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Utilization of Soymilk in Flavoured Milk Preparation

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Manuscript Received: 20 May 1974

The possibility of utilising soymilk as milk extender in the form of flavoured milk has been explored. Flavoured milk prepared from soymilk admixed with 30 per cent standardized buffalo or recombined milk was acceptable to the judging panel. Amongst the different synthetic flavours used in the milk, rose flavour was preferred to others. The keeping quality of such flavoured milk was on an average 7 days at 4°C, though slight sediment was observed after two days.

The daily per capita milk consumption in India is low, being 110 g as against the recommended level of 280 g. In order to augment the milk supply and make it available at reasonable prices, toned milk, recombined milk and standardised milk have appeared in the Indian markets. Owing to the restricted availability and high cost of animal milk proteins, efforts are being made to supplement the protein supply through the use of plant proteins. Miltone, a vegetable toned milk has been introduced by CFTRI, Mysore utilising groundnut protein¹. Attempts have also been made in using soymilk as milk extender at JNKV, Jabalpur and GBPAU, Pantnagar, since soybeans are rich in good quality proteins. The history of soymilk in China dates back to more than two millennia². Soymilk prepared by the traditional method has however an objectionable beany flavour.

Investigations reported in this paper relate to (i) acceptability of blends of standardised buffalo milk or recombined milk and soymilk, in the form of flavoured milk, (ii) preference of the type of synthetic flavour in such flavoured milk, and (iii) keeping quality of the product.

Materials and Methods

Raw materials: Soybeans were procured from GBPAU, Pantnagar. Standardised milk and fresh ghee were drawn from the Experimental Dairy of NDRI, Karnal.

Preparation of soymilk: 300 g of soybeans were soaked in 900 g of tap water and left overnight at room temperature. The soak water was drained and the beans washed with fresh water. The beans were then put in boiling water to which formaldehyde (0.5 per cent of boiling water) and sodium bicarbonate (0.1 per cent of boiling water) were added. Boiling was continued for 10 min and the beans were washed with hot water.

The hulls were then removed by rubbing. Approximately 100 g of dehulled beans were taken at a time in an electrically operated Waring blender and ground with 400 g of boiling water for 10 min. The dispersion was filtered through a muslin cloth to get soymilk.

Preparation of flavoured milk: 1.5 kg of soymilk were prepared by the method described above. Ghee was added to adjust the fat of soymilk to 3 per cent. Standardised buffalo milk (3 per cent fat, 8.5 per cent SNF) was admixed with soymilk at 10, 20, 30 and 40 per cent levels. Sugar was added at 8 per cent level to each of the admixed samples. The samples were heated to 63°C and then homogenised at pressures of 17.5 kg/cm² in the first stage and 35 kg/cm² in the second stage using a Rannie piston type homogeniser. The samples were then pasteurised at 63°C for 30 min under laboratory conditions and cooled to 4°C. Colour and flavour were added to these samples.

The procedure was same as above excepting that the admixing was done with recombined milk (3 per cent fat, 8.5 per cent SNF) prepared from low-heat spray dried skim milk powder and ghee.

Vanilla, lemon, rose, pineapple, mango, banana, chocolate and Kewra synthetic flavours were used in the preparation of flavoured milk from soymilk admixed with 30 per cent standardised buffalo milk.

Analysis: Total solids and fat in soymilk were determined by Mojonnier gravimetric method. Acidity was determined as per ISI⁴. Specific gravity was determined by using specific gravity bottle. Surface tension was determined using the method described⁵ and as adopted for detergents. Relative viscosity was determined as per the method described by Sommer⁶ using the following formula:

$$\frac{\text{Density of liquid}}{\text{Density of water}} \times \frac{\text{Flow time of liquid}}{\text{Flow time of water}}$$

TABLE 1. ACIDITY, SPECIFIC GRAVITY, SURFACE TENSION AND RELATIVE VISCOSITY OF SOYMILK AT 20°C

Total solids %	Fat %	Acidity*	Sp. gravity	Surface tension	Relative viscosity
8.2	2.05	0.08	1.010	50.14	1.28
8.6	2.04	0.09	1.012	49.20	1.34
8.5	2.04	0.08	1.011	49.29	1.31
8.2	2.05	0.08	1.011	50.09	1.26
8.3	2.05	0.08	1.010	50.01	1.28

*ml of O in NaOH used

The organoleptic evaluation of the flavoured milk samples was done by a panel of 8 members drawn from the staff of NDRI. Judging was restricted to flavour, body, sedimentation and keeping quality.

Results and Discussion

The total solids, fat, acidity (in terms of ml of 0.1 N NaOH used), specific gravity, surface tension and relative viscosity of soymilk ranged between 8.2-8.6, 2.04-2.05, 0.08-0.09, 1.010-1.012, 49.20-50.14 and 1.26-1.34 respectively as observed from Table 1. These values excepting for surface tension which lies in the normal range as that for cow milk are lower than those for cow milk. This can be attributed to lower efficiency of extraction of total solids from soybeans by the method adopted.

It is observed from Table 2 that the flavoured soymilk having admixture of 30 per cent and above of standardised milk is acceptable to majority of the judges. The same conclusion can be drawn for the flavoured milk based on a mixture of soymilk and recombined milk as seen in Table 3.

Cooked flavour from the recombined milk could not make any noticeable improvement in the flavour of such milk. In all the cases some slight sedimentation was

TABLE 2. ORGANOLEPTIC EVALUATION OF FLAVOURED SOYMILK PREPARED FROM SOYMILK AND BUFFALO MILK (6 TRIALS)

Buffalo milk admixed %	Flavour	Body & texture	Sedimentation	Acceptability**
0	+++	Less viscous*	Very slight	0/8
10	+++	"	"	2/8
20	++	Good	"	3/8
30	+	"	"	7/8
40	+	"	"	7/8

*Compared to natural milk;

**Number of judges who liked the product / no. of total judges

+ Acceptable; ++ Improved still unacceptable;

+++ unacceptable.

TABLE 3. ORGANOLEPTIC EVALUATION OF PASTEURISED FLAVOURED SOYMILK AND RECONSTITUTED MILK (6 TRIALS)

Reconstituted milk admixed %	Flavour	Body & texture	Sedimentation	Acceptability**
0	+++	Less viscous*	Very slight	0/8
10	+++	"	"	3/8
20	++	Good	"	4/8
30	+	"	"	7/8
40	+	"	"	7/8

**Number of judges who liked the product/number of total judges.

*Compared to natural milk.

+++ Unacceptable ++ Improved but still unacceptable
+ Acceptable

TABLE 4. ACCEPTABILITY OF FLAVOURED SOYMILK CONTAINING 30 PER CENT BUFFALO MILK

Synthetic flavour	Milk flavour	Body & texture	Sedimentation	Acceptability
Vanilla	Acceptable	Acceptable	Very slight	++
Lemon	"	"	"	++
Rose	"	"	"	+++ +
Pineapple	"	"	"	+++
Mango	"	"	"	++
Banana	"	"	"	+++
Chocolate	"	"	"	+
Kewra	"	"	"	+

+++ First preference; ++ Second preference;

+ Third preference; + Fourth preference.

TABLE 5. REPORT OF THE JUDGES ON ACCEPTABILITY OF PASTEURISED FLAVOURED SOYMILK HAVING 30% ADMIXTURE OF STANDARDISED BUFFALO MILK, AFTER DIFFERENT PERIODS OF STORAGE AT 4°C (4 TRIALS)

Storage period (days)	Flavour	Body & texture	Sedimentation	Acceptability
1	Acceptable	Good	Very slight	Acceptable
2	"	"	Slight	"
3	"	"	Present	"
4	"	"	"	"
5	"	"	"	"
6	"	"	"	"
7	"	"	"	"
8	Medicinal	"	"	Unacceptable
9	"	"	Curdled	"

observed which was not objectionable. The body and texture of milk was found to improve by admixing standardised/recombined milk at levels over 20 per cent.

Data on the preference of flavours by the judges for flavoured milk prepared from soymilk blended with 30 per cent standardised buffalo milk is presented in Table 4. It is observed that majority of the judges gave prime preference to the use of rose flavour which helped to mask the beany flavour of soymilk to a considerable extent.

Studies on the keeping quality of flavoured milk prepared from soymilk having 30 per cent admixture of standardised buffalo milk revealed that on an average the keeping quality was 7 days at 4°C. The sedimentation rate however increased after 2 days of storage as is observed in Table 5.

Acknowledgement

The help given by Shri M. R. Srinivasan, Dairy Technologist, NDRI, Karnal is acknowledged.

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Pathogenic *Staphylococci* Associated with Contamination of Market Eggs

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Manuscript received: 27 July 1974

Investigations carried out on the incidence of pathogenic *Staphylococci* from the surface of market shell eggs revealed that 30.9 per cent of the staphylococcal strains isolated from 1490 eggs were positive for the same. Out of this around one third of the strains was found to be of human origin. A good correlation was observed among the isolates with respect to their biochemical reactions like coagulase production, haemolysin activity, salt resistance, nitrate reduction and pathogenicity to mice. Most of the strains that showed fibrinolytic activity also reduced mannitol and caused opacity of egg yolk.

Association of staphylococcal organisms with food borne intoxications have been documented by Dack¹, Kraft², Longree³, Hobbs⁴, Pivnick *et al*⁵, and Dack⁶. Dack⁷ has listed 29 types of foods that have been implicated with staphylococcal food poisoning. Keeping this in view, the present investigation attempted to determine the incidence of pathogenic *Staphylococci* through contaminated shell eggs marketed in the country.

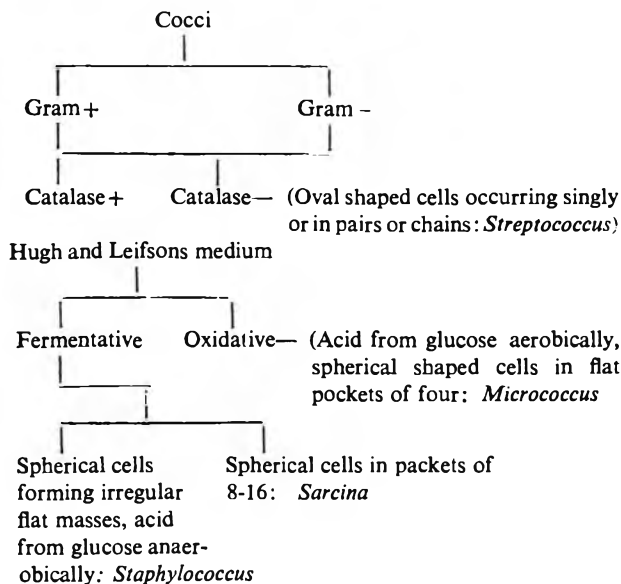
Materials and Methods

Shell eggs offered for sale in the retailing channel of the country located in the northern, southern, western

and central zones (based on their geographical situation) were collected in lots of 450 to 500 in three different seasons of the year (summer, rainy and winter) and transported to the laboratory in clean containers. Following their arrival at the laboratory, these were washed with light hand brushing, using sterile rubber gloves while allowing a steady stream of sterile saline solution to flow as per procedure described by Panda and Panda⁸. The said wash water acted as the inoculum for further culturing in tryptose agar and cocci isolated in the above medium were checked as per the scheme drawn below.

A portion of the doctoral thesis of the first author submitted to the University of Agra.

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By the above process, altogether 165 strains of *Staphylococci* were isolated from 1490 eggs. Out of these isolates 51 came from clean, 49 from slightly soiled and 65 from dirty shelled eggs. In addition, as pathogenicity of staphylococcal organisms have been correlated with metabolic activities like coagulase production, haemolysin production, chromogenesis, mannitol fermentation, gelatin liquefaction, fibrinolysin production, phosphatase production, nitrate reduction, egg yolk opacity test and salt resistance tests were carried out on all the strains isolated. Coagulase test, chromogenesis studies, fermentation of mannitol, liquefaction of gelatin and fibrinolysin tests were performed as per the procedure described by Malik *et al.*⁹, haemolysin test according to Baily and Scott¹⁰, nitrate reduction test according to Wilson and Miles¹¹ and egg yolk opacity test as outlined by Gillespie and Alder¹². Animal inoculation studies with mice were also carried out to confirm the pathogenicity of strains isolated. The criteria for pathogenicity in mice was detection of specific lesions or death within a period of 8 days on inoculating a 18 hr old broth culture and subsequent isolation of the organism from the heart blood that proved positive.

Results and Discussion

Out of the 165 strains of staphylococcal organisms isolated, 51 were coagulase positive and the rest negative. The distribution of these isolates with respect to summer, rainy and winter season were 18, 24 and 9 respectively. Further, maximum number of coagulase positive strains were obtained from southern and eastern regions of the country during summer and rainy seasons, perhaps due to the hot and humid climatic conditions of the regions in addition to the prevailing egg marketing practices.

The coagulase positive isolates were further charac-

TABLE 1. CORRELATION OF COAGULASE POSITIVE TEST WITH OTHER PATHOGENICITY STUDIES

No. of strains examined	Test	Number +ve	Percentage
51*	Animal inoculation	51	100
	Haemolysin test	48	94.04
	Chromogenesis	41	80.39
	Mannitol positive	33	64.70
	Gelatin liquefaction	20	39.20
	Fibrinolytic activity	25	49.02
	Salt resistance	47	92.95
	Egg yolk opacity	27	52.95
	Nitrate reduction	44	86.27

*All coagulase positive

terised by other pathogenicity tests. The results are shown in Table 1.

Above results show good correlation between animal inoculation studies and coagulase production. Coagulase positive strains were also pathogenic for mice as observed by Moss *et al.*¹³, Kourilsky and Mercier¹⁴ and Elek and Levy¹⁵. Around 94.01 per cent of the coagulase positive strains showed haemolysin activity as observed by Malik *et al.*⁹ in their studies on staphylococcal strains isolated from bovine udder where 80.39 per cent coagulase positive strains produced pigmentation. 64.7 per cent of isolates fermented mannitol consistent with the findings of Cowen¹⁶. Only 49.02 per cent of the coagulase positive strains exhibited fibrinolytic activity and 52.95 per cent egg yolk opacity. Most of the coagulase positive strains were salt resistant, but with respect to gelatin liquefaction, only a very few exhibited this activity. Hence, this criterion cannot be applied as a reliable test for pathogenic strains isolated from shell eggs. Malik *et al.*⁹ have also expressed the same view.

Cruickshank¹⁷ has indicated that *Staphylococci* showing fibrinolytic activity and mannitol positive character can be taken as of human origin. Hence, attempts made to distinguish isolates that would fall under this category, revealed that out of 25 isolates showing fibrinolytic activity and coagulase positive character, only 18 exhibited mannitol fermentation, and as such they may be considered to be of human origin. Perhaps these strains have gained entry to the surface of shell eggs while manual handling in the retailing channel. Rest of the isolates might have been of animal or poultry origin. Whatever may be the source, there are enough evidences in the literature to show that under favourable conditions, coagulase positive strains with the other characters detailed earlier can give rise to health hazards when allowed to contaminate with food items.

Results of the present study has shown that the pathogenic *Staphylococci* in shell eggs of market sample is as

high as 30.9 per cent based on total number of staphylococcal strains isolated. Out of this one third was of human origin. Perhaps this was due to their ubiquitous nature and common occurrence in dust, soil, cloth, floor, animal and human body and lack of hygienic handling of the product in the trade practice. Retailing of these products in proper egg cartons without exposing directly to the market atmosphere and adoption of strict hygienic handling during collection, grading,

packing and retailing might prove beneficial in reducing the incidence of spoilage.

Acknowledgement

Our sincere thanks are due to Dr H. A. B. Parpia, Director, CFTRI, Mysore and Dr C. M. Singh, Director, Indian Veterinary Research Institute, Izatnagar for providing necessary facilities to carry out the investigation at their respective laboratories.

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Evaluation of Mutton Quality Traits of Four Indian Carpet Type Sheep Breeds

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Manuscript Received: 4 March 1975

Qualitative evaluation of mutton of Nali, Lohi, Magra and Hissardale × Magra sheep was undertaken to compare their fleshing index, fat status, and meat-bone ratio, using least square procedure. Effects of age, body weight, height, length, heart girth and paunch girth were also studied on these traits. Differences observed were not significant for the effects studied on fleshing index and fat status except for body height on the former trait. Significant differences due to breed and age of animal were observed for meat-bone ratio.

Improvement of traits determining the quality of mutton has to be taken up along with improvement of sheep for dressed carcass weight. No published information on the fleshing index, fat status, and meat bone ratio is available on Indian breeds of sheep. Therefore, the present study was carried out to evaluate the standards for these traits and also to study on these, the effect of certain identifiable factors i.e. breed, age, body

weight, length, height, heart and paunch girth of the animal at the time of slaughter.

Materials and Methods

This experiment was conducted on four breeds of sheep namely 'Nali' (32), 'Lohi' (16), 'Magra' (15) and 'Hissardale' × 'Magra' (7), maintained under semi-stall fed conditions. These animals were allowed 6-8 hr

TABLE 1. LEAST SQUARE CONSTANTS AND MEANS FOR THE DIFFERENT GENETIC AND NON-GENETIC FACTORS AFFECTING FLESHING INDEX AND FAT STATUS

	No. of observa- tion	Fleshing index		Fat status	
		L.S.C.	L.S.M.	L.S.C.	L.S.M.
Mean	70	24.23	5.47	0.91	22.07
Nali	32	-0.09	5.38	1.40	23.46
Lohi	16	0.74	6.25	2.78	19.29
Magra	15	-0.43	5.06	2.91	24.98
Hissardale × Magra	7	-0.22	5.25	-1.53	20.54

Partial regression of trait on

Age (days)	0.000	-0.011
Body wt (kg)	0.000	-0.281
Body length (cm)	0.021	0.180
Body ht (cm)	-0.371	-0.033
Heart girth (cm)	0.057	0.356
Paunch girth (cm)	-0.036	-0.021

Represents overall population value for least squares constant.
L.S.C. = Least square constants; L.S.M. = Least square means.

of grazing (including stubble grazing). After slaughter, data on the quality traits were collected in the following manner:

- (i) *Fleshing index*: The gross fleshing index of the carcass was obtained according to the method suggested by Yeates¹.
- (ii) *Fat status*: This is the ratio of lean meat to depth of sub-cutaneous fat measured over the transverse process of first lumbar vertebra. The thickness of loin eye muscle and sub-cutaneous fat over it was measured with the help of a calliper. The fat status was calculated as

$$\frac{(B+C) - C}{C}$$

Where, B = the depth of loin eye muscle; C = the depth of subcutaneous fat over loin eye muscle.

- (iii) *Meat-bone ratio*: The carcass was cut medially and the left side was used for studying the meat-bone ratio. The muscles were separated from the bones manually for fore and hind saddles separately. Muscles and bones, then were weighed to calculate the ratio.

Since the number of observations in the subclasses were unequal and disproportionate, influence of the various factors on the mutton quality traits using the least square procedure was evaluated. The following statistical model was chosen:

$$Y_{ij} = \alpha + B_i + b_1A_{ij} + b_2W_{ij} + b_3L_{ij} + b_4H_{ij} + b_5G_{ij} + b_6P_{ij} + e_{ij}$$

TABLE 2. ANALYSIS OF VARIANCE FOR FACTORS AFFECTING FLESHING INDEX AND FAT STATUS

Source	d.f.	Fleshing index		Fat status	
		M.S.	R ²	M.S.	R ²
Between breeds	3	2.87	1.56	63.24	5.07
Partial regression of trait on					
Age (days)	1	11.26	2.05	86.07	2.30
Body wt. (kg)	1	0.00	0.00	26.20	0.70
Body length (cm)	1	0.33	0.06	23.68	0.63
Body ht. (cm)	1	81.75**	14.87	0.65	0.02
Heart girth (cm)	1	1.44	0.26	55.49	1.48
Paunch girth (cm)	1	1.15	0.21	0.38	0.01
Error	60	7.42	30.99	46.00	89.79

**P < 0.01

Where,

Y_{ij} = j th observation in the i th B class

α = theoretical population mean with equal subclass frequencies when deviations of the covariate from their mean values is zero, and

$$\mu = \alpha + b_1A_{ij} + b_2W_{ij} + b_3L_{ij} + b_4H_{ij} + b_5G_{ij} + b_6P_{ij}$$

B_i = effect of the i th genetic group.

