins in Food Health and Industry' (ISPROFHI) was conducted during 7-11 January 1986 at Loyola College, Madras as part of its Diamond Jubilee Celebrations. The Symposium was co-sponsored by COSTED, the Madras Chapter of AFST (I) and DST and CSIR, New Delhi. The focal theme was: "The Emerging Role of Proteins in Developing Society".

A one-day Seminar on 'Export Potential of Food Industry' was organised by the Small Industries Service Institute, Madras and the Madras Chapter of AFST (I) on 26 September 1986 at Madras. More than 125 delegates from Tamil Nadu and adjoining States participated.

The SISI, Madras in collaboration with M/s. Thermodyne Company, Bangalore organised a Workshop-cum-demonstration on Cooking Gas Bakery ovens on 1st October 1986 at Madras. Dr. P. G. Adsule, Secretary of the Madras Chapter hignlighted the advantages of such ovens. More than 150 existing and prospective bakers from Andhra Pradesh, Kerala, Pondicherry and Tamil Nadu participated in the Workshop.

VII ICFOST Convention

This was held during February 27 to March 1 at CFTRI, Mysore. About 250 delegates attended the convention. The theme of the convention was "Present Status and Future Perspectives in the Technology of Foodgrains". Dr. B. L. Amla, Director, CFTRI welcomed the delegates and Dr. T. R. Sharma, the President of the Association made introductory remarks. Dr. K. R. Bhattacharya, presented the theme of the convention. Dr. A. P. Mitra, Director-General of CSIR and Secretary DSIR inaugurated the convention and released the souvenir brought out on the occasion. The keynote address was delivered by Dr. M. V. Rao, Special Secretary to Government of India and Director, Technology Mission on Oilseed Production DARE, New Delhi. The function ended with a vote of thanks by the Secretary, AFST (I).

Symposium sessions on the focal theme of foodgrains were held on all the three days. In all 27 papers were presented, which were discussed in five sessions under the heads: Primary processing of foodgrains; Utilization of grain by-products; Grain quality as affected by structure, Composition and processing; Grain-based industries; and Storage, marketing and distribution of foodgrains. A special lecture on 'Some perspectives in comparative cereal science and technology' was delivered by Dr. H. S. R. Desikachar on this occasion. Plenary session of the symposium was held on March 1 afternoon and recommendations were drafted.

Poster presentations on various topics in food science and technology were held simultaneously with the symoposium sessions on all the three days. The topics were: Basic; product development; Applied; quality assessment, assurance and control; Storage, handling and preservation and safety, adulteration and assay. About 160 posters were presented by scientists from all over the country and abroad.

IFCON 88
II INTERNATIONAL FOOD CONVENTION
MYSORE (INDIA)
18-23 FEBRUARY 1988

Organisers

The Association of Food Scientists and Technologists (India) and the Central Food Technological Research Institute have decided to organise the II International Food Convention during 18-23 February 1988.

A. Objectives

The major objective of the convention is to review the developments in food science and technology that have taken place in India as well as in other countries, since the last convention held at Bangalore in 1982. It will also highlight the importance of the interphase areas having a bearing on food science and technology. The convention will provide an opportunity for technology exposition and information transfer amongst participants.

B. Participation

The convention is open to R&D personnel, industry professionals, teaching faculties of universities, technocrats, quality assurance personnel from national and international institutions and organisations dealing with supply of food products. technology and machinery and those having an abiding interest in the production, handling, storage, processing and marketing of food. About 1500-2000 participants are expected to attend the convention.

C. Topics and Subjects

The convention will have general and parallel sessions on subjects of topical interest. General sessions will cover subjects on frontier technologies, food quality and safety, packaging, food machinery, food policy, human resource development, pollution, international trade, energy management and futurology. Parallel sessions will focus on foodgrains, animal products, edible oils, horticultural processing, dairy products, institutional foods, surplus food management, sociology and anthropology of foods, food distribution and marketing, traditional food technology, rural development and biotechnology. Eminent invitee speakers will spearhead discussions in these sessions.

Poster sessions will be arranged on specific dates to cover all contributed papers based on original work in all areas of food science and technology.

D. Special Presentations

A series of special lectures by eminent scientists and technologists are planned to highlight the State-of-the-art in a few frontier areas.

E. Food Exposition

Exhibition and display facilities for highlighting a spectrum of food products, services, packaging material, food adjuncts and protectants, and food machinery at the convention site are available for about 200 participants. The exhibition will promote active interaction between buyers and sellers and provide a source of information to participants and visitors.

Registration

The registration fee will be Rs. 350/- for an AFST(I) member, Rs. 600/- for a non-member in India, US$100 for international participants.

Secretariat

The Secretariat of the Convention is located at CFTRI, Mysore, India. All correspondence should be addressed to Dr. B. L. Amla, Chairman, National Steering Committee, IFCON-88, CFTRI, Mysore-570 013. India. Please supersubscribe IFCON-88 on top of the envelope for prompt delivery.

Telex: 0846-241. *Telephone:* 22660
Cable: FOODSEARCH Mysore.

UNIVERSITI SAINS MALAYSIA
SCHOOL OF INDUSTRIAL TECHNOLOGY

Applications are invited for the post of Professor in Food Technology.

Qualifications and Experience: Candidates should have a Masters or Ph.D. degree with severa years of teaching and/or research experience in any of the following areas:—

Food Processing and Manufacture;

Food Engineering;

Biotechnology as applied to food production and manufacture;

Treatment/Utilisation/Disposal of food processing wastes.

Salary and Allowances: Salary and entertainment allowances will range from M$51,720 to M$58,920 per annum. In addition, candidates will also be provided furnished accomodation up to the value of M$1,400 per month. Candidates will also be paid gratuity at 17½% of last drawn basic salary for each completed month of service at the end of the contract.

Other Benefits: Return passage for appointee and family (wife and 3 children); excess baggage assistance; 30 days paid annual leave, free medical benefits for staff and family.

Provisions for Research: Selected candidates will also be allocated an initial capital sum of M$250,000 for purchase of equipment for research and also a research budget of $20,000 to employ a research assistant and to purchase consumables for research work.

Tenure: Selected candidate will be appointed on contract for 3 years initially which is renewable.

Detailed Curriculum Vitae with complete list of research publications should be sent to Registrar, Personnel Section, Universiti Sains Malaysia, 11800 USM PENANG, Malaysia. Enclose personal biodata and references.

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, since only minor corrections are allowed at the proof stage. The submitted paper should not have been published or data communicated for publication anywhere else. Only invited review papers will be published.

2. Short communications in the nature of Research Notes should clearly indicate the scope of the investigation and the salient features of the results.

3. Names of chemical compounds and not their formulae should be used in the text. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Superscripts and subscripts should be legibly and carefully placed. Foot notes especially for text should be avoided as far as possible.

4. **Abstract:** The abstract should indicate the principal findings of the paper. It should be about 200 words and in such a form that abstracting periodicals can readily use it.

5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. They 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) $\times$16 cm (ox axis). The lettering should be such that they are legible after reduction to column width. Photographs must be on glossy paper and must have good contrast; *three copies* should be sent.

7. Abbreviations of the titles of all scientific periodicals should strictly conform to those cited in the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.

8. **References:** Names of all the authors along with title of the paper should be cited completely in each reference. Abbreviations such as *et al.*, *ibid*, *idem* should be avoided.

   The list of references should be included at the end of the article in serial order and the respective serial number should be indicated in the text as superscript.

   Citation of references in the list should be in the following manner:

   (*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.

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

REG. NO. 24918/64

JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Vol. 24. No. 4 **Contents of forthcoming issue** **July/August 1987**

Research Papers

Research Notes

Printed and Published by T. S. Satyanarayana Rao, Secretary, AFST(India), CFTRI, Mysore-570 013, at Sharada Press, Mangalore-575 001