$b_1, b_2, b_3, b_4, b_5,$ and b_6 are partial regression coefficients of the trait on six covariates i.e. age (A_{ij}), body weight (W_{ij}), body length (L_{ij}), body height (H_{ij}), heart girth (G_{ij}), and paunch girth (P_{ij}).

Results and Discussion

Fleshing index and fat status: The least squares constants and means for fleshing index and fat status for the various breeds and nongenetic factors fitted in the model given above are presented in Table 1. Analysis of variance of these traits for the various factors are presented in Table 2.

Differences due to breeds, age, body weight and various physical measurements were statistically non significant for fleshing index and fat status except the body height which influenced the fleshing index significantly ($P < 0.01$). The negative value of the partial regression indicated that leggy animals had lower fleshing index and the low set sheep put on more flesh. Although the breed effect on fat status was not significant yet the R^2 value of more than 5.0 per cent was indicative of the trend that 'Magra' had the highest fat status while 'Lohi' were the poorest in this regard.

Meat-bone ratio: The least square constants and means of meat-bone ratio taken separately for fore saddle (portion of carcass to the fore of thoracolumbar joint), hind saddle (portion of carcass to the rear of thoracolumbar joint) and pooled are presented in Table 3. Analysis of variance for this trait is presented in Table 4. The results revealed that the breed and age effect were significant ($P < 0.01$) for fore-saddle, hind-

TABLE 3. ESTIMATES OF LEAST SQUARE CONSTANTS AND MEANS OF MEAT BONE RATIO

	No. of observations	Meat bone ratio mean		Meat bone ratio fore saddle		Meat bone ratio hind saddle	
		L.S.C.	L.S.M.	L.S.C.	L.S.M.	L.S.C.	L.S.M.
Mean*	70	1.17	2.45	0.22	2.33	2.72	3.14
Nali	32	0.46	2.91	0.26	2.60	0.55	3.69
Lohi	16	0.51	2.96	0.49	2.82	0.59	3.73
Magra	15	-1.04	1.41	1.12	1.22	-0.85	2.28
Hissardale × Magra	7	0.07	2.53	0.37	2.67	-0.28	2.86

Partial regression of trait on

Age (days)	0.001	0.002	0.001
Body wt (kg)	0.043	0.040	0.058
Body length (cm)	-0.002	0.016	-0.015
Body ht (cm)	-0.015	0.021	-0.015
Heart girth (cm)	-0.011	0.004	-0.038
Paunch girth (cm)	0.019	0.000	0.040

*Represents overall population value for least square; constant.

L. S. C. = Least square constant L. S. M. = Least square means.

TABLE 4. ANALYSIS FOR FACTORS AFFECTING MEAT-BONE RATIO

Source	d.f.	Meat bone ratio mean		Meat bone ratio fore-saddle		Meat bone ratio hind-saddle	
		M.S.	R ²	M.S.	R ²	M.S.	R ²
Between breeds	3	2.14**	29.45	1.93**	27.86	2.50**	15.52

Partial regression of trait on

Age (days)	1	1.52**	7.01	2.37**	11.39	0.53	1.10
Body wt (kg)	1	0.61	2.81	0.54	2.57	1.12	2.31
Body length (cm)	1	0.00	0.00	0.18	0.87	0.16	0.33
Body ht (cm)	1	0.13	0.61	0.27	1.28	0.13	0.26
Heart girth (cm)	1	0.05	0.23	0.01	0.03	0.63	1.30
Paunch girth (cm)	1	0.31	1.42	0.00	0.00	1.37	2.83
Error	60	0.21	58.45	0.19	56.00	0.62	76.35

**P < 0.01; *P < 0.05

saddle and the pooled data with the exception of age for hind-saddle. Breeds contributed 29.5 per cent to the total sum of squares for the pooled meat-bone ratio.

TABLE 5. AGE AFFECTING THE MEAT BONE RATIO

Age group	No. of animals	Muscle bone ratio
166-278 days	10	2.1
279-410 "	18	2.9
411-542 "	21	3.1
543-674 "	9	2.5
675-806 "	9	2.9
807-938 or above	3	2.8

Highest meat-bone ratio

Apparently 'Lohi' has a definite edge over the other breeds for the average meat-bone ratio. 'Nali' and 'Lohi' had higher meat-bone ratio both for fore-saddle and hind-saddle. It may be further concluded that the proportion of bones to that of meat are higher in the fore-saddle than that of hind-saddle in all the breeds of sheep.

As the age affected the meat-bone ratio significantly, the animals were grouped into six age groups starting

from 146 days to 930 days or more, with each class of 132 days. The meat-bone ratio was observed to be low in case of very young animals as well as in the old ones (Table 5). It was highest in animals ranging from 411 to 542 days of age. Significant breed differences in the present investigation are in conformity to the observations made on some exotic breeds by Hacatran² and Brannang and Nilsson³. 'Lohi' and 'Nali' had the maximum meat-bone ratio (2.96 and 2.91) followed by 'Hissardale' × 'Magra' crossbreds (2.53) and 'Magra' (1.41) in the descending order. Since 'Nali' is a predominant breed of North-Western plain of India, it can be most efficaciously exploited for lean meat production.

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Microbiological Evaluation of Raw, Pasteurised and Flavoured Milk

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Unlike pasteurised milk, the pH of raw milk was abnormal. The results of the alcohol test showed that the raw milk lacks stability and mineral balance. The raw milk had higher bacterial counts than the pasteurised milk whereas flavoured milk had negligible microbial load. Preponderance (83%) of *E. coli* in raw milk indicated that it is unsatisfactory for human consumption. The flavoured milk was free from microbial contamination.

There is the risk of milk being contaminated with microorganisms because of insanitary conditions of collection and storage. Much of the milk sold in our country, is unpasteurised. The purpose of this study has been to determine the bacterial load of raw or unpasteurised milk samples collected from separate sources in the city of Bombay and to compare the results with those of pasteurised milk. In addition, microbiological analyses of certain flavoured milk beverages have also been carried out. Some data are available on the keeping quality of raw milk¹ and infant milk foods². However, very little is known about the microbiological status of flavoured milk.

Materials and Methods

Since the bulk of the distribution took place early in the morning, milk samples for the study were collected in the morning. Pasteurised milk was obtained from the daily stock received in the Institute's store. Samples of flavoured milk (bottles) were obtained from local vendors. The number of samples of pasteurised, raw and flavoured milk analysed was 9, 12 and 50 respectively.

The samples of milk were collected and stored in the refrigerator at 4°C. The tests were performed within 4 hr of collection. The pH of the samples was estimated using a pH meter. The effect of processing on the stability and mineral balance of milk was assessed by the alcohol test³. The methylene blue reduction test was performed according to the procedure of the American Public Health Association⁴. The total bacterial load was estimated by the agar plate method using nutrient agar as the medium⁴.

Indicator organisms: The presumptive test was done employing lactose broth⁴ to detect the presence of coliforms. Loopfulls from tubes showing positive to gas and acid formation were streaked on eosin-methylene

blue-agar and endo agar. Typical colonies were picked out for confirmation by the IMViC tests⁵. Samples of flavoured and pasteurised milk were subjected to the methylene blue reduction test. The total bacterial load was estimated by the agar plate method using nutrient agar as the medium. The coliform group of bacteria was enumerated using violet red-bile-agar and MacConkey's agar⁴.

Results and Discussion

The normal pH of cows' and buffaloes' milk ranged from 6.6 to 6.8⁵. Two thirds of the raw milk samples examined showed pH lower than normal (pH 6.1 to 6.5). It has been reported that the pH of milk from animals suffering from *mastitis* show an alkaline pH⁵. One third of the raw milk samples showed higher than normal pH (pH 6.9 to 7.0). However, all the pasteurised milk samples showed a pH range of 6.6 to 6.8.

Results of the alcohol test indicate that 50 per cent of pasteurised milk samples show tiny flake formation

TABLE 1. METHYLENE BLUE REDUCTION TEST ON PASTEURISED AND RAW MILK

No of samples	Decolourisation time (hr)	Class*
Pasteurised milk		
1	7.5	II
1	8	II
1	10	I
1	9	I
Raw milk		
5	1 to 1.5	IV
7	3.5	III

*Salle 1961

TABLE 2. STANDARD PLATE COUNT OF PASTEURISED AND RAW MILK

Sample no.	Colonies/dilution		Count ratio*	Standard plate count/ml at 37°C
	1:1000	1:10000		
Pasteurised milk				
1	156	78	5.0	1.56×10^5
2	237	128	5.4	2.37×10^5
3	143	64	4.4	1.43×10^5
4	169	98	5.2	1.69×10^5
Raw milk				
1	148	25	—	1.48×10^6
2	190	96	5.1	1.90×10^6
3	194	140	7.6	1.94×10^6
4	139	94	6.8	1.39×10^6
5	102	64	6.2	1.02×10^6
6	146	65	4.5	1.46×10^6
7	134	108	8.1	1.34×10^6
8	140	18	—	1.40×10^6
9	141	93	6.6	1.41×10^6
10	138	102	7.3	1.38×10^6
11	129	67	5.2	1.29×10^6
12	156	75	4.8	1.56×10^6

*Count ratio is the ratio of the greater to the lesser plate count, as applied to plates from consecutive dilutions having between 30 and 300 colonies.

TABLE 3. THE IMVIC TEST ON PASTEURISED AND RAW MILK

No. of samples	Indole	Methyl red	Voges Proskaur	Citrate
Pasteurised milk				
2	—	—	+	+
2	—	—	—	—
Raw milk				
10	+	+	—	—
2	—	—	+	+

+ = positive - = negative

TABLE 4. THE METHYLENE BLUE REDUCTION TEST ON FLAVOURED AND PASTEURISED MILK

No of samples	Decolourisation time (hr.)
Flavoured milk	
8	10 to 20
19	21 to 30
15	31 to 40
8	> 40
Pasteurised milk	
2	7 to 8
3	9 to 10

TABLE 5. TOTAL PLATE COUNT OF FLAVOURED AND PASTEURISED MILK

No of samples	No of organisms/ml
Flavoured milk	
24	0
17	1 to 10
7	11 to 20
2	> 20
Pasteurised milk	
5	$1.0-2.0 \times 10^5$

TABLE 6. THE COLIFORM COUNT OF FLAVOURED AND PASTEURISED MILK

No of samples	No of organisms/ml
Flavoured milk	
48	0
2	1-3
Pasteurised milk	
5	35-40

while all the raw milk samples showed large flake formation. This indicates the lack of stability and mineral balance of the latter.

From the methylene blue reduction test it is evident that nearly 40 per cent of the raw milk samples reduced the dye in 1.5 hr where as the remaining samples took 3.5 hr (Table 1). The methylene blue reduction time of pasteurised milk samples ranged from 7.5 to 10 hr (Table 1). It is evident from the results that the raw milk decolourised methylene blue within shorter periods of time. The time taken by milk to decolourise methylene blue is accepted as a fairly good measure of its bacterial content and therefore its sanitary and keeping qualities⁶. Based on a qualitative classification of milk, the raw milk samples fell into Class III and IV and pasteurised milk into Class I and II⁵. The results indicate the poor quality of raw milk.

There was a wide variation in the plate count (Table 2). In the case of the raw milk samples, this may be attributed to the fact that the samples had been collected from different sources. Nearly all of the raw milk samples and half of the pasteurised samples were presumptive coliform positive. The results (Table 3) indicate that 83 per cent of raw milk had *E. coli*. *Aerobacter aerogenes* was present in 17 per cent of the raw milk and 50 per cent of pasteurised milk samples. Because coliforms usually do not survive pasteurisation, a positive coliform test, suggests recontamination⁴. These results indicate a high degree of contamination of raw milk with microorganisms of faecal origin.

Results in Tables 4, 5 and 6 show that there was little or no bacterial load in the samples of flavoured milk. The low bacterial count cannot be attributed solely to the effect of pasteurisation since the results on flavoured and pasteurised milk were widely different. Besides, pasteurised milk has a limited shelf-life at ambient temperature unlike flavoured milk which was observed to keep for many days. It is likely that the flavoured milk is made from milk powder or groundnut milk, which in combination with heat sterilisation may account for the low bacterial count. The addition of chemical preservatives may be another factor. The coliform contamination has been observed in only 4 per cent of the samples. Since coliforms reflect the general bacterial contamination, it is apparent that the flavoured milks were in good sanitary condition.

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Effect of Modern Packaging Materials on the Keeping Quality of Khoa

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A study was conducted to investigate the effect of less costly and easily available laminated packages, polyethylene and parchment paper on the keeping quality of khoa. The preirradiated laminates consisted of (i) cellophane/low density polyethylene; (ii) poster paper/low density polyethylene/0.015 mm aluminium foil/low density polyethylene; and (iii) poster paper/low density polyethylene/0.02 mm aluminium foil/low density polyethylene. The changes occurring in khoa packs during storage at 37°C, 8±1°C and -20°C were studied in respect of organoleptic properties, moisture, lactic acidity, peroxide value, free fat acidity and tyrosine content; microbiological changes in terms of differential count, spores, yeast and moulds and lipolytic counts at 37°C and 8±1°C were also recorded. Khoa samples packed in parchment paper and polyethylene remained acceptable upto five days at 37°C and upto 14 days at 8±1°C. Laminate packed samples were acceptable for more than 5 days at 37°C, 30 days at 8±1°C and more than 75 days at -20°C. Four ply aluminium coated laminates proved to be the best for packaging of khoa followed by two ply packs, high density polyethylene and parchment paper.

Khoa occupies a prominent place in the diet of Indians, as it provides a base for the preparation of variety of sweets. Of the 25 million tons of milk produced in the country nearly 7 per cent is converted into khoa¹. The keeping quality of khoa is affected by the extent of contamination after manufacture and subsequent handling. Various types of microorganisms growing in khoa produce off flavours and changes in physico-chemical attributes^{2,3} which lower the shelf life to a few days. Packaging of khoa in deterged butter paper was suggested as a possible method for improving the keeping quality⁴⁻⁷. The feasibility of using tin containers as suggested by Rudreshappa and De⁸,

appears to be impracticable because of their very high cost. In view of this, less costly materials such as flexible films, laminates, aluminium foil and plastic pouches have successfully been tried in Western dairy products. Cooke⁹ observed that foils of 0.0015 in. thickness or heavier which are devoid of any pinholes give zero water vapour permeability. The properties of thinner foils are improved by combination with one or more plastics in the form of coatings or laminates¹⁰⁻¹¹. Although many of the film sachets utilize a single ply material with heat-seal characteristics for most types of food products, the laminates offer all the barrier and functional values and also meet other physical require-

ments including climatic resistance, weldability and machineability¹². In view of the above considerations the present studies were planned to investigate the effect of (a) laminated packs; (b) high density polyethylene; and (c) parchment paper on the keeping quality of khoa at different temperatures of storage.

Materials and Methods

a) *Preparation of khoa*: Fresh cow and buffalo milks were mixed in equal proportions and standardised to 5 per cent fat. Khoa was made from 5-lit lots of milk in a stainless steel kettle. The different lots were mixed thoroughly while still hot (in the same kettle) before being distributed into the various packs.

b) *Packaging of khoa*: 500-g lots of khoa were packaged while hot in the following pouches: (i) parchment paper—(1 P); (ii) 300 MSDAT cellophane/50 g low density polyethylene—(2 MSDAT); (iii) high density food grade polyethylene—(3 HDP); (iv) 40 GSM poster paper/100 g low density polyethylene/0.015 mm aluminium foil/150 g low density polyethylene—(4 P); (v) 50 GSM poster paper/100 g low density polyethylene/0.02 mm aluminium foil/150 g low density polyethylene (5 P).

The presterilized 2 MSDAT, 4P and 5P laminates were procured from Bhabha Atomic Research Centre, Bombay. 1 P and 3 HDP procured locally were sterilized by conventional method. The packages containing khoa were sealed, labelled and stored at 37°C, 8±1°C and -20°C.

Sensory evaluation: This was carried out to assess the flavour, colour, body and texture of khoa. *Gulab-jamun* (a type of sweetmeat prepared from khoa) was prepared from khoa stored at three different temperatures for different durations. *Gulabjamuns* were evaluated by a panel of judges for ascertaining the suitability of fresh and stored khoa for sweet meat making on hedonic scale¹³.

Chemical methods: The moisture and fat per cent in khoa were determined by ISI methods¹⁴. Per cent lactic acidity was determined by titrating 10 g of reconstituted khoa with 0.1 NaOH. Peroxide value expressed as ml of 0.002 N sodium thio-sulphate per gram of khoa was determined on fat extracted from 10 g khoa soaked in 30 ml chloroform for 12 hr in an airtight flask. The chloroform extract was used for estimation of peroxide value by AOAC¹⁵ method. Free fatty acid (as per cent oleic acid) was estimated by the method of Thomas *et al*¹⁶. Tyrosine (mg/100 g khoa) was measured by the method of Hull¹⁷ after reconstituting the product in warm distilled water.

c) *Microbiological analysis*: The stored samples of khoa were opened by schematic random process under aseptic conditions in an inoculation chamber sterilized

TABLE 1. CHEMICAL QUALITY OF KHOA AT THE TIME OF PACKAGING

Constituents	Range %	Average %
Moisture	31.04 - 37.44	33.84
Total solids	62.55 - 69.00	66.13
Fat	21.60 - 23.50	22.40
Protein	18.58 - 21.10	19.54
Lactose	19.82 - 21.03	20.29
Ash	2.55 - 3.42	3.00
Acidity (% lactic)	0.423 - 0.450	0.432
Tyrosine (mg/100 g khoa)	15.8 - 16.5	16.13
Peroxide value (ml of 0.002N Na ₂ S ₂ O ₃ /g khoa)	0.05 - 0.25	0.15
Free fatty acids (% oleic)	0.0141 - 0.0578	0.03251

by ultraviolet radiation. Khoa samples stored at deep freeze temperature (-20°C) were not used for microbiological studies since microbes are not expected to show appreciable growth at this temperature.

Khoa suspension for analysis was prepared according to the method of Foster *et al*¹⁸ for cheese. Appropriate dilutions were made from homogeneous suspension of khoa using 2 per cent sodium citrate.

Total plate count: Appropriate dilutions of khoa suspension were plated in duplicate using China blue agar⁶ and colonies counted after 48 hr of incubation at 37±1°C.

Differential counts: The plates used for total plate count were also used for counting acid producing, proteolytic and chromogenic colonies. Acid producing organisms were either surface or sub-surface colonies which had a light to deep blue colour. The proteolytic organisms had a clear zone around the colonies. The chromogenic group of organisms showed a variety of colours which ranged from golden yellow, orange to pink. The chromogenic count was taken after 5 days at 30°C.

Spore count: 1:10 dilution of khoa suspension was heated at 80°C for 10 min (Foster *et al*)¹⁸. Appropriate dilutions were then plated using Tryptone Dextrose agar. Plates were incubated for 48 hr at 37±1°C.

TABLE 2. BACTERIOLOGICAL QUALITY OF KHOA AT THE TIME OF PACKAGING

Microorganisms	Count/g	Average count/g
Total bacterial counts	8,000 - 21,000	12,000
Acid producers	3,000 - 11,000	7,500
Proteolytic types	3,000 - 5,000	3,000
Chromogenic types	2,000 - 3,000	2,000
Lipolytic counts	900 - 1,000	600
Spore count	50 - 300	200
Yeasts & moulds	10 - 30	20

TABLE 3. EFFECT OF MODERN PACKAGING MATERIALS ON CHEMICAL QUALITY OF KHOA

Type of package	Storage period (days)	Storage temp. (°C)	Moisture %	Acidity (% lactic)	Tyrosine (mg/100 g khoa)	Peroxide value	FFA (%oleic)
1 P	5-10	37	25.9 - 20.9	0.58 - 0.82	17.6 - 32.0	0.40 - 0.95	0.345 - 0.451
	14-30	8±1	32.5 - 23.9	0.54 - 0.62	20.0 - 25.2	0.03 - 0.77	0.113 - 0.155
	30-75	-20	32.6 - 27.0	0.51 - 0.67	—	0.20 - 0.75	0.113 - 0.212
2 MSDAT	5-10	37	32.0 - 28.5	0.56 - 0.74	17.7 - 27.4	0.32 - 0.78	0.254 - 0.367
	14-30	8±1	33.5 - 31.2	0.56 - 0.61	17.8 - 24.3	0.32 - 0.65	0.092 - 0.141
	30-75	-20	33.8 - 32.1	0.51 - 0.64	—	0.10 - 0.40	0.085 - 0.115
3 HDP	5-10	37	30.2 - 26.3	0.59 - 0.79	18.1 - 29.7	0.37 - 0.90	0.338 - 0.430
	14-30	8±1	33.1 - 30.2	0.56 - 0.62	19.2 - 24.0	0.34 - 0.70	0.075 - 0.169
	30-75	-20	35.1 - 31.0	0.51 - 0.67	—	0.10 - 0.55	0.113 - 0.197
4 P	5-10	37	31.6 - 30.1	0.57 - 0.73	17.3 - 25.8	0.34 - 0.70	0.212 - 0.303
	14-30	8±1	33.6 - 31.8	0.54 - 0.59	18.6 - 24.0	0.33 - 0.60	0.113 - 0.155
	30-75	-20	36.0 - 33.6	0.49 - 0.59	—	0.14 - 0.65	0.099 - 0.169
5 P	5-10	37	32.9 - 30.5	0.54 - 0.69	17.3 - 25.8	0.29 - 0.73	0.212 - 0.289
	14-30	8±1	33.7 - 31.7	0.54 - 0.58	18.3 - 23.8	0.28 - 0.60	0.092 - 0.141
	30-75	-20	35.7 - 32.8	0.49 - 0.58	—	0.14 - 0.55	0.085 - 0.141

Yeast and mould count: These were determined by plating suitable dilutions of khoa on potato dextrose agar¹⁹. The plates were incubated for 3-5 days at 22±1°C and counts made as per APHA²⁰.

Lipolytic counts: Suitable dilutions of khoa samples were plated on tributyrin agar medium²¹. The plates were incubated at 30±1°C for 3-5 days.

Results and Discussion

The chemical and microbiological quality of khoa before packaging have been presented in Tables 1 and 2. The moisture per cent in khoa samples varied from 31.0 to 37.0 per cent and fat from 21.6 to 23.5 per cent with averages of 33.8 and 22.4 per cent respectively. The average values for total acidity, tyrosine, peroxide value and free fatty acids were 0.432 per cent, 16.13 mg/100g of khoa, 0.15/g khoa and 0.032 per cent respectively for fresh samples. The chemical and microbiological changes of differently packed and stored khoa corresponding to various storage temperatures are given in Table 3 and 4 respectively.

Organoleptic quality: The suitability of khoa samples for the preparation of *Gulabjamun* before and after storage was studied. *Gulabjamun* were prepared by standard procedure. Local ghee was used for frying; it was fried till a deep brown colour was obtained. *Gulabjamun* immersed in sugar syrup were served to a panel of judges for sensory evaluation. The acceptability scores were classified on a nine point hedonic scale¹³

(Table 5). Khoa samples packed in parchment paper and polyethylene packs were rated as "satisfactory" upto five days at 37°C and good upto 14 days at 8±1°C. Good quality *Gulabjamun* were obtained from khoa samples packed in 5P upto 30 days at 8±1°C. At -20°C storage all the samples packed in parchment paper, polyethylene and laminates produced good quality *Gulabjamun* upto 75 days of storage. Storage studies beyond 75 days were not conducted.

Moisture: The loss of moisture during storage varied with the type of package used and the temperature of storage. Khoa samples packed in parchment paper and stored at all temperature showed the maximum loss of moisture, while 5P packed khoa registered a minimum loss at all temperatures of storage. Although the loss of moisture at 8±1°C and -20°C was considerably lower than those of the samples stored at 37°C, the trend of loss of moisture in all the packages was more or less the same. It is evident that the aluminium coated laminates provide better protection against loss of moisture due to their superior moisture barrier properties.

Acidity: The average total lactic acidity of khoa before storage was 0.432 per cent. The acidity of khoa samples stored at 37°C in three laminates did not show a significant increase upto 5 days of storage except 1P and 3HDP where it rose to 0.58 and 0.59 per cent respectively. The values for lactic acidity on 7th and 10th day of storage at 37°C showed a steep rise in all the

TABLE 4. EFFECT OF MODERN PACKAGING MATERIALS ON MICROBIOLOGICAL QUALITY OF KHOA

Type of package	Storage period (days)	Storage temp. (°C)	Total plate count	Acid producers/g	Proteolytic count/g	Chromogenic count/g	Lipolytic count/g	Y & M count/g	Spore count/g
1 P	5-10	37	$6.7 \times 10^5 - 143.5 \times 10^5$	$1.2 \times 10^5 - 22 \times 10^5$	$0.88 \times 10^5 - 18 \times 10^5$	$19 \times 10^3 - 400 \times 10^3$	$44 \times 10^3 - 90 \times 10^3$	$0.52 \times 10^2 - 18 \times 10^2$	$8 \times 10^2 - 18 \times 10^2$
	14-30	8 ± 1	$91 \times 10^3 - 320 \times 10^3$	$31 \times 10^3 - 80 \times 10^3$	$21 \times 10^3 - 70 \times 10^3$	$17 \times 10^3 - 34 \times 10^3$	$18 \times 10^3 - 30 \times 10^3$	$1.9 \times 10^2 - 30 \times 10^2$	$4 \times 10^2 - 8 \times 10^2$
2 MSDAT	5-10	37	$5.6 \times 10^5 - 115.5 \times 10^5$	$1.0 \times 10^5 - 13 \times 10^5$	$0.81 \times 10^5 - 11 \times 10^5$	$17 \times 10^3 - 300 \times 10^3$	$36 \times 10^3 - 80 \times 10^3$	$0.75 \times 10^2 - 13.2 \times 10^2$	$6 \times 10^2 - 17 \times 10^2$
	14-30	8 ± 1	$86 \times 10^3 - 230 \times 10^3$	$24 \times 10^3 - 63 \times 10^3$	$20 \times 10^3 - 58 \times 10^3$	$9 \times 10^3 - 26 \times 10^3$	$12 \times 10^3 - 23 \times 10^3$	$0.95 \times 10^2 - 19 \times 10^2$	$3 \times 10^2 - 7 \times 10^2$
3 HDP	5-10	37	$8.95 \times 10^5 - 148.5 \times 10^5$	$1.5 \times 10^5 - 21 \times 10^5$	$1.10 \times 10^5 - 15 \times 10^5$	$18 \times 10^3 - 400 \times 10^3$	$38 \times 10^3 - 120 \times 10^3$	$0.6 \times 10^2 - 26 \times 10^2$	$9 \times 10^2 - 19 \times 10^2$
	14-30	8 ± 1	$91.5 \times 10^3 - 320 \times 10^3$	$29 \times 10^3 - 67 \times 10^3$	$18 \times 10^3 - 61 \times 10^3$	$11 \times 10^3 - 35 \times 10^3$	$13 \times 10^3 - 28 \times 10^3$	$2.70 \times 10^2 - 39 \times 10^2$	$3 \times 10^2 - 6 \times 10^2$
4 P	5-10	37	$4.95 \times 10^5 - 99.5 \times 10^5$	$0.94 \times 10^5 - 16 \times 10^5$	$0.76 \times 10^5 - 12 \times 10^5$	$19 \times 10^3 - 200 \times 10^3$	$30 \times 10^3 - 80 \times 10^3$	$0.5 \times 10^2 - 21.7 \times 10^2$	$7 \times 10^2 - 13 \times 10^2$
	14-30	8 ± 1	$77 \times 10^3 - 240 \times 10^3$	$25 \times 10^3 - 58 \times 10^3$	$19 \times 10^3 - 51 \times 10^3$	$12 \times 10^3 - 24 \times 10^3$	$14 \times 10^3 - 20 \times 10^3$	$0.80 \times 10^2 - 18.5 \times 10^2$	$4 \times 10^2 - 5 \times 10^2$
5 P	5-10	37	$3.85 \times 10^5 - 101 \times 10^5$	$0.98 \times 10^5 - 17 \times 10^5$	$0.78 \times 10^5 - 13 \times 10^5$	$17 \times 10^3 - 300 \times 10^3$	$36 \times 10^3 - 80 \times 10^3$	$0.35 \times 10^2 - 14.8 \times 10^2$	$6 \times 10^2 - 14 \times 10^2$
	14-30	8 ± 1	$73.5 \times 10^3 - 215 \times 10^3$	$21 \times 10^3 - 51 \times 10^3$	$12 \times 10^3 - 42 \times 10^3$	$11 \times 10^3 - 23 \times 10^3$	$13 \times 10^3 - 21 \times 10^3$	$0.75 \times 10^2 - 19 \times 10^2$	$3 \times 10^2 - 5 \times 10^2$

TABLE 5. RATING OF GULABJAMUNS PREPARED FROM STORED KHOA SAMPLES (AVERAGE OF FIVE JUDGES)

Storage temp °C	Storage period (days)	Type of packaging material				
		1P	2MSDAT	3HDP	4P	5P
37±1	5	7	8	7	8	9
	7	4	4	4	5	6
	10	3	4	3	4	4
8±1	14	9	9	9	9	9
	24	6	7	6	8	8
	20	3	4	3	5	7
-20	30	9	9	9	9	9
	45	9	9	9	9	9
	60	9	9	9	9	9
	75	8	9	7	9	9

Scoring

Like extremely-9; Like very much-8; Like moderately-7; Like slightly-6; Neither liked nor disliked-5; Dislike slightly-4; Dislike moderately-3; Dislike very much-2; Dislike extremely-1.

samples. The increase in the acidity upto 14 days at 8±1°C was insignificant but later it also showed an increase by 0.1 per cent. After 75 days of storage at -20°C the acidity of khoa increased to 0.67 per cent in 1P and 3HDP, 0.64 in 2MSDAT, 0.59 in 4P and 0.58 in 5P. Lactic acidity on 30th day of storage at 8±1°C corresponded to the acidity on 75 days of storage at -20°C. The rate of change in acidity in different packages showed the same trend at all temperatures of storage. On the basis of lactic acid development, 4P and 5P may be considered to afford better protection for storage of khoa.

Protein breakdown: Tyrosine values were intended to serve as a marker for determining the extent of protein breakdown in stored khoa. The initial tyrosine value of 16.13 mg/100g khoa in the present experiment is lower than those reported by Rudreshappa and De⁸, although on dry matter basis they would be identical. According to Rudreshappa and De⁸ the khoa tested gave 75 per cent total solids as against 66 per cent in the present study. The enquiries with khoa dealers reveal that product with 75 per cent total solids does not meet their specific requirements for the preparation of sweets. A maximum proteolytic activity was observed in khoa samples stored at 37°C in parchment paper. Although, differences in tyrosine values as affected by packages were statistically significant at 5 per cent level, the duration of storage was significant both at one and 5 per cent levels at 37°C and 8±1°C. The aluminium coated laminates provided better protection against protein hydrolysis as compared with cellophane/polyethylene laminate and single polyethylene films.

Peroxide value: Oxidative changes in fat were markedly higher at 37°C than at 8±1°C and -20°C storage. Khoa samples packed in parchment paper and polyethylene showed maximum formation of peroxides at

37°C. A 50 to 100 per cent increase in the peroxide value took place between 5 and 7 days in parchment paper packs at 37°C. At 8±1°C also the parchment paper packed product showed maximum peroxide value (0.775) upto 30 days of storage. Two ply laminates afforded better protection against oxidation as compared with 1P or 3HDP but the former proved inferior to aluminium coated laminates. Low temperature storage significantly retarded the oxidative spoilage of khoa. The statistical analysis showed that peroxide values were significantly affected both by the packages and periods of storage, the test of significance being valid at 1 per cent and 5 percent levels.

Free fatty acids: Free fat acidity (per cent oleic acid) was measured to quantify hydrolytic changes of fat during storage of khoa. Khoa packed in parchment paper showed a rapid increase in the production of free fatty acids at all the three temperatures of storage, the maximum oleic acid (0.4512) being at 37°C after 10 days of storage. The minimum acidity (% oleic) at 37°C was produced in khoa samples packed in aluminium coated laminates on all days of analysis. 2MSDAT proved better than polyethylene but inferior to fourply laminates as judged by per cent oleic acidity. Similar observations were recorded at 8±1°C and -20°C. The four-ply laminates coated with aluminium were superior over the two-ply lamination or polyethylene and parchment paper for storage at all temperatures.

Total plate count: At the time of packaging of fresh khoa the total plate count ranged from 8000 to 21000/g of khoa with an average of 12000/g. Naidu and Ranganathan²² reported S.P.C. to range from 13000 to 15,00,000/g of khoa in the market samples of khoa. The values for S.P.C. in stored khoa samples at 37°C temperature on 5, 7 and 10 days and at 8±1°C on 14th day onward showed a rapid increase. The multiplication of microorganisms in khoa samples stored at 37°C was faster than at 8±1°C. The maximum plate count was observed after 10 days of storage at 37°C (1,48,50,000/g khoa) whereas the highest count (3,20,000/g) was reached at 8±1°C after 30 days of storage (Table 4). Further, the growth of microorganisms during storage of khoa was also affected by the type of package used. On the 5th day of storage at 37°C khoa samples packed in 3HDP showed highest plate count (8,95,000/g khoa) followed in descending order by 1P, 2MSDAT, 4P and 5P. On 30th day of storage at 8±1°C khoa samples packed in 3HDP and 1P showed maximum plate count (320×10³/g) while 5P showed the least count (215×10³/g). It may be possible that parchment paper and high density polyethylene films with high moisture and gas transmission properties allow oxygen to enter the packs and help the growth and multiplication of bacteria.

Differential counts: The differential counts comprising of acid producers, proteolytic and chromogenic types also increased during storage of khoa as compared with the initial differential count (Table 2). Differential counts were also affected by the package and temperature of storage (Table 4). The initial number of acid producers in khoa before packaging was only 7500/g but after 7 days at 37°C and 30 days at 8±1°C it rapidly rose to 22×10⁵/g and 8×10⁴/g respectively in 1P packets. The increase in the number of acid producers was accompanied with increased lactic acid production in khoa. The lowest number of acid producers was found in 5P. The initial proteolytic count of khoa before packaging was 3000/g. Proteolytic counts increased in stored khoa at 37°C and 8±1°C. The maximum number of proteolytic organism reached was 18×10³/g in 1P packed khoa samples at 37°C after 7 days of storage. The highest and lowest values recorded for proteolytic counts after 30 days at 8±1°C were 70×10³ and 42×10³/g for 1P and 5P packed khoa samples. The increases in the number of proteolytic organisms during storage of khoa samples at 37°C and 8±1°C are also accompanied by higher tyrosine values (Table 3). Although the chromogenic counts were low in comparison to acid formers and protein hydrolyzers their numbers also progressively increased during storage (Table 4).

Spore count: The spore count of fresh khoa samples taken before packaging was 200/g. Naidu and Ranganathan²² have reported 324 spores/g in khoa samples collected from market. In our study a minor increase in the spore count was observed both at 37°C and 8±1°C during storage (Table 4). The effect of packages on spore count was negligible.

Yeast and mould count: The initial yeast and mould count in khoa was 20/g. A distinct mould growth appeared on the surface and body of khoa samples packed in 3HDP after 30 days storage at 8±1°C and less so at 37°C. The presence of yeast and moulds in khoa is objectionable since they produce discolouration defects and lipolytic changes causing off flavour development in the product. The number of yeast and moulds increased progressively during storage at 37°C as well as 8±1°C in all the stored khoa samples. The differences in the yeast and mould counts due to period of storage, temperature of storage and type of packages were found to be statistically significant.

Lipolytic count: The lipolytic organisms in khoa before packaging were 600/g. Their number showed an increasing trend in all khoa samples on storage at 37°C and 8±1°C. 1P packed khoa samples showed the maximum lipolytic count of 44×10³/g while the least count of 30×10³/g was observed in 4P after 5 days of storage at 37°C. Similarly at 8±1°C after 30 days storage the maximum number of lipolytic organisms were 30×10³ in 1P and only 20×10³/g in 4P packed samples. It was of interest to note that increase in the lipolytic counts both at 37°C and 8±1°C storage were accompanied by higher values of free fatty acids in khoa.

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Development of High Protein Bread. Part II. Soya Flour Utilisation

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The optimum level of soya flour which can be added to a new high yielding Indian wheat variety, namely *Sharabati sonara* and processing conditions for obtaining high protein bread have been discussed. Soya fortified (10%) bread can be prepared by straight dough method using 100 min fermentation time. Addition of fat and oxidising agent improves the quality of the bread. Sodium stearoyl-2-lactylate increases the mixing tolerance, loaf volume, grain texture and crumb score.

It has long been recognised that the soya flour is an excellent means of increasing the protein content of cereal foods and also enhancing their nutritive value, as the amino acid of soya protein and cereals supplement each other. However soya fortification of baked products creates many technological problems, with reference to water absorption, mixing, fermentation, loaf volume and the acceptability of the final product. Various workers have also suggested that the baking performance of soya fortified flour can be improved by adopting certain modifications in the processing technique. Ofelt *et al.*¹, Finney *et al.*^{2,3} and Tsen *et al.*⁴, and Tsen and Tang⁵ have shown that improvement in the baking performance of soya fortified flour can be achieved by increasing water absorption, decreasing mixing time, reducing fermentation time, increasing oxidant and adding dough conditioner. No systematic work on the soya fortification of Indian wheat for the preparation of bread has been reported. The work was therefore, undertaken to standardise conditions for preparation of soya fortified bread and to understand the effects of different levels of soya flour and improvers on the quality of fortified bread.

Materials and Methods

The straight grade flour used in this study was obtained by milling *Sharabati sonara* wheat in a Brabender junior experimental mill. Defatted solvent extracted commercial soya flour (100 mesh) and wheat flour used in the study were analysed for moisture, protein and crude lipids according to Cereal Laboratory Methods and the values are given in Table 1.

Baking tests: Three different methods were used to examine the influence of various formulae and pro-

cedural changes on the relative quality and loaf volume of finished bread. The baking formula and procedure employed included: (i) a short straight dough method with 100 min fermentation time; (ii) a 70 per cent sponge dough method with 4 hr fermentation time; and (iii) a salt sugar delayed method with 1 hr fermentation time.

Short straight dough method: The following formula was used.

	Quantity (g)
Soya fortified (10%) flour	200
Water	140
Yeast	6
Shortening	4
Salt	4
Sugar	10

Sodium stearoyl-2-lactylate (SSL) or potassium bromate was used in variable quantities. All the ingredients are mixed at room temperature; dough temperature was kept at 27°C. The dough was rounded by hand and fermented at 27°C and 85 per cent R.H. upto selected period. Fermented dough was moulded using National moulder. Moulded dough was placed in the greased pan and proofed at 45°C and 85 per cent R.H. upto appropriate time. Baking was done for 20 min at 220°C. The volume was measured by the rape seed displacement method within 10 min after removing loaf from the oven and scored after 18 hr.

Seventy per cent sponge dough method: The following formula was used.

	Quantity (g)
Sponge	140
Water	84
Yeast	6
Shortening	4

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TABLE 1. COMPOSITION OF WHEAT AND SOYA FLOURS

Ingredient	Moisture %	Protein %	Crude lipid %
Wheat flour	14.0	9.8*	0.80
Defatted soya flour	8.0	50.0**	1.50

*14% moisture basis; **on dry basis.

All the ingredients of sponge were mixed for 2 min in National mixer and sponge was kept for 4 hr fermentation at 27°C and 85 per cent R.H. Sponge was taken out and remixed upto appropriate mixing time with the following in a National mixer and dough was kept at 27°C.

	Quantity (g)
Dough	60
Water	52
Salt	4
Sugar	10

Floor time given was 45 min. Scaling, and intermediate proofing for 10 min. Dough was moulded and kept in greased pan and proofed upto appropriate proofing at 50°C and 85 per cent R.H., baked at 220°C for 20 min.

Salt sugar delayed method:

	Quantity (g)
Sponge	200
Water	140
Yeast	6
Shortening	4

All the ingredients of sponge were mixed for 1 min in National mixer. Sponge fermented for 1 hr at 27°C and 85 per cent R.H.; taken out and remixed with salt-4 g; and sugar-10 g for appropriate time.

Dough was kept at 27°C temperature. Floor time given was 45 min. Scaling, and intermediate proofing for 10 min. Dough was moulded and kept in greased pan for proofing at 50°C and 85 per cent R.H. for appropriate proofing. Proofed dough was baked at 220°C for 20 min.

Results and Discussion

Mixing studies: Farinographic studies were conducted to determine the effect of addition of different levels of defatted soya flour and SSL on the mixing characteristics of wheat flour/wheat soya flour blends. The effect of addition of soya flour and SSL is shown in the resulting farinograms presented in Fig. 1 and the interpretation of these farinograms are given in Table 2. Farinographic studies indicated that water absorption of control flour was 56.6 per cent and by the addition of 5, 10 and 15 per cent soya flour the water absorption increased to 58.8, 61.6 and 65.0 per cent respectively. The stability, MTI, TMD was usually less than the wheat flour alone.

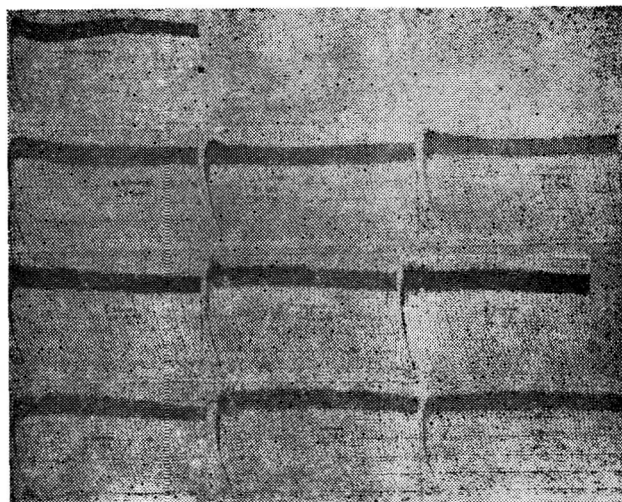


Fig. 1. The effect of addition of soya flour with and without SSL on farinograph mixing characteristics of the *Sharabati sonara* wheat variety flour.

Top: wheat flour control bread. From left to right: first line, addition of 5% soya flour, with 0.5% SSL, with 1.0% SSL; 2nd line from left to right, addition of 10% soya flour, with 0.5% SSL, with 1.0% SSL; 3rd line—addition of 15% soya flour, with 0.5% SSL and with 1.0% SSL.

The narrow farinograms width at the addition of different levels of soya flour showed the poor stability. Addition of SSL at 0.5 and 1.0 per cent did not effect the water absorption but could delay dough development, increase in dough stability and tolerance which are well comparable with wheat flour farinograms.

The increase in water absorption of soya fortified flour mainly depends upon the type of processed soya flour used. Generally over heated flour absorbs high percentage of water than the under heated one.

TABLE 2. EFFECT OF ADDITION OF DEFATTED SOYA FLOUR AND SSL ON THE FARINOGRAPH CHARACTERISTICS OF WHEAT FLOUR/WHEAT SOYA FORTIFIED FLOUR

Soya flour (%)	SSL added (%)	WA (%)	STD (min)	TMD (B.U.)
Control	—	56.6	17.25	40
5	—	57.8	14.0	60
5	0.5	57.8	19.5	30
5	1.0	57.8	19.5	20
10	—	61.8	11.5	70
10	0.5	61.8	19.5	40
10	1.0	61.8	19.5	20
15	—	65.0	11.5	80
15	0.5	65.0	19.5	30
15	0.1	65.0	19.5	30

WA = Water absorption %
 STD = Stability time in min
 TMD = 20 min drop

Baking studies: It is well known that addition of soya flour to wheat flour effects adversely the water absorption, fermentation time, volume, grain, texture score, colour of crumb, flavour and finally acceptability of the baked products. To overcome these adverse effects certain modification in the mixing and fermentation are adopted to minimise the damage to gluten structure so that the dough can hold the gas bubbles produced during fermentation.

Effect of fermentation time: To shorten the straight dough procedure, evaluation was first carried out on the effect of fermentation time. Ten per cent soya fortified flour was fermented at 27°C for 100–180 min with the difference of 20 min. Fig. 2 shows the bread produced with different fermentation times and the results are summarised in the Table 3. When the dough was fermented for 160–180 min the resulting bread has slightly decreased volume, open thick wall grain, slightly harsh texture with dull crumb colour. The dough



Fig. 2. Effect of fermentation time on the baking quality of 10% soya fortified flour.

Left to right: 100 min, 120 min, 140 min, 160 min and 180 min fermentation time.

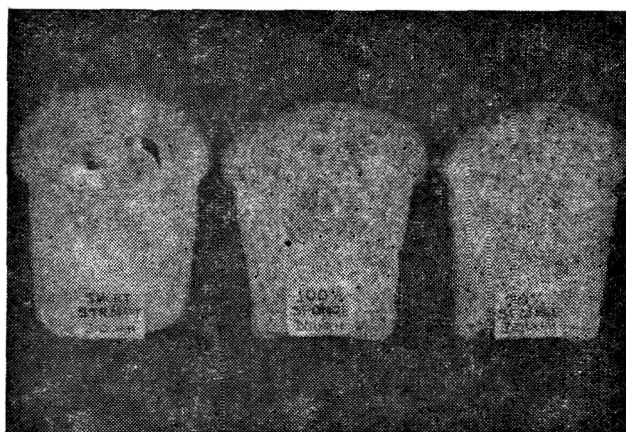


Fig. 3. Comparison of three different methods for the production of 10% soya fortified bread.

Left to right: bread produced by short straight dough method, salt sugar delayed method and 70% sponge dough method.

TABLE 3. EFFECT OF FERMENTATION TIME ON THE BAKING QUALITY OF 10 PER CENT SOYA FORTIFIED FLOUR

Fermentation time (min)	Volume (100 g flour loaf)	Grain score	Texture score	Crumb colour score
Wheat flour	530	8.0	10.0	8.0
100	500	7.0	8.0	6.5
120	500	6.5	7.5	6.5
140	500	6.0	7.0	6.0
160	480	6.0	6.75	6.0
180	480	5.0	6.75	6.0

Grain and texture scores were out of 15, and crumb colour score was out of 10.

TABLE 4. EFFECT OF VARIOUS FORMULAE AND PROCEDURES ON THE BAKING QUALITY OF 10 PER CENT SOYA FORTIFIED FLOUR

Method	Volume (100 g flour loaf)	Grain score	Texture score	Crumb colour score
1. Short straight dough with 100 min fermentation	500	7.0	8.0	6.5
2. 70% sponge dough with 4 hr fermentation	500	6.25	8.0	6.0
3. Salt sugar delayed method with 1 hr fermentation	500	6.25	7.5	6.0

Grain and texture scores were out of 15 where as crumb colour score was out of 10.

TABLE 5. EFFECT OF ADDITION OF SOYA FLOUR AT DIFFERENT LEVELS WITH AND WITHOUT SSL ON THE BAKING QUALITY OF WHEAT FLOUR

DSF %	SSL added %	Volume (100 g flour loaf)	Grain score	Texture score	Crumb colour score
100%	wheat flour	520	8.0	10.0	8.0
5	—	510	8.0	9.0	7.0
5	+0.5	560	8.0	9.0	7.0
5	+1.0	570	8.5	9.5	7.5
5	+2.0	630	8.5	9.75	7.5
10	—	480	7.0	8.0	6.5
10	+0.5	530	7.25	8.25	6.75
10	+1.0	560	7.5	8.5	7.0
15	—	380	6.0	6.5	6.0
15	+0.5	430	6.0	7.0	6.0
15	+1.0	470	7.0	7.25	6.5
15	+2.0	510	7.0	7.5	6.75

DSF = Defatted soya flour. Grain and texture scores were out of 15 where as crumb colour score was out of 10.



Fig. 4A. The effect of addition of 5% soya flour with and without SSL.

Left to right: wheat flour-control; 5% soya fortified flour with 0.5% SSL; 1.0% SSL and 2.0% SSL.



Fig. 4B. Effect of addition of 10% soya flour with and without SSL.

Left to right: wheat flour-control; 10% soya fortified flour alone, and with 0.5% SSL and 1.0% SSL.

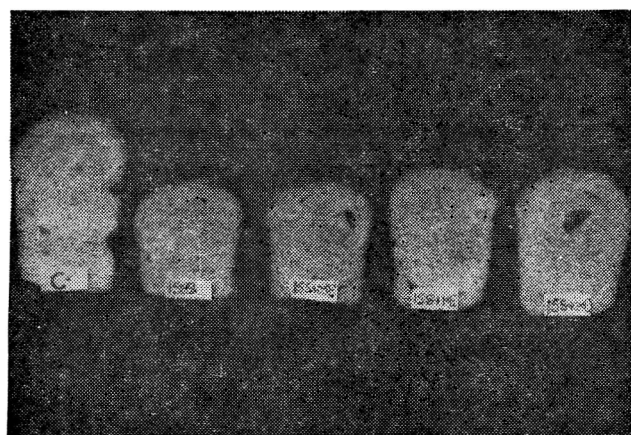


Fig. 4C. The effect of addition of 15% soya flour with and without SSL.

Left to right: wheat flour-control, with 15% soya fortified flour alone, and with 0.5% SSL; 1.0% SSL and 2.0% SSL.

TABLE 6. EFFECT OF ADDITION OF POTASSIUM BROMATE ON THE BAKING QUALITY OF 10 PER CENT SOYA FORTIFIED FLOUR

Pot. bromate (ppm)	Volume (100 g flour loaf)	Grain score	Texture score	Crumb colour score
20	500	7.0	8.0	6.5
30	500	7.0	8.0	6.5
40	500	7.25	8.0	6.5
50	520	7.50	8.2	6.5
60	540	7.75	8.3	6.75

Grain and texture scores were out of 15 and crumb colour score was out of 10.

TABLE 7. EFFECT OF ADDITION OF REFINED SOYA BEAN OIL ON THE BAKING QUALITY OF 10 PER CENT SOYA FORTIFIED FLOUR

DSF (%)	Soybean oil (%)	Volume (100 g flour loaf)	Grain score	Texture score	Crumb colour score
10	0	430	6.5	7.5	6.5
10	5	490	7.0	8.0	6.0
10	10	510	7.25	8.25	6.0
10	15	510	7.50	8.50	6.0
10	20	530	8.00	8.75	6.0

DSF = Defatted soya flour. Grain and texture scores were out of 15 and crumb colour score was out of 10.

TABLE 8. EFFECT OF BLEACHING AGENT ON THE BAKING QUALITY OF 10 PER CENT SOYA FORTIFIED FLOUR

DSF (%)	Bleaching agent* (%)	Volume (100 g flour loaf)	Grain score	Texture score	Crumb colour score
10	—	500	7.0	8.0	6.5
10	0.03	500	8.0	8.0	7.5
10	0.06	500	8.0	8.0	8.0

*Oxylite type B B bleaching agent. Grain and texture scores were out of 15 and crumb colour score was out of 10.

fermented for 100–140 min showed same volume but 100 min fermented dough was better in grain, texture and crumb colour than the 120 and 140 min fermented dough. Fermentation for 100 min was appropriate for the development of 10 per cent soya fortified bread, and hence this was selected for further studies.

Effect of different methods on the loaf volume: The effect of three different methods (i) short straight dough with 100 min fermentation time; (ii) 70 per cent sponge dough with 4 hr fermentation time; and (iii) salt sugar delayed method with 1 hr fermentation time, on the bread quality of 10 per cent soya fortified flour is shown in Fig. 3 and the results are summarised in Table 4. Figure 3 shows that very acceptable bread was produced



Fig. 5. Effect of addition of potassium bromate on the 10% soya fortified flour.

Left to right: 20 ppm, 30 ppm, 40 ppm, 50 ppm and 60 ppm.

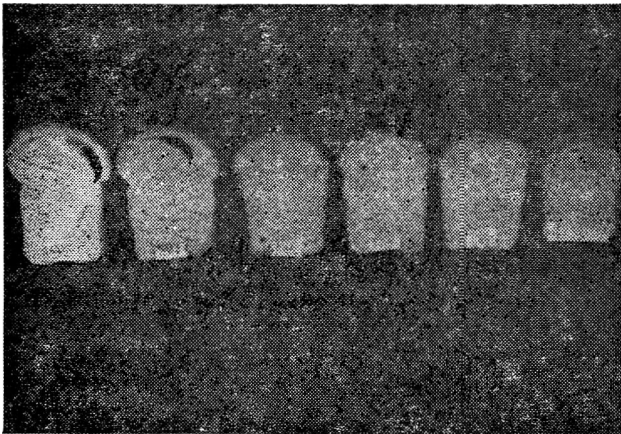


Fig. 6. Effect of addition of refined soya bean oil on the baking quality of 10% soya fortified flour.

Left to right: wheat flour-control, 10% soya fortified flour bread with 20% oil, 15% oil, 10% oil, 5% oil and 10% defatted soya flour bread.

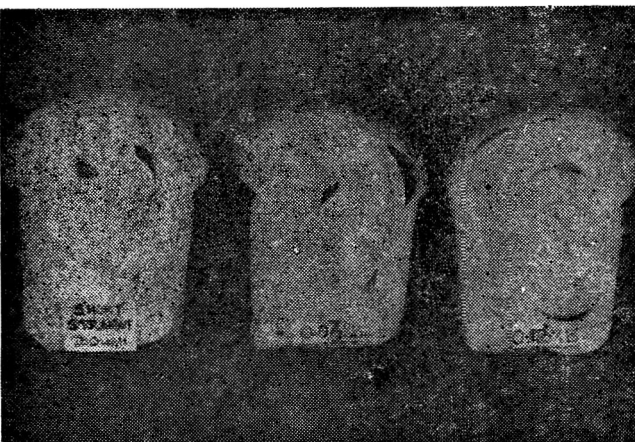


Fig. 7. Effect of bleaching agent on the 10% soya fortified bread. Left to right: 10% soya fortified bread with 0.03% and 0.06% bleaching agent.

by the 10 per cent soya fortified flour by all the three methods. The bread produced by the salt sugar delayed method was slightly inferior than the bread produced by using the other methods. The choice was the short straight dough method with 100 min fermentation time. Therefore the short straight dough method was selected for further studies.

Effect of addition of defatted soya flour with and without SSL on baking quality: Addition of soya flour adversely affects the loaf volume, grain, texture and crumb score of the finished bread and the adverse effects intensify as soya flour is increased, particularly at 10–15 per cent levels (Table 5 and Fig. 4 A,B,C). Results of the baking study showed that the addition of 5 per cent defatted soya flour to wheat flour did not affect significantly the loaf volume, grain score but the texture and crumb colour score decreased. By the addition of 0.5, 1.0 and 2.0 per cent SSL the volume, grain and texture was increased than control flour bread. By the addition of 10 per cent soya flour the volume of the bread decreased about 50 ml than the control flour bread and the bread was of acceptable quality. With the addition of 0.5 per cent SSL the volumes of 10 per cent defatted soya fortified bread and control flour were the same and the addition of 1.0 per cent SSL increased the volume of 10 per cent soya fortified flour by about 30 ml more than the control flour. With the addition of 15 per cent soya flour the volume was reduced for about 150 ml and the grain texture and crumb colour score was very poor. By the addition of SSL at the level of 2.0 per cent, the volume increased but the grain and texture score were not significant and the bread was of unacceptable quality.

Effect of potassium bromate on the baking quality: As the addition of potassium bromate is allowed in the country, the suitability of potassium bromate for improving the baking characteristics of 10 per cent soya fortified flour was tested. Potassium bromate was added at 20, 30, 40, 50, 60 ppm to the 10 per cent soya fortified flour. The breads produced are shown in Fig. 5 and the results are summarised in Table 6. The results indicated that the 20, 30, 40 ppm potassium bromate addition did not improve the volume, but grain score was slightly improved. But at 50 and 60 ppm there was an increase in volume by 40 ml and grain score was equivalent to the wheat flour bread.

Effect of addition of refined soya bean oil on the baking quality: Studies were conducted to determine the effect of addition of soya bean oil on the baking quality of 10 per cent soya fortified flour. Fig. 6 shows the bread produced by the addition of 5, 10, 15 and 20 per cent refined soya bean oil and the results are summarised in Table 7. These show that a very poor quality of bread is produced with the 10 per cent soya fortified

flour, but with the addition of increasing quantities of soya bean oil the volume, grain and texture are improved. The colour of the crumb showed dull appearance with the addition of refined soya bean oil. The best bread was produced with the 10 per cent soya fortified flour with the addition of 20 per cent refined soya bean oil.

Effect of bleaching agent (oxylite type BB) on the baking quality: Addition of soya flour to wheat flour gives dull colour to the crumb due to which it is unacceptable by the consumer. To overcome this problem flour was treated with flour bleaching agent namely oxylite type BB at the concentration of 0.03-0.06 per cent. The bread produced is shown in Fig. 7 and

data are summarised in Table 8. These show that addition of bleaching agent did not affect the texture score and volume, the grain and colour of crumb was very much improved. With the addition of 0.06 per cent oxylite type BB the crumb colour was equal to the 100 per cent wheat flour bread.

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Effect of Addition of Formaldehyde on the Dietary Value of Milk for Mice and Its Quality

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Raw milk preserved with 0.075, 0.15 and 0.21 g formaldehyde per 100 ml sample was tested to detect any toxicities while used as a diet for mice. Possible variations in acidity and milk fat content in these milk samples were also studied. No significant differences in weights of mice fed fresh raw whole milk and those fed milk treated with 0.075 or 0.05 g formaldehyde were noted. However, a significant difference in weight at 5 per cent confidence level was noticed between mice fed fresh raw whole milk and those fed milk treated with 0.21 g formaldehyde. Per cent lactic acid values revealed that formaldehyde preserved samples were not significantly different from fresh untreated raw milk. Milk fat levels remained the same in control and formaldehyde treated milk samples. Fat content in all samples remained constant throughout the one week storage. All samples retained normal texture, cowy odour, and normal taste throughout the length of storage. They exhibited formaldehyde flavour which became less perceptible with longer storage.

Formaldehyde is widely used as a preservative of milk for chemical analysis¹⁻⁴. The purpose of this study was to determine whether raw milk preserved with different levels of formaldehyde would be toxic, or would affect growth and well being of mice. Variations in fat contents of formaldehyde treated milks also were studied to identify possible confounding effects on the test animals from such variations.

Materials and Methods

Twenty healthy weanling mice were selected without regard to strain and breed from a colony maintained in a research laboratory of the biology department of the university. After a thorough inspection of general health and performance, the mice were distributed randomly into 4 separate cages with 5 mice per cage. One group served as control and received raw

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whole milk containing no chemical preservative. The other three groups received chemically treated raw milk. Each cage group was identified by a tag indicating the type of chemically treated raw milk with which the mice would be fed. Individual mice were identified by ear notching and were weighed at the beginning and on the 14th day of the experiment.

Dietary regimen: Individual mice were fed two weeks with samples of raw whole milk treated with 0.075, 0.15, and 0.21 g of formaldehyde per 100 ml sample respectively. Mice in the control group were fed untreated milk samples. To balance other nutrient requirements, mice also were supplied a commercial chow *ad libitum*. Mice in each cage group were examined daily for their performance and/or changes in normal activities.

Determination of milk quality: An approximate 4 liter composite milk sample was prepared by mixing aliquots of milk from different cows in one sterilized container. All of the fresh raw milk was collected in an afternoon milking for the experiment at the University Dairy Center. The milk was stirred to achieve thorough mixing and care was taken to avoid shattering fat globules. Approximately 40 ml of the raw milk was distributed uniformly into test tubes containing various levels of formaldehyde; 0.075, 0.15, and 0.21 g of formaldehyde per 100 ml of raw milk were used. Seven tubes were prepared for each treatment and control group. All test tubes were stoppered, labelled, and kept in a clean, dry cabinet in the laboratory under ambient conditions. The samples were stirred daily to assure uniform distribution of fat. Acidity, fat content determinations and organoleptic evaluation were carried out each day on a sample from each chemical treatment and level grouping a one week experimental period. Standard acidity procedure⁵ and Babcock test⁶ were used for these determinations.

Results and Discussion

Average body weights of mice fed the various milk diets for fourteen days are shown in Table 1. Mice fed untreated raw whole milk had an average increase in

TABLE 1. AVERAGE BODY WEIGHTS OF MICE FED MILK DIETS FOR FOURTEEN DAYS.*

Milk Diet**	Initial wt	Final wt	Av gain in wt (g)
	(g)	(g)	
Control	32.500	32.875	0.375
HCHO 0.075 g	31.825	31.900	0.075
HCHO 0.15 g	25.875	26.000	0.125
HCHO 0.21 g	29.000	30.825	1.825

*Average weights of five individuals

**Raw milk fed *ad libitum* including a regular Purina Chow diet.

TABLE 2. ACIDITIES OF CHEMICALLY PRESERVED RAW MILK SAMPLES DURING STORAGE AT AMBIENT TEMPERATURES FOR SEVEN DAYS.*

Treat- ment	Chemical g/100 ml milk	Acidity produced at indicated days of storage (% lactic)						
		1st	2nd	3rd	4th	5th	6th	7th
Control	0.00	0.19	0.21	0.37	0.82	1.02	1.05	1.08
HCHO	0.075	0.23	0.24	0.24	0.25	0.26	0.27	0.28
HCHO	0.15	0.26	0.26	0.26	0.28	0.29	0.30	0.31
HCHO	0.21	0.28	0.28	0.28	0.29	0.32	3.20	0.32

*Figures in the table represent averages for three replications.

body weight of 0.375 g over a two-week feeding period while the mice that received 0.075, 0.15, and 0.21 g formaldehyde treated milk showed an average increase in body weight of 0.075, 0.125, and 1.825 g respectively during that period.

Statistical analysis⁷ indicated that there were no significant differences in weight gains after two weeks between mice fed fresh whole milk and those containing formaldehyde at the 0.075 or 0.15 g levels. Compared with the control mice, the ones fed 0.21 g formaldehyde in milk were found significantly different at 5 per cent level. The average gains of mice fed milk treated with 0.21 g level of formaldehyde were significantly greater than the control group. This difference in weight may be due to the improved quality of milk and out of the high concentration of the preservative used.

Behaviour of mice fed fresh or formaldehyde preserved milk was quite normal. All mice in each group remained active throughout the period of the dietary experiment.

Acidities which developed in the milk samples during storage at ambient temperature of 23.3–25.5°C for seven days are shown in Table 2. Control samples in which no chemical preservatives were used, increased in acidity an average of 0.89 per cent during the seven day interval. Acidities in raw milk samples preserved with 0.075, 0.15, and 0.21 g of formaldehyde increased

TABLE 3. ACIDITY, TEXTURE, ODOUR, FLAVOUR, AND TASTE QUALITY OF CHEMICALLY PRESERVED RAW WHOLE MILK DURING STORAGE AT AMBIENT TEMPERATURE; FOR SEVEN DAYS.*

HCHO g/100 ml milk	Criteria of milk quality				
	Acidity	Texture	Odour	Flavour	Taste
0.00 g	—	—	—	—	—
0.075 g	+	+	+	+	+
0.05 g	+	+	+	+	+
0.21 g	+	+	+	+	+

*Results based on evaluation over three replications

+ denotes acceptability

— denotes unacceptability

0.05, 0.05 and 0.04 per cent respectively. Statistical analysis reveals that formaldehyde preserved samples were not significantly different from fresh untreated raw milk.

A summary of results of milk quality tests are shown in Table 3. On the basis of acidity, texture, odour, flavour, and taste of raw milk samples, formaldehyde was judged acceptable for preserving the quality for seven days at ambient temperatures of 23.3–25.5°C. A slight formaldehyde "off-flavour" was associated with samples thus preserved, but the defect is not deemed severe enough to render formaldehyde unacceptable as a preservative of raw whole milk.

Milk fat levels in raw milk samples that contained 0.075, 0.15 and 0.21 g formaldehyde were the same as those analyzed in control samples throughout seven days of storage. It became evident that formaldehyde does not

effect the milk fat level when milk is preserved with 0.075, 0.15, or 0.21 g formaldehyde per 100 ml raw milk.

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Changes in Coconut Lipids during Desiccation

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Coconut lipids were fractionated by silicic acid column chromatography into neutral and polar lipid fractions which amounted to 94 and 6 per cent respectively. The fatty acid composition of total lipids and of the neutral lipid fractions was similar to that of coconut oil; desiccation did not alter this. In contrast, the polar lipid fraction was rich in unsaturated fatty acids which accounted for 35–45 per cent. On desiccation, the unsaturated fatty acids decreased considerably indicating that they were oxidised even under the mild conditions of desiccation. It was concluded that these changes and the differences in composition between coconut oil and coconut lipids would explain problems of rancidity encountered with desiccated coconut and foods incorporating it.

Coconut oil is composed predominantly of saturated fatty acids and is considered one of the most stable food fats. Yet problems of rancidity have been encountered with desiccated coconut and also with other food formulations incorporating desiccated coconut. Such behaviour on storage is not compatible with the known fatty acid composition of coconut oil^{1,2}. This may be due to the fine differences in lipid composition between coconut oil and the total lipids of coconut. While there are several reports regarding the fatty acid^{1,2} and the triglyceride compositions^{3,4} of coconut oil, little is known about the lipid class composition of coconut and their individual fatty acid make-up. These aspects were studied and the findings are reported in this communication.

Materials and Methods

Preparation of samples: Freeze-dried and dehydrated coconuts were prepared as described elsewhere⁵.

Extraction of lipids: Moisture-free samples were extracted with petroleum ether⁶ and then with hexane-ethanol (79:21) in a Soxhlet apparatus. Extraction with chloroform-methanol (2:1) was done by packing 5 g sample in a glass column (0.5 cm × 12 cm) and allowing the solvent to flow at about 3 ml per min; 250 ml of solvent was needed for adequate lipid recovery. Non-lipid matter in the extract was removed by the Folch procedure⁷.

Silicic acid column chromatography: Separation of total lipids into neutral, glyco- and phospho-lipid fractions was achieved by chromatography on silicic acid⁸. The quantity of lipid in the neutral and glycolipid fractions was determined by evaporating an aliquot and weighing. Percentage of phospholipids was calculated from phosphorus content estimated after perchloric acid digestion⁹ using cephalin as standard.

Gas chromatography: Total and neutral lipids were converted to fatty acid methyl esters using sodium

methoxide². Glycolipids and phospholipids were pooled together, saponified with alcoholic KOH and the liberated fatty acids were esterified with diazomethane. Fatty acid methylesters were separated on a Varian Aerograph model series 1400 instrument equipped with FID, conditions being as follows: A stainless steel column $5' \times \frac{1}{8}"$ packed with 15 per cent DEGS on chromosorb W 60-80 mesh; temperature programme-70 to 195°C started at 70°C, temperature rise 10°C per min injector and detector 230°C; carrier gas-N₂ 15 ml/min; H₂-20 ml/min; air 300 ml/min; range-10⁻¹⁰ amps/mV; attenuation 16× to 64× manipulated as required and chart speed 1 min/cm. Each analysis was done in triplicate. The resolved peaks were identified by comparing the retention time with those of a standard mixture of the methyl esters of laurate, myristate, palmitate, stearate, oleate, linoleate (all from V.P. Chest Institute, Delhi, India) and arachidonate (Sigma Chemical Co., USA). Quantitation was by triangulation of peaks.

Results and Discussion

The extractability of lipids from coconut by three solvent systems is shown in Table 1. Extraction with chloroform-methanol was better than with petroleum ether followed by hexane-ethanol. The normally advocated procedures of blending the sample with chloroform-methanol or of occasional shaking with the solvent did not result in satisfactory extraction because the quantity of lipid extracted was found to be less than that extractable by petroleum ether. The procedure involves repeated extractions and use of large volume of solvent for washing the residue. Chloroform-methanol 2:1 by volume and the sample-solvent ratio 1:20 W/V was used for blending. About 200 ml solvent mixture was used for washing the residue. The total lipids obtained by this procedure⁷ was only 71.0 per cent. Though extraction in a Soxhlet extractor gave a higher lipid content, this procedure was not used as the non-lipid matter was high and the extracts were invariably deep yellow or brown. Also, one of the purposes of the present study was to examine the effect of desiccation on the lipids. It was found that packing the coconut sample in a column and eluting it with chloroform-methanol gave an extract that was (a) satisfactory in

TABLE 1. COCONUT LIPIDS EXTRACTED BY DIFFERENT SOLVENTS

Sample	Lipids* (%) extracted by		
	Pet. ether	Hexane: ethanol after pet. ether	Chloroform: methanol & Folch ⁷ wash
Coconut, freeze-dried	71.6±1.18	0.98±0.16	75.25±0.96
Coconut, desiccated	70.5±1.20	1.10±0.23	72.58±0.82

*Mean±S.D. of 5 determinations.

terms of lipid recovery and (b) very low in non-lipid matter. There was no difference in the extractability of lipids between freeze-dried and desiccated coconuts.

The lipids of coconut were composed of about 94 per cent neutral lipids and 6 per cent polar lipids. Phospholipids constituted only 0.2 per cent of the total lipids (Table 2). This type of composition was not unexpected as nuts are almost entirely composed of triglycerides. No difference was observed between desiccated and freeze-dried coconut with regard to the lipid class composition.

In Table 3 is shown the fatty acid composition of the lipid fractions of freeze-dried and desiccated coconut. The total lipids and the neutral lipids fraction contained a fatty acid not hitherto reported in coconut oil. It was tentatively identified as arachidonic acid. Whereas a chloroform-methanol extract of coconut showed the presence of this fatty acid, it was not present in the press oil obtained from the same sample of freeze-dried coconut. This clearly delineated the difference in composition between the oil and the total lipids of coconut. But for this special feature, the fatty acid composition

TABLE 2. LIPID CLASSES OF COCONUT LIPIDS

Sample	Neutral lipid* %	Glycolipid* %	Phospholipid* %
Coconut, freeze-dried	94.34±0.39	5.47±0.37	0.20±0.06
Coconut, desiccated	93.5±0.47	6.28±0.49	0.19±0.03

*Mean±S.D. of 3 determinations; Represents per cent of total lipid.

TABLE 3. FATTY ACID COMPOSITION OF THE TOTAL, NEUTRAL AND POLAR LIPIDS OF COCONUT

Fatty acid**	Fatty acid* as per cent by weight of total in					
	Total lipids		Neutral lipids		Polar lipids	
	F.D.	D.C.	F.D.	D.C.	F.D.	D.C.
C _{6:0}	0.4	0.4	0.4	0.4	—	—
C _{8:0}	8.2	8.5	7.9	8.1	—	—
C _{10:0}	6.0	5.5	5.4	5.8	0.9	1.4
C _{12:0}	49.0	48.7	48.4	49.7	14.7	22.6
C _{14:0}	17.9	19.7	20.1	19.2	9.5	7.1
C _{16:0}	7.4	7.3	7.7	7.2	20.4	23.3
C _{18:0}	1.5	1.3	1.5	1.7	9.7	10.0
C _{18:1}	5.7	5.8	5.9	5.2	8.1	8.1
C _{18:2}	1.0	0.5	0.9	0.6	1.5	3.0
C _{18:3}	—	—	—	—	3.0	3.3
C _{20:4}	2.8	2.0	1.9	2.1	33.5	20.8
Total saturated	90.5	91.5	91.3	92.1	55.2	64.4
Total unsaturated	9.5	8.4	8.7	7.9	46.1	35.1

*Each value is the mean of three determinations.

**The first number is the total number of carbon atoms and the second the number of double bonds in the fatty acid.
F.D. Freeze-dried D.C. Desiccated coconut.

of the total lipids or the neutral lipids fractions was comparable to that of coconut oil^{2,3}. Desiccation did not alter the fatty acid make-up of neutral lipids.

The polar lipid fractions of both desiccated and freeze-dried coconut were rich in unsaturated fatty acids, particularly in arachidonic acid. Whereas in the total lipids and in the neutral lipids fraction, the unsaturated fatty acids amounted to only 10 per cent, in the polar lipids, they amounted to 35–45 per cent. Linolenic acid which was not detectable in either the total lipids or the neutral lipids fraction was present to the extent of 3 per cent in the polar lipids. The ratio of saturated to unsaturated fatty acids of 55/45 in freeze-dried coconut changed to 64/35 on desiccation indicating considerable destruction of the unsaturated fatty acids. The loss was greatest in arachidonate changing from 33 per cent in the freeze-dried to 21 per cent in the desiccated sample. This considerable decrease in unsaturated fatty acids during even the mild conditions of desiccation is evidently indicative of their oxidation. Although the quantity of phospholipids and total polar lipids in coconut is indeed small, it is well known that even ppb concentrations of degradation products of fatty acids impart rancid flavour to food products¹⁰. This would explain why the storage behaviour of desiccated coconut and foods incorporating it may not be interpretable on the basis of the fatty acid composition of coconut oil.

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Parameters Affecting the Viscosity of Chitosan from Prawn Waste

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A process is described for the preparation of highly viscous chitosan solution from prawn waste. The process involves demineralization of the material with 5% HCl at room temperature, followed by deproteinization with 5% NaOH at 100°C for 30 min. Finally deacetylation is effected with 60% (w/w) KOH solution 1:65 parts at 100°C for 1 hr. Bleaching of the material affects the viscosity of chitosan.

Chitosan (2-amino-2-deoxy-D glucose)_n, a deacetylated product of chitin, finds wide application in textile and paper industries as sizing and adhesive material. Earlier work¹ on preparation of chitosan shows that 55 per cent (w/w) KOH was used for deacetylating the chitin at 100°C. Demineralization of the material was effected with 10 per cent HCl² for 2-3 days and deproteinisation

was carried out with 10 per cent (w/v) NaOH at 103–105°C. In another process, first deproteinization was done by boiling with aqueous NaOH solution for 30 min followed by bleaching with 0.5 per cent H₂O₂ overnight and then it was demineralized with dilute HCl (5-15 per cent) overnight; chitin was deacetylated with 50 per cent (w/w) NaOH and alcohol in the ratio 1:1 and

the maximum viscosity claimed was 1.78 poise³. In a recent work⁴, 0.5 to 3 per cent NaOH has been used for deproteinization followed by bleaching for 30 min with 0.5 per cent hypochlorite solution and then demineralizing it with 1.25 N HCl for one hour; deacetylation of chitin was effected by using 50 per cent (w/w) NaOH for 2 hr at 100°C.

The viscosity of chitosan solution is an important factor. There are many parameters affecting the viscosity of chitosan such as the choice of alkali, its concentration, volume used for deacetylation, concentration of HCl and time of demineralisation, the stage at which the material is bleached, etc. A study has been made therefore to prepare chitosan under different conditions of processing and their effect on viscosity.

Materials and Methods

Prawn waste in fresh condition was obtained either from *Penaeus indicus* or *Metapenaeus dobsoni* variety or the waste from both the varieties.

Unless otherwise stated, 5 per cent HCl was used for demineralization of prawn waste for 1 hr at room temperature (20–22°C).

Deproteinization of prawn waste was effected with 5 per cent NaOH (w/v) for 30 min at 100°C (open steam.) Bleaching was effected by immersing the material with 0.5 per cent H₂O₂ overnight. Deacetylation was effected by treating chitin with NaOH or KOH (as described in the text) for one hour at 100°C (open steam.) Chitosan (1 g) was dissolved in 100 ml of 1 or 2 per cent acetic acid (w/v) or 0.1 M sodium acetate buffer of pH 4.5.

Viscosity was determined by Ostwald technique

$$\text{Relative viscosity} = \frac{d_1 t_1}{t_2}$$

Where, d_1 = density of chitosan solution,

t_1 = time of flow for chitosan solution,

t_2 = time of flow for same volume of water.

Absolute viscosity = relative viscosity × 0.00895 poises where 0.00895 is the absolute viscosity of water at 25°C.

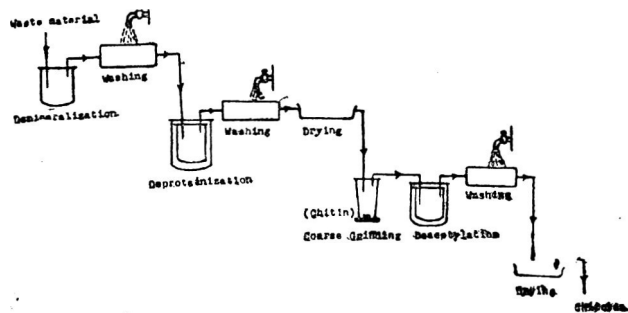


Fig. 1. Flow sheet for processing of chitosan

Results and Discussion

Table 1 shows that as the period of demineralization prolongs, the viscosity of final product gets decreased and also demineralization with 1.57 N (5 per cent) HCl gives better values of viscosity than that treated with 1.25 N HCl.

The results in Table 2 show that the sequence in which the product is demineralised, deproteinized, bleached and deacetylated plays a considerable part in affecting the viscosity of chitosan. Bleaching after deacetylation considerably reduces the viscosity of the product (Table 3). It is desirable not to bleach the material at any stage as it affects the viscosity of chitosan. Chitin deacetylated (Table 4) with KOH of optimum strength and proportion gives a highly viscous solution of chitosan as compared to deacetylation with NaOH. For dissolving chitosan, 2 per cent (w/v) acetic acid is better than other solvents mentioned (Table 5) to get highly viscous solution. Chitosan of 3.42 poises absolute viscosity is obtained

TABLE 1. EFFECT OF TIME AND CONCENTRATION OF HCL USED FOR DEMINERALIZATION OF PRAWN WASTE

Demineralization time (min)	Ash in chitin (%)	Rel. viscosity	Abs. viscosity* (Poises)
HCl conc. 1.57N (5% HCl)			
60	1.92	151.5	1.356
120	1.397	148.3	1.326
180	0.3147	140.5	1.257
HCl conc. 1.25 N			
60	5.667	139.5	1.248

1% Chitosan in 2% w/v acetic acid;

*Chitin bleached after demineralisation, deacetylated with 60% (w/w) NaOH

TABLE 2. EFFECT OF SEQUENCE OF DEMINERALIZATION AND DEPROTEINIZATION

Sequence of process ^a	Rel. viscosity	Abs. viscosity* (poises)
1. Demineralization ↓ Bleaching ↓ Deproteinization	151.5	1.356
2. Deproteinization ↓ Demineralization ↓ Bleaching	114.5	1.024

^a = Demineralization with 5% HCl for 1 hr and chitin deacetylated with 60% (w/w) NaOH

* 1% Chitosan in 2% (w/v) acetic acid.

TABLE 3. EFFECT ON VISCOSITY OF CHITOSAN WHEN BLEACHING IS CARRIED OUT AT DIFFERENT STAGES

Bleaching stage	Rel. viscosity	Abs. viscosity* (poises)
After demineralization	151.500	1.3560
After deproteinization	98.220	0.8790
After deacetylation	1.354	0.0121
Unbleached	226.1	2.0230

a = The sequence of the process is demineralization (with 5% HCl for 1 hr), deproteinization and deacetylation (with 60% (w/w) NaOH

*1% Chitosan in 2% (w/v) acetic acid.

TABLE 4. EFFECT OF DEACETYLATED CHITIN WITH POTASSIUM HYDROXIDE AND SODIUM HYDROXIDE

Chitin : Alkali	Alkali concentn (w/w)	Rel. viscosity	Abs. viscosity* (poises)
1 : 60	60%KOH	Chitosan not fully dissolved in acetic acid	
1 : 65	"	344.8	3.086
1 : 70	"	309.4	2.769
1 : 100	"	381.5	3.414
1 : 60	60%NaOH	226.1	2.023

Note: The material is not bleached at any stage

*1% Chitosan in 2% (w/v) acetic acid.

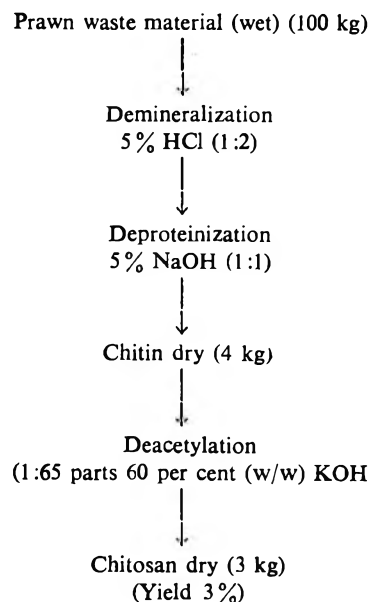
TABLE 5. EFFECT OF CONCENTRATION OF ACETIC ACID AND SODIUM ACETATE BUFFER USED FOR DISSOLUTION OF CHITOSAN

Solvent	Rel. viscosity	Abs. viscosity of 1% chitosan* (poises)
2% (w/v) acetic acid	151.5	1.356
1% (w/v) acetic acid	107.0	0.9579
0.1 M sodium acetate buffer pH 4.5	90.28	0.8079

*Chitin deacetylated with 60% (w/w) NaOH

if the ratio of chitin to KOH solution is 1:100 while viscosity of 3.09 poises is obtained if the ratio of chitin to KOH solution is 1:65. The above values of viscosity for chitosan are higher than the viscosity of 1.78 poise reported earlier.

Accordingly, the following process is suggested for the preparation of highly viscous solution of chitosan.



Fresh prawn waste material is washed with water and kept immersed in 5 per cent HCl (1.57N) (2 lit for 1 kg material) for one hour at room temperature. As material floats on the top due to evolution of CO₂, some weight is applied to ensure complete contact with the acid. The demineralized material is then thoroughly washed free from acid and it is then deproteinized with 5 per cent NaOH for 30 min at 100°C (steam). After washing the material free from alkali, it is dried and coarsely ground. Chitin thus obtained is deacetylated with 60 per cent (w/w) potassium hydroxide solution (1:65 parts) at 100°C for one hour, thoroughly washed to free it from alkali and perfectly dried. One per cent solution of chitosan, thus obtained, is prepared in 2 per cent (w/v) acetic acid by stirring vigorously to get a highly viscous solution. The solution can be passed through a mull cloth to separate any suspended material. The yield of chitosan is 3 to 3.5 per cent of the starting material.

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Studies on the Chemical Composition of Market Samples of Shrikhand

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A study on the chemical composition of market samples of *Shrikhand* collected from fifteen different manufacturers of four different cities of Gujarat was made. The product exhibited the following ranges in percentage of respective constituents: moisture 34.48 to 35.66, total solids 64.34 to 65.52, fat 1.93 to 5.56, protein 5.33 to 6.13, reducing sugar 1.56 to 2.18, and non-reducing sugar 52.55 to 53.76. Statistically a greater uniformity was observed in the composition of the product from the manufacturers of two cities.

Shrikhand is a fermented milk product, mainly popular in Gujarat, Maharashtra and part of Karnataka States. The product has a distinct taste, richness, delicacy, diversity and a fairly long shelf life. Scanty information is available on the composition aspects of the market samples of this product. A need, therefore, was felt to analytically survey the commercial samples and to determine the extent of variations as influenced by manufacturers, lots and cities.

The chemical composition of the laboratory made chakka, the raw material for *Shrikhand* making, was studied by Ganguly *et al*¹ while the effects of the levels of fat and sugar on the organoleptic quality of laboratory made *Shrikhand* were investigated by Puntambekar². He also suggested the modified Gerber test for estimating the fat content of *Shrikhand*. Bhattacharya *et al*.³ conducted the preliminary laboratory trials for the manufacture of *Shrikhand* and its powder.

Materials and Methods

Samples (45) of plain market *Shrikhand* were collected from 15 different manufacturers from Gujarat, namely, Ahmedabad, Anand, Nadiad and Vododara and duplicate samples were analysed.

The moisture and total solids of the product were gravimetrically determined by using the method of ISI prescribed for milk⁴. The fat content of the product was determined by using Gerber fat test as modified by Puntambekar².

The protein content was estimated by Kjeldahl method as prescribed for proteins in milk⁴, whereas the sugars (reducing and nonreducing) were determined by using the method of ISI prescribed for use with ice-cream mix⁵.

The average values of the duplicate samples were subjected to statistical analysis⁶.

Results and Discussion

Moisture and total solids: The results of moisture and total solids presented in Table 1 and 2 respectively reveal that the product of different cities did not differ significantly whereas significant variation was observed among the manufacturers and different lots of the product collected for the above two components. The manufacturers and the lots of cities 2 and 4 were statistically uniform and the same was also true for the lots collected from city 1.

From the above results, it is evident that even in the absence of a scientifically standardized method of preparing *Shrikhand*, some manufacturers could make the product with a fairly constant moisture and total solids content.

The average values for moisture and total solids of the market samples ranged from 34.48 to 35.66 per cent and from 64.34 to 65.52 per cent respectively. The higher range of total solids observed in the market *Shrikhand* as compared to the range selected by Bhattacharya *et al*³, in their trials on the laboratory made product could be attributed to the high rate of addition of sugar observed.

Fat: The results presented in Table 3 reveal that the cities and the manufacturers differed significantly for the fat content of *Shrikhand*, but no significant variation was observed in the fat content of different lots of *Shrikhand* ignoring the cities. The product of cities 2 and 4 was found to be uniform statistically as regards to the fat content, both for manufacturers and lots.

A comparison of the observed levels of fat in the market samples could not be made in the absence of any available compositional standards for the product. Compared with the levels of fat, 4.8 and 12 per cent used by Puntambekar² and levels of fat, 6 to 8 per cent used by Bhattacharya *et al*³ in their laboratory trials,

TABLE 1. MOISTURE CONTENT OF SHRIKHAND IN PER CENT

		Cities			
		1	2	3	4
Manufacturers	1	33.48	32.44	33.30	34.80
	2	35.72	34.33	38.33	34.21
	3	38.46	35.87	36.59	34.45
	4	31.09	37.57	34.44	—
Lots	1	35.06	35.86	35.75	33.20
	2	35.46	36.44	37.33	36.37
	3	33.54	32.84	33.91	33.88
Mean for cities		34.69	35.05	35.66	34.48
S. Em	Manufacturers	0.804	1.005	0.522	1.247
	Lots	0.698	0.870	0.452	1.247
C. D.	Manufacturers	2.782	ns	1.806	ns
	Lots	ns	ns	1.564	ns
C. V.		4.02	4.08	0.80	6.26
		Pooled			
		S. Em		C. D.	
Among cities 1, 2 and 3		0.450		ns	
City 4 vs cities 1, 2 & 3		0.485		ns	
Manufacturers/city		0.900		2.639	
Lots/city		0.780		2.287	

Mean for cities = 34.97

C.D. is at 5% level; C.V. = 4.45

TABLE 2. TOTAL SOLIDS CONTENT OF SHRIKHAND IN PER CENT

		Cities			
		1	2	3	4
Manufacturers	1	66.52	67.56	66.70	65.20
	2	64.28	65.67	61.67	65.79
	3	61.54	64.13	63.41	65.55
	4	68.91	62.43	65.56	—
Lots	1	64.94	64.14	64.25	66.80
	2	64.54	63.56	62.67	63.63
	3	66.46	67.16	66.09	66.12
Mean for cities		65.31	64.95	64.34	65.52
S. Em.	Manufacturers	0.804	1.005	0.522	1.247
	Lots	0.698	0.870	0.452	1.247
C. D.	Manufacturers	2.782	ns	1.806	ns
	Lots	ns	ns	1.564	ns
C. V.		2.13	2.66	1.40	3.29
		Pooled			
		S. Em		C.D.	
Among cities 1, 2 and 3		0.450		ns	
City 4 vs cities 1, 2 and 3		0.485		ns	
Manufacturers/city		0.900		2.639	
Lots/city		0.780		2.287	

Mean value (cities) = 65.03

C.D. is at 5% level; C.V. = 2.39

TABLE 3. FAT CONTENT OF SHRIKHAND IN PER CENT

		Cities			
		1	2	3	4
Manufacturers	1	4.08	6.60	0.23	5.25
	2	1.92	4.46	2.38	6.67
	3	3.17	1.85	0.58	4.75
	4	5.84	2.19	4.54	—
Lots	1	3.34	3.27	1.94	6.46
	2	3.44	3.44	1.85	4.88
	3	4.44	4.63	2.00	5.34
Mean for cities		3.75	3.78	1.93	5.56
S. Em	Manufacturers	0.294	1.218	0.863	0.652
	Lots	0.255	1.055	0.748	0.652
C. D.	Manufacturers	1.017	ns	2.986	ns
	Lots	0.882	ns	ns	ns
C. V.		13.60	56.20	7.71	20.35
		Pooled			
		S. Em		C. D.	
Among cities 1, 2 and 3		0.363		1.064	
City 4 vs cities 1, 2 and 3		0.391		1.147	
Manufacturers/City		0.726		2.129	
Lots/City		0.628		ns	

Mean for cities = 3.75

C.D. is at 5% level; C.V. = 34.62

TABLE 4. PROTEIN CONTENT OF SHRIKHAND IN PER CENT

		Cities			
		1	2	3	4
Manufacturers	1	5.03	5.10	6.28	5.19
	2	6.85	5.53	5.62	5.15
	3	5.14	5.06	6.25	5.91
	4	7.00	5.62	6.39	—
Lots	1	5.97	5.30	6.15	5.38
	2	5.88	5.47	6.18	5.54
	3	6.17	5.22	6.07	5.33
Mean for cities		6.00	5.33	6.13	5.41
S. Em	Manufacturers	0.222	0.249	0.293	0.409
	Lots	0.192	0.216	0.254	0.409
C. D.	Manufacturers	0.768	ns	ns	ns
	Lots	ns	ns	ns	ns
C. V.		6.41	8.12	8.29	13.06
		Pooled			
		S. Em		C. D.	
Among cities 1, 2 and 3		0.140		0.410	
City 4 vs cities 1, 2 and 3		0.151		0.493	
Manufacturers/City		0.280		0.821	
Lots/City		0.242		ns	

Mean for cities = 5.72

C.D. is at 5% level; C.V. = 8.85

TABLE 5. LACTOSE CONTENT OF SHRIKHAND IN PER CENT

		Cities			
		1	2	3	4
Manufacturers	1	2.38	2.64	2.73	1.37
	2	1.70	1.77	1.68	1.85
	3	2.13	1.90	1.53	1.46
	4	2.53	2.02	2.35	—
Lots	1	2.16	2.24	1.72	1.50
	2	2.56	1.84	1.68	1.61
	3	1.83	2.17	2.82	1.57
Mean for cities		2.18	2.08	2.07	1.56
S. Em	Manufacturers	0.252	0.201	0.366	0.155
	Lots	0.218	0.174	0.317	0.155
C. D.	Manufacturers	ns	ns	ns	ns
	Lots	ns	ns	ns	ns
C. V.		20.32	16.73	30.63	17.20
		Pooled			
		S. Em		C. D.	
Among cities 1, 2 and 3		0.132		0.387	
City 4 vs cities 1, 2 and 3		0.142		0.416	
Manufacturers/City		0.263		0.771	
Lots/City		0.278		0.815	
Mean value (cities) = 1.97					
C.D. is at 5% level; C.V. = 22.46					

TABLE 6. PER CENT SUCROSE CONTENT OF SHRIKHAND

		Cities			
		1	2	3	4
Manufacturers	1	54.54	52.81	56.96	53.01
	2	53.39	53.41	51.57	51.70
	3	50.51	54.89	54.72	52.94
	4	53.10	52.05	51.80	—
Lots	1	52.95	52.92	53.97	52.84
	2	52.14	52.33	52.51	51.30
	3	53.56	54.62	54.79	53.51
Mean for cities		52.88	53.29	53.76	52.55
S. Em	Manufacturers	0.934	0.791	0.947	0.586
	Lots	0.808	0.685	0.820	0.586
C. D.	Manufacturers	ns	ns	3.277	ns
	Lots	ns	ns	ns	ns
C. V.		3.59	2.55	3.06	1.92
		Pooled			
		S. Em		C. D.	
Among cities 1, 2 and 3		0.422		ns	
City 4 vs cities 1, 2 and 3		0.456		ns	
Manufacturers/City		0.845		2.478	
Lots/City		0.732		ns	
Mean value (cities) = 53.12					
C.D. is at 5% level; C.V. = 2.82					

the range of fat content observed in the market samples was 1.93 to 5.56 per cent.

Fat being the costliest milk constituent, its lower levels observed in the market product could easily be understood by the profit making attitude of the manufacturers.

Protein: The data presented in Table 4 reveal that the protein content of the product from different cities varied significantly. Similarly, the product from different manufacturers also differed significantly, ignoring the cities. Within cities, the protein content of the product did not vary significantly both for lots and manufacturers except in case of manufacturers of city 1.

The range of protein in the product was observed to be 5.33 to 6.13 per cent. In the absence of any standards for comparison for the protein content of the product, it is not possible to critically evaluate the samples. However, it is definitely felt that the protein content of *Shrikhand* can increase beyond the levels found during the study, provided the comparatively much higher amount of sugar added in the product is reduced.

Reducing sugar (lactose): Pooled analysis of the data presented in Table 5 reveal that the reducing sugar content of *Shrikhand* collected from different manufacturers, lots and cities varied significantly. However, within individual cities the variation was not significant both for manufacturers and lots.

The range for the reducing sugar observed in the product was 1.56 to 2.18 per cent, which is very near to the reducing sugar content of other acidified and drained products such as chhana and cheese.

Non reducing sugar (sucrose): The results presented in Table 6 reveal that the nonreducing sugar content of

Shrikhand did not differ significantly for cities and lots. The product from different manufacturers showed significant variation for this component. Within cities there were no significant differences, both for manufacturers and lots except the product from one city.

The average nonreducing sugar content of the samples analysed ranged from 52.55 to 53.76 per cent, which was very near to the upper levels, of the sugar used by Puntambekar² in his trials. However, it was quite higher as compared to the levels of 18 to 20 per cent used by Bhattacharya *et al*³, this may probably be due to the local consumer preference, the high acidity of the chakka used and also the profit making attitude of the manufacturers.

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RESEARCH NOTES

PRELIMINARY OBSERVATIONS ON THE SURVIVAL OF SALMONELLA IN CURRY, SAMBAR, CORIANDER AND RED-CHILLY POWDERS

Microbiological examination of curry, sambar, coriander and red-chilly powders inoculated with a type culture of *Salmonella typhimurium*, and stored at 37°C and R. T., revealed that *Salmonella* diminished in curry, sambar coriander and red-chilly powders. In coriander powder, *Salmonella* survived longer. In the initial stages of storage, destruction of *Salmonella* was slightly accelerated at 37°C.

The most important problem in food poisoning is the *Salmonella* outbreak.¹ The most virulent type of *Salmonella* causing food poisoning is *Salmonella typhimurium*,² several food infections being traced to it.³ Vegetable products other than desiccated coconut⁴ are rarely carriers of salmonella. *Salmonella* was absent in processed chappathies and parotas.⁵ Bactericidal activity was observed when *S. typhimurium* was inoculated into freshly reconstituted dehydrated onion and garlic powders⁶. *Salmonella* was absent on black-pepper and other spices^{7,8}. Several physiological and chemical characteristics of growth medium seem to affect the growth and virulence of *Salmonella* on foods^{9,10}.

The object of the study reported here was to examine the survival of *Salmonella* in certain spices.

Cells of type culture of *S. typhimurium* (KASAULT) grown on brain-heart infusion broth (DIFCO) at 37°C for 48 hr were harvested by centrifugation and resuspended in the same broth. Curry, sambar, coriander and red-chilly powders (initially free from *Salmonella*) were aseptically infected with the above culture preparation at levels of 10⁷ to 10¹⁰ cells/g (viable count) with a glass atomiser. The experimental materials were packed in heat-sealed polythene bags (300 gauge) and stored at 37°C and R.T. The rate of survival of *Salmonella* was examined at weekly intervals as per standard procedures.¹¹ The presumptive presence of *Salmonella* was confirmed by positive reaction of selected typical colonies (on bismuth sulfite agar) on Gilles and lysine decarboxylase media¹². The rates of survival of *Salmonella* of infected spices are given in Fig. 1.

It is seen from the figure that in curry powder, from an initial load of 7 log numbers, the *Salmonella* population gradually declined during storage (37°C and R.T.) and absent in 42 days. In sambar powder, the initial load (9 log numbers) became absent in 49 (37°C) and 56 (R.T.) days, respectively. *Salmonella* population in coriander powder from a load of 10 log numbers declined to 5 (37°C) and 5.5 (R.T.), log numbers respectively, in 70 days.

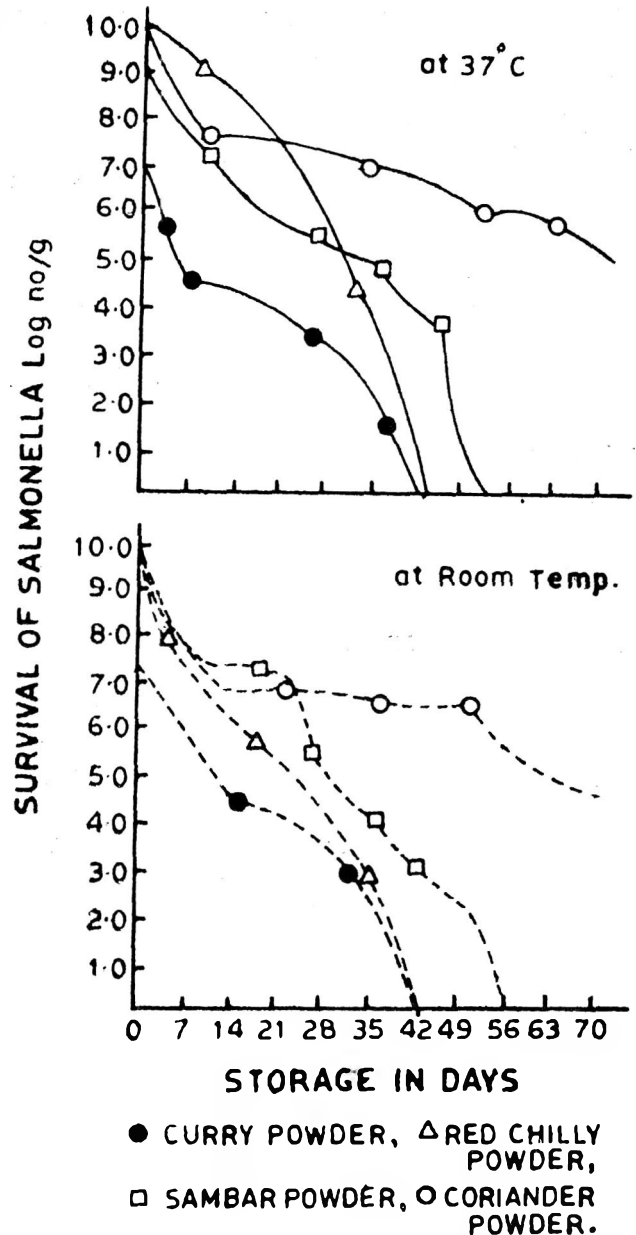


Fig. 1. Survival of *Salmonella typhimurium* in spices

The initial load (10 log, numbers) of *Salmonella* population in red chilly powder was destroyed completely in 42 days of storage. It will be seen from the results that the rate of destruction of *Salmonella* in the early stages of storage at 37°C was slightly higher than at R.T. in all the spices. It is also observed that in coriander powder, *Salmonella* survived longer than in other spices examined.

The growth and virulence of *S. typhimurium* in stored foods vary with factors like temperature, pH, etc. It has been observed¹⁰ that virulence of *S. typhimurium* increased during growth in an acid medium whereas in near alkaline medium, the virulence decreased. The pH of the spices studied were near neutral without significant

variations during storage, causing reduction of growth of the test organism. The growth and virulence of *S. typhimurium* in different foods under identical conditions may markedly vary¹³. This preliminary study has shown that spices may not be suitable substrates for the growth of *S. typhimurium*.

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COMPARATIVE EFFICIENCY OF TRIPHENYL TETRAZOLIUM CHLORIDE REDUCTION TEST FOR GRADING OF MILK

Triphenyl tetrazolium chloride reduction test for milk has been found as reliable as other conventional tests for grading of raw market milk.

Bacteriological testing of milk is one of the several uses of 2, 3, 5 triphenyl tetrazolium chloride (TTC).¹ It has been described as an alternative to the methylene blue reduction test^{2,3}. Mustakillio *et al.*⁴, have reported that reduction of TTC in milk with 4×10^6 bacteria/ml or less became visible earlier than the reduction of methylene blue. Usefulness of TTC in the

indirect determination of the number of bacteria has also been noted⁵. In the present work an attempt has been made to assess the applicability of triphenyl tetrazolium chloride reduction test (TTCRT) in grading of raw market milk by comparing it with other conventional tests like standard plate count (SPC), methylene blue reduction test (MBRT) and resazurin reduction test (RRT).

A total of 50 milk samples were analysed during summer (April to June) and winter (November to January) months. Fifteen milk samples each of buffaloes and cows milk in summer and 10 samples each of buffaloes and cows in winter were processed. These samples originated from different sources within Hissar city representing various stages of milk production. Samples were chilled and stored at 4°C and processed within 2 to 4 hr for TTCRT, MBRT, RRT and SPC.

The test procedures and grading for SPC and MBRT were as per ISI⁶ methods whereas the test procedure and grading for RRT was similar to MBRT. TTCRT was conducted as per Schonberg⁵ and grading was done as per ISI⁶ for MBRT. The red formazan appeared within 30 min to 9 hr depending upon bacterial concentration of milk.

Grading of milk through various tests (Table 1) indicated that TTCRT has a tendency to "overgrade" the raw milks of buffaloes and cows i.e. poor quality of milk was graded as fair more frequently than other reduction tests. However, SPC also showed a slight tendency to overgrade fair quality of buffalo milk as good and good quality cow milk as very good. The classification of good quality milk as fair or *vice versa* does not reflect a serious problem either to consumer or producer because fair quality of milk is also accepted for processing in milk industry⁷. Grading of good or fair quality milk as poor concerns producers (Producer's risk) whereas *vice versa* concerns to consumer (consumer's risk).

TABLE 1. GRADING OF COW AND BUFFALO MILKS THROUGH VARIOUS TESTS

Tests	Very good	Good	Fair	Poor
Cow milk				
MBRT	1	4	12	8
RRT	—	3	11	11
TTCRT	3	8	12	2
SPC	7	4	8	6
Buffalo milk				
MBRT	3	4	9	9
RRT	3	3	10	9
TTCRT	8	4	13	—
SPC	9	12	1	3

TABLE 2. COMPARATIVE EFFICIENCY OF VARIOUS TESTS USED FOR GRADING MILK SAMPLES

Season	Cow milk				Buffalo milk			
	MBRT	RRT	TTCRT	SPC	MBRT	RRT	TTCRT	SPC
Summer	7/15	4/15	13/15	13/15	6/15	5/15	15/15	14/15
Winter	10/10	10/10	10/10	6/10	10/10	10/10	10/10	10/10

Figures in numerator indicate number of samples accepted for human consumption; denominator figures indicate total number of samples examined.

The comparative efficiency of various tests employed for milk grading is shown in Table 2. All reduction tests exhibited similar efficiency during winter months when bacterial counts were low but TTCRT appeared to yield better results than MBRT and RRT during summer months when bacterial count in milk increased and milk grading became significant from consumer's view. SPC possessed same efficiency as that of TTCRT but latter might be substituted for SPC because of its simplicity.

The present investigation suggests that TTCRT can be adopted for evaluating bacterial quality of milk. This is in conformity with the earlier report of Laxminarayana and Iya⁸. It has added advantage over other reduction tests that the formazan produced is stable in atmospheric oxygen where as methylene blue may be reoxidized and resorufin, a product in RRT, is reversible to dihydroresorufin.

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PREDICTION OF TIME OF HYDRATION DURING PARBOILING OF PADDY FROM ACTIVATION ENERGY

This note emphasises the importance of accuracy of activation energy in the prediction of soaking time for parboiling of paddy using published reports on soaking of some Indian paddy varieties. It has also been pointed out that the Arrhenius line for the soaking process has distinct break for most of the varieties already reported in the literature.

Some comprehensive studies reported by earlier workers^{1,2} on hydration characteristics of paddy during parboiling indicate that water uptake of paddy can be related to time and temperature of soaking by diffusion equation as follows:

$$\bar{x} - x_o = \frac{2}{\sqrt{\pi}} (x_s - x_o) \left(\frac{S}{V} \right) \sqrt{D_m \theta + x_t} \quad \dots(1)$$

$$\bar{x} - x_o = k_m \sqrt{\theta + x_t} \quad \dots(2)$$

$$k_m = k \sqrt{e^{-E/RT}} \quad \dots(3)$$

where, $k_m = \frac{2}{\sqrt{\pi}} (x_s - x_o) \left(\frac{S}{V} \right) \sqrt{D_m}$

$$k = \frac{2}{\sqrt{\pi}} (x_m - x_o) \left(\frac{S}{V} \right) \sqrt{D_o}$$

- where, x_o = initial, uniform moisture content, g/g, dry basis.
- \bar{x} = average moisture content at given hydration time, g/g, dry basis.
- x_s = effective surface moisture content at the bounding surface at times, greater than zero, g/g, dry basis.
- x_t = moisture content corresponding to initial hydration, g/g, dry basis.
- θ = time, sec.
- $\left(\frac{S}{V} \right)$ = surface to volume ratio of paddy
- D_m = diffusion coefficient, cm²/sec.
- D_o = diffusion constant (in Arrhenius equation), cm²/sec.
- E = energy of activation, cal/mole.
- H = gas constant, cal/mol. °K.
- T = absolute temperature, °K.
- k_m, k = constants.

To test the validity of the above equations for soaking of some Indian paddy varieties and to predict their soaking characteristics the authors of the said references plotted the values of log k_m , obtained from experimental data on soaking, against reciprocal of absolute temperature and calculated activation energy from the Arrhenius lines for all the varieties. While reanalysing the data of the said references, the present authors noted clear breaks in the Arrhenius lines at about 75°C, except for the 'Patnai' and 'Aus' varieties, in the data reported by Bandopadhyay and Ghose¹ and for the 'Patnai' variety in the data reported by Biswas and Ghose² as shown in Fig. 1, 2 and 3. This peculiarity of the Arrhenius plot was not reported in either of the publications.

Moreover, the values of activation energy calculated from the above figures have been found to be much

TABLE 1. COMPARATIVE VALUES OF ACTIVATION ENERGY

Paddy variety	Activation energy, cal/g. mole					Reported in Ref. 2
	Calculated using data of Ref. 1		Reported in Ref. 1	Calculated using data of Ref. 2		
	Temp. range°C			Temp. range°C		
	65 - 75	75 - 80		65 - 75	75 - 85	
Sitalal	14,300	28,030*	11,150	—	—	—
Jhingasal	—	—	—	11,600	22,000	12,480
Rupsal	17,450	27,200*	10,630	15,100	23,200	11,860
Patnai	17,150**	17,150	9,630	19,300**	19,300	9,956
Aus	17,450**	17,450	9,125	16,400	23,100	9,940

*Activation energies calculated may be unreliable, as the Arrhenius lines were drawn between two points only.

**There were no break in the lines of these varieties.

TABLE 2. PREDICTION OF SOAKING TIME

(A) Variety : Sitalal

Desired hydration: $(x) = 0.41$ at 70°C ; $(x_0) = 0.118$; $(x_f) = 0.046$

	Energy of activation, (E) cal/g. mole	(K) g/g (sec.) ^{1/2}	Soaking time	
			Experimental	Predicted
Values reported in Ref. 1	11,150	5,820	4 hr	0.0225 sec.
Values recalculated from data of Ref. 1	14,300	77.62	—	3.5 hr.

(B) Variety : Jhingasal

Desired hydration: $(x) = 0.55$ at 80°C ; $(x_0) = 0.032$; $(x_f) = 0.046$

	Energy of activation (E) cal/g. mole	(K) g/g (sec.) ^{1/2}	Soaking time	
			Experimental	Predicted
Values reported in Ref. 2	12,480	89.35	3 hr	0.32 hr
Values recalculated from data of Ref. 2	22,000	2.291×10^4	—	3.7 hr

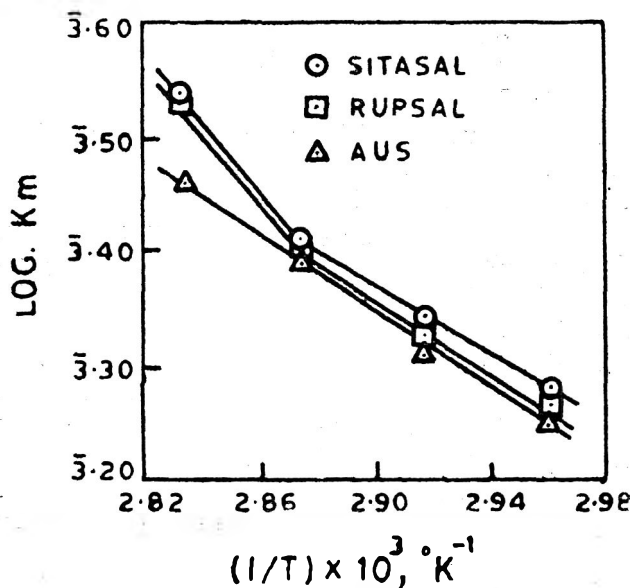


Fig. 1. Arrhenius plot for the temperature range 65-80°C

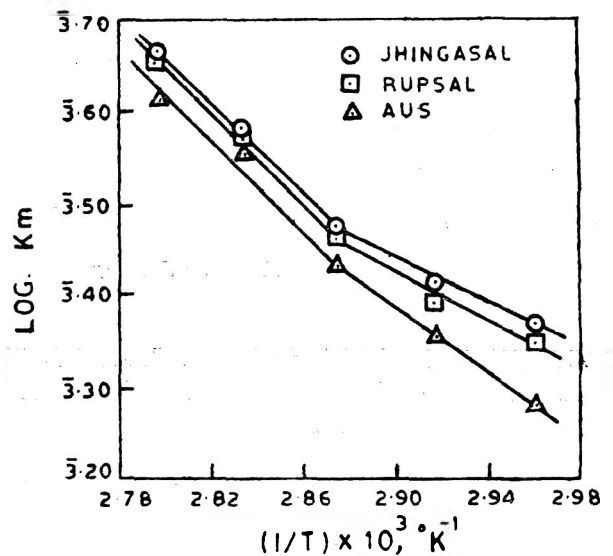


Fig. 2. Arrhenius plot for the temperature range 65-85°C

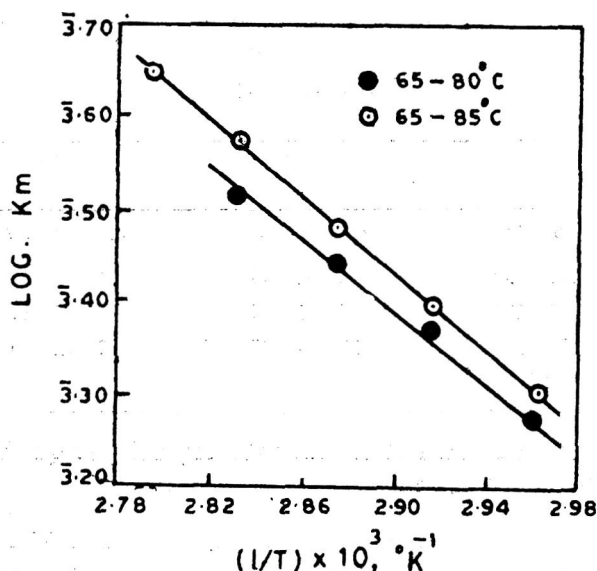


Fig. 3. Arrhenius plot for the Patnai variety

different from the values reported earlier. A comparative picture is presented in Table 1.

From the values of activation energy it is clearly evident that even if mean straight lines would have been drawn considering all the points, the values would not conform to those reported in the references.

For prediction of time of hydration (soaking time) from equations(2) and (3), it has been found also that values of activation energy reported in the references give absurd results, whereas with recalculated values time of hydration becomes fairly predictable, as shown in Table 2.

It may be concluded that above diffusion equations give fair prediction of hydration if only the activation energy is accurate enough.

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STUDIES ON KEEPING QUALITY OF COMMERCIAL MALT EXTRACT

Studies on the keeping quality of commercial malt extract showed that while the untreated malt had a microbial contamination, the number of microorganisms were 60×10^3 , 74×10^3 and 50×10^3 with pH 3.2, 3.6 and 4.0. Sodium bisulphite when used as a source of sulfur dioxide at 50, 100 and 150 ppm, helped in decreasing the number of microorganisms in the treated malt extract stored over a period of 6 months.

A considerable portion of the malt produced from barley and other grains by various malting industries is utilized for the preparation of malt-extract. Because of its poor keeping quality, most often, it undergoes spoilage. Hence preservation of malt extract under suitable conditions deserves attention. The spoilage is brought about by microbial contamination. There are quite a few of the bacterial genera known to grow in malt extract, e.g. gram positive genera are *Lactobacillus* and *Pediococcus* and gram negative genera are *Aerobacter*, *Acetobacter*, *Acetomonas* and *Zymomonas*. As early as 1922, Hampshire¹ reported that acetic acid bacteria may also produce a dextranous 'rope' in substantial quantities in beer. Frequently, acetic acid bacteria grow as greasy pellicle in order to increase the exposure to atmospheric oxygen. Vaughn² observed that the growing vinegar bacteria are responsible for the development of sour taste, off flavour and spoilage. Many wild strains of yeast e.g. *S. cerevisiae* and *S. carlsbergensis* are also known to cause off flavour, fermentation of dextrin and turbidity in beers. Gjersten *et al.*³ showed that in moist growing seasons barley may become infected with *Fusarium* spores that grow during steeping process, producing substances which cause gashing in beer.

A number of treatments to check the bacterial growth in various brewing processes have been adopted. Dupuy⁴ found pH to be the most important factor influencing the growth of *Acetobacter*. The use of sulphur dioxide and pH^{5,6} in the control of spoilage organisms have been stressed. Rainbow⁷ observed the growth of foetid bacteria occurring on the husks during steeping and these can be eliminated in part by changing the steep liquor frequently. Mold growth during steeping is reduced by raising the pH of steep liquor with lime. Since long a generalized treatment of SO₂ as an antimicrobial agent has been in use. The most common sources of SO₂ nowadays is potassium metabisulphite or sodium metabisulphite. Amerine⁸ reported that SO₂ in malt extract exists as sulphurous acid, bisulphite, sulphite ions and some as free SO₂ gas.

Thus, in the present investigation, the effect of different concentrations of SO₂ at various pH levels on the microbial growth of commercial malt has been assessed. The keeping quality of malt was also studied under these

TABLE 1. EFFECT OF SODIUM METABISULPHITE TREATMENT ON MICROBIAL POPULATION OF COMMERCIAL MALT

SO ₂ ppm	Microbial counts		
	pH 3.2	pH 3.6	pH 4.0
50	50×10^3	39×10^3	27×10^3
100	20×10^3	31×10^3	30×10^3
150	7×10	2×10	15×10

(Average of 4 readings)

TABLE 2. EFFECT OF SODIUM METABISULPHITE TREATMENT ON MICROBIAL POPULATION IN THE COMMERCIAL MALT AFTER STORAGE

Microbial counts ($\times 10$) on indicated days and pH

SO ₂ ppm	Days											
	15			30			90			120		
	pH											
	3.2	3.6	4.0	3.2	3.6	4.0	3.2	3.6	4.0	3.2	3.6	4.0
50	65	108	225	58	140	190	12	31	43	4	5	11
100	52	104	155	42	92	135	15	51	45	2	4	35
150	45	100	125	44	88	118	22	24	27	2	12	10

(Average of 4 readings)

treatments over a period of 6 months. Malt extract was obtained from Malt Extract Co. India (Pvt.) Ltd., Gurgaon, Haryana. The pH of original sample was 5.0 which was lowered by addition of calculated amount of tartaric acid. The storage effect was observed at room temperature over a period of 6 months using sodium metabisulphite as a source of SO₂ at 50, 100 and 150 ppm.

Table 1 shows the effect of SO₂ concentration at three pH levels on microbial population. It was observed that the sample whose pH was 3.6 had least number of micro-organisms i.e. 20 at 150 ppm concentration of SO₂ as compared to 70 and 150 at pH 3.2 and 4.0 respectively. Moreover, the reduction in microbial population was also maximum at pH 3.6 i.e. from 74×10^3 to 20 with increasing concentration of sodium metabisulphite, while at pH 3.2 the reduction was from 60×10^3 to 70 and at pH 4.0, 53×10^3 to 150. This Table also shows that pH 3.2 is more effective as compared to pH 4.0. Both the pH as well as concentration of SO₂ are important in order to control bacterial population of malt extract. When the effect of various concentrations of sodium metabisulphite on storage was seen (Table 2), it was found that the number of micro-organisms decreased in all the three concentrations of sodium metabisulphite at three different pHs under trial over a period of 6 months. Most effective concentration was 100 ppm at both pH 3.2 as well as pH 3.6. At pH 4.0 the cell counts were relatively high at all the three concentrations of sodium metabisulphite. The present study thus suggested that the malt extract can be protected from bacterial contamination at low pH ranging from 3.2 to 3.6 and using 100 ppm of sodium metabisulphite.

The present study thus recommends one more factor other than pH, recommended by Dupuy⁴ namely, the concentration of sodium metabisulphite in the control of bacterial growth during storage of malt extract. The decrease in the number of micro-organisms with storage may possibly be due to the slow effect of sodium metabisulphite which becomes conspicuous in course of time. This in turn increases the shelf-life of malt extract. It

would be of interest to study the organoleptic quality of malt thus treated.

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RESIDUES OF CARBARYL IN RIDGE GOURD (*LUFACUTANGULA ROXB.*: CUCURBITACEA)

Residues of carbaryl were estimated on ridge gourd (*Lufacutangula Roxb*) by both spectrophotometric and enzymatic assay. The residues of carbaryl dissipated by about 94% in 6 days and below detectable level after 14 days of application. However, it reached below tolerance level of 3 ppm after 6 days of application.

Experiments conducted at this University have shown that three applications of carbaryl, first as 5 per cent dust at the rate of 0.75 kg a.i./ha and second and third as suspensions 50 per cent w.p. at the rate of 1.0 kg a.i./ha at two weeks interval starting at the stage when plants were 4-6 weeks old, were most effective out of the five insecticidal schedules tried against pumpkin beetles (*Aulacophora* sp.) and fruitfly (*Dacus cucurbitae*) pests of cucurbits¹. Investigations have now been undertaken on the extent of carbaryl residues in/on ridge gourd.

TABLE 1. RESIDUES OF 0.1 PER CENT CARBARYL SUSPENSION IN/ON RIDGE GOURD

Days after treat.	Spectrophotometric method			Enzymatic method		
	Residue (ppm)	Av. (ppm)	Reduction (%)	Residue (ppm)	Av. (ppm)	Reduction (%)
0	33.67	27.95	—	33.97	27.50	—
	28.74			26.86		
	21.45			21.66		
2	10.12	8.02	71.31	12.13	9.05	67.09
	8.64			8.95		
	5.30			6.07		
4	9.18	7.03	74.85	11.27	8.05	70.73
	1.29			7.22		
	4.62			5.66		
6	2.12	1.61	94.25	2.02	1.62	94.10
	1.44			1.54		
	1.26			1.30		
11	0.41	0.39	98.61	0.51	0.42	98.48
	0.41			0.41		
	0.34			0.33		
14	BDL	BDL	100.00	BDL	BDL	100.00
	„			„		
	„			„		

BDL - Below detectable level; The half life was 2.62 days and coefficient of correlation was 0.998

Seeds of ridge gourd var. 'Pusa nasdar' were sown at the rate of 5 kg a.i./ha at a distance of 50 cm on both sides of 1m wide sub-irrigation channel which divided the subplot of 7m × 4m into two halves, on July 1974 at the Horticultural Farm of the Rajasthan College of Agriculture. Three applications of carbaryl were given as indicated above. The edible fruits (10-15 cm long) were harvested at definite intervals and were chopped to small pieces. A 50 g sample was taken and extracted by blending with 150 ml distilled methylene chloride. The extract was filtered through a filter paper Whatman No. 1 and was analysed by both spectrophotometric and enzymatic methods. The spectrophotometric method² used is based on the hydrolysis of carbaryl by KOH which on reaction with p-nitrobenzene diazonium fluoroborate in alkaline medium produces yellow colour. This can be measured at 477 m μ . The method of Zweig and Archer³ based on the inhibition of cholinesterase was used for determining microquantities of carbaryl. The recoveries of carbaryl by spectrophotometric and enzymatic methods were 86.09 and 87.48 per cent respectively.

Perusal of results in Table 1 indicate that there is a high degree of correlation (0.998) between both the methods used and the carbaryl deposit of about 27.5 ppm on ridge gourd dissipated by about 94 per cent in 6 days and below detectable level after 14 days of application. Dewan *et al.*⁴ reported nil residues of carbaryl on marketable bhendi fruits on 4th day. The carbaryl residues on tomato were lost completely in 11-15 days⁵.

The half life obtained was about 2.6 days on ridge gourd. Similar half lives of 2.91 days on cabbage head⁶ and 3.71 days on tomato⁵ were also reported. If this

was taken as an index of rate of dissipation, the carbaryl deposit would take about 8 days to reach the tolerance level of 3 ppm⁷. This assumption has been corroborated in the present study and the carbaryl residues have reached below tolerance level after 6 days of application. Thyagarajan and Khan⁸ also suggested a lapse of 5 and 3 days for tomato and brinjal respectively before they could be consumed safely.

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CHEMICAL QUALITY OF MARKET ICE CREAM

A survey of the chemical quality of ice cream available in the market was made. Of the 46 samples analysed for percentages of fat, acidity, total solids, protein and sucrose as well as weight/litre only 12 samples satisfied the prescribed standards of all parameters. Failure to conform to the prescribed acidity was greatest and total solids was the least.

Ice cream occupies unique position among the milk products consumed in our country. Excepting the well established manufacturers, the majority of ice cream manufacturers do not seem to maintain the required hygienic quality during the time of preparation, storage and distribution, which poses a health hazard to the consumers; besides the standard chemical quality as prescribed by the PFA Act¹ and the ISI² is not strictly maintained. In order to study these aspects, a survey of the chemical quality of ice cream available in Bangalore City was undertaken.

Ice cream samples available in the city from different sources as supplied by organised manufacturers and their distributors, and those made by individual concerns like hotels and restaurants, were collected in collaboration with the Dairy Bacteriology Section of the Institute. These samples were tested for their chemical quality as prescribed by ISI and PFA regulations. The factors determined were weight/litre, fat, acidity as lactic acid, total solids, sucrose and protein. Methods of

analysis prescribed by ISI were followed in carrying out these determinations.

Forty six samples of ice cream collected over a period of one year from various sources available in Bangalore City were analysed to assess their chemical quality. A few of the typical analytical results presented in Table 1 represent samples conforming to the standards and those which fail in one or more parameters. The results were analysed statistically to obtain the mean, standard error and coefficient of variation of each parameter and compared with PFA Act and ISI standards.

It is found that out of the 46 samples, only twelve samples satisfied the prescribed standards with respect to all the parameters, whereas the number of samples which were substandard in one, two, three and four of the six parameters were 12, 13, 5 and 4 respectively. It is also observed that in the order of failing standards, the following percentages of failure were observed in the descending order; acidity (38.2), sucrose (37.0), weight/litre (30.4), protein (23.9), fat (19.4) and total solids (13.04). This indicates that most of the samples were satisfactory in the total solid content while acidity was the least satisfactory, the others being in the intermediate range. It is hoped that these findings would be helpful to both the manufacturers and consumers regarding the status of the quality of ice cream available in the market and also to impress upon the concerned enforcing authorities to exercise stricter quality control checks. This would help in making available quality ice cream in the market.

TABLE 1. ANALYTICAL RESULTS OF MARKET SAMPLES OF ICE CREAM

	Wt./lit (g)	Fat (%)	Acidity (% lactic)	Total solids (%)	Sucrose (%)	Protein (%)
ISI/PFA standards	Min. 525.0	Min. 10.0* Min. 8.0**	Max. 0.25	Min. 36.0	Max. 15.0	Min. 3.5
Type sample 1	625.0	5.5	0.27	35.84	23.50	2.50
„ 2	440.0	10.5	0.16	37.62	19.50	2.25
„ 3	830.0	18.5	0.40	42.45	19.58	5.18
„ 4	540.0	12.8	0.19	38.94	12.37	4.94
Maximum	830.0	18.5	0.40	44.75	23.50	7.74
Minimum	410.0	3.0	0.12	34.92	10.50	2.21
Mean	570.0	11.1	0.24	38.20	15.20	4.05
Standard deviation	±97.53	±2.7247	±0.0624	±2.4677	±2.9826	±1.0858
S.E. of mean	14.38	0.4017	0.0107	0.3638	0.4398	0.1601

*For plain; **for fruity.

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A PRELUDE TO PESTICIDE RESIDUES IN DIFFERENT DIAGNOSTIC SITUATIONS

The end point of toxicity of pesticides in human population cannot be regarded as safe. The gradual accumulation in minute quantities have some significance to the ensuing pathological situation in the long term. As the action of pesticide residues in the total body dynamics is not clear, there is need for definite criteria to be adopted in clinical diagnosis of population exposed to pesticides.

The pesticides revolution started in 1942 when DDT was first made available in the market. Since then an ever increasing number of synthetic organic chemicals in innumerable formulations have appeared all over the world. Intensification of facilities for scientific storage will demand use of more pesticides.

The pesticide initially added to the surface becomes residue as a result of weathering and metabolic degradations. The residue is often present in trace concentrations (ppm) which make their isolation, separation and analysis very difficult.

According to Marth¹ pesticide residues can be found wherever plant or animal life exists and the significance of trace residues in food supply over an extended period of time on human health is debatable. Pesticides have not caused any demonstrable illness, but there is a potential threat of accumulation of minute quantities of pesticides in human tissues. Fortunately, there is limitation to the transfer of accumulated pesticide from adult to the offspring; probably the metabolic changes during pregnancy accelerate the metabolism of pesticide.²

The route of absorption of pesticides into the human body is mainly through the gastrointestinal tract. But there is lack of definition of human exposure to pesticides; studies based on defined oral exposures have their limitations like the storage characteristics of individuals to pesticides in tissues and length of exposure⁴⁻⁶, and also the nutritional intake⁷.

The dietary intake of DDT of an adult in India is about 0.2664 mg/day⁸; the residue in adipose tissue ranges from 12.8 to 31 ppm⁹, which is high as compared to other developed countries¹⁰. The adipose tissue residue levels may be considered as the body burden in the light of reports³, assuming only one way transport of pesticides.

Pesticides have also scope to traverse to other tissues of the human body and has been located in spleen, bone-marrow and lung,¹¹ gall stones¹², in milk of lactating humans¹³, brain, liver and kidney¹⁴, human placenta, cord and blood²³. Casarett *et al.*¹¹ Randozski¹⁵ and de Vliger *et al.*¹⁴ have screened for pesticide residues in wide ranging age groups and in different diagnostic situations like carcinoma cases, myocardial infarction, cerebral thrombosis, sepsis, leukemia, nephritis,

local and generalized pathological conditions of liver. The pesticide residues found mostly are DDT, DDE, DDD, BHC, Lindane, and Dieldrin; the proportion of total DDT residues was higher. There was also a tendency for higher residue levels in adults, probably explaining the greater exposure period and the cumulative property of these organochlorine compounds in the human body. The preferential levels in some tissues perhaps is due to their fat solubility and lipophilic character too. Such preferential absorption in the brain tissues developed electroencephalogram abnormalities¹⁶. Pesticide exposures have also been incriminated in the renal tubular dysfunction, amino acidemia and amino aciduria¹⁷⁻¹⁸, hematologic abnormalities and associated effects²⁰.

The above points support the view that pesticide residues in the human body may be one of the contributory factors for various diseases apart from their beneficial aspect of controlling some disease vectors²¹. Further it is clear that the end point of pesticide toxicity as death cannot be regarded as safe. The gradual accumulation in minute quantities have some significance to the ensuing pathological state. It is also not clear at this stage, to explain the action of the pesticide residues in the total body dynamics. There is need for definite criteria to be adopted in the clinical diagnosis of population exposed to pesticides. Such an approach, may give additional support to diagnose diseases of unknown etiology and considered as enigma.

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BOOK REVIEWS

Storage of Cereal Grains and their Products: Edited by C. M. Christensen, American Association of Cereal Chemists Inc., St. Paul, Minnesota, USA, 1974, pp. 549.

This second revised and expanded edition of a very useful publication is most welcome to grain storage men, research scientists and everybody connected with agricultural produce in one way or the other. Three more additional chapters deal with chemical control of the stored grain insects and associated micro and macro organisms, air-tight storage and chilling. Chapters on respiration and heating, country storage and terminal elevator storage of the earlier edition have been omitted and chapters on aeration and grain drying and whole grain storage have been included.

Chapter I written by W. Hunt and S. W. Pixton deals all aspects concerning with grain moisture—its significance and measurement. Methods of determination of 'true moisture' by gas chromatography, vacuum, NMR, Karl Fisher Titration and Near-infrared absorption techniques are discussed.

Chapter II by Y. Pomeranz deals with the biochemical, functional and nutritive changes during storage. The subject is covered under seven headings. Measurement of respiration is described but no mention is made of gas chromatography as a tool for measuring respiration. The importance that moisture has on respiration of dormant grain is well brought out. Storability of grain can be predicted by making several tests. Whereas mold counts and viability tests are good indices of incipient deterioration, fat acidity and non-reducing sugar content are useful measures of the extent of the existing damage.

Chapter III by G. R. Baumin and H. L. Ryans deal with sampling infestation and grading of grain. It is a well illustrated and documented chapter. Accuracy and precision in applying the various parameters of inspection are emphasised. A combination of physical and chemical factors are to be employed to evaluate the quality of the constituents of grain (protein, starch, oil, minerals and vitamins).

Chapter IV by C.M. Christensen and H.H. Kaufmann is more or less an echo of what is written in the book entitled "Grain Storage—The Role of Fungi in Quality Loss" by the same authors, published by the University of Minnesota press in 1969.

Chapter V by R. T. Cotton and D. A. Wilbur is on insects. Insects damaging stored cereals and products are described and evaluated as to the damage they cause. Storage losses from insects in the U. S. averaged

\$ 471, 417,000 a year. The ecological conditions favouring insect life in stored produce are discussed.

The damage caused by insects has been well presented by way of examples from published work. 25 per cent of the weight of the wheat kernel was consumed by the rice weevil during its development to quote an example from Kansas University studies.

Under indirect insect damage are included topics on micro-organisms including toxigenic ones, consumer resistance to unsanitary products, insect fragments and excreta. A short account on insecticidal treatments and detection of insect infestation in grain and the methods used conclude this very informative and useful chapter.

P. K. Harein and E. D. L. Casas have dealt with chemical control of stored grain insects and associated micro and macro organisms in Chapter VI.

The review summarises the venue of insect control by chemicals with material on integrated control using the chemical methods.

'Prophylactic' measures using methoxychlor, pyrethrins combined with piperonyl butoxide or malathion are suggested as surface treatments. Inspection of grain by sieving at 30 day intervals is recommended along with spotting of abnormal odour, webbings or hot spots. Permissible grain protectants to be used mixed with grain are discussed. Only a few insecticides are approved for being mixed directly with grain the most important being malathion and pyrethrin. Inert dusts, however, have limitations in their usefulness. The insecticides discussed include Diazinon, Fenitrothion, Fenchlorphos, Bromodan Bromphos, Phoxin, Gardona and Lindane. Dichlorvos has received a wider coverage. The residual levels in treated grain are discussed but no mention is made of any rat-feeding experiments on Dichlorvos treated grain to prove its total safety in diets.

Under the caption of potential protectants are included sorbic acid, butylated hydroxyanisole and hydroxy toluene as ovicides to *T. confusum*. No mention is made of tricalcium phosphate as a protectant [Majumder & Bano, *Nature* (4939, 1359, 1964)] although it has shown promise as a safe protectant for flour and enriched foods.

Mention is made of sex pheromones, juvenile hormone analogues and some of the drawbacks in their use, as also the problem of resistance.

Under fumigant characteristics, methyl bromide, hydrogen cyanide, phosphine and dichlorvos are discussed separately. A paragraph on potential fumigants includes morpholine and acetate of dimethyl 2, 2-dichloro-1-hydroxyvinyl phosphonate, tribromopropane, ethylenimine and crotyl bromide.

Residues after fumigation are discussed in greater detail. Methyl bromide, ethylene dibromide, ethylene dichloride and aluminium phosphide are the fumigants that are discussed.

Maintaining fumigant concentrations by using gas-analyzer instruments, detection tubes, halide lamps and gas chromatography are also dealt in brief. Mention of the bio-assay method of monitoring fumigant concentration (Muthu *et al*, *Int. Pest Control*, **13**, 11, 1971) would have rounded off the review well, but has apparently escaped the attention of the authors.

Fumigation under tarpaulin is yet another important topic that has been discussed. Disinfestation in mills producing flour using pyrethrums, spot fumigation and fumigation of the entire mill are discussed. Dichlorvos is suggested as a potential vapor toxicant for controlling insects that infest packaged cereal products.

Treatment of packaging material with insecticides is also reviewed. Pyrethrin, methoxychlor, Lindane and malathion, carbaryl resmethrin, tetramethrin, d-trans-allethrin have protection for 12 months. Anti-feeding chemicals as laminates of packaging materials is suggested.

Fumigants like methyl bromide, hydrogen cyanide and phosphine are mentioned as being useful for empty containers. Methyl bromide however is still the fumigant of choice.

Sub chapter 9 on micro and macro pests includes the role of bacteria in infestation and attractancy of fungi for certain insects.

Insect control by pathogenic micro organisms heralded by *B. thuringiensis* is a field of insect control that needs more research. Mycotoxins from fungal infestations have also the possibility of being used against insects.

Control of microbes and their metabolites by chemical treatments including fumigation is discussed next. Control of mites forms the last section of this very illuminative chapter. Among fumigants mention is made of methyl bromide and phosphine but strangely enough no mention is made of ethylene dibromide which shows very high toxicity to storage mites (Muthu *et al*, *J. Stored Prod. Res.* **6**, 93, 1970).

The VIIIth Chapter is on Rodents by K. L. Harris. The importance of rodent control, its economics and assessment of the problem form the introductory section.

The author feels that the indigenous storage structures still have a useful role to play due to their many advantages like porosity that keeps grains dry and economy in construction. A careful watch kept to ward off rodents will prove easier than the 'alien' metal structures.

Different types of rodents are described with a chart showing the identification key. Among control measures are included sanitation, rodent-proofing, poisoning with bait formulations, fumigation, and trapping.

J. E. Bailey writes on Whole grain storage in Chapter VIII. Different types of storage facilities are mentioned. Under the title Structural Requirements of Storage. Bins are discussed silo bins and flat bins.

Bulk storage of flour forms Chapter IX by Donald E. Schaetzel. Important considerations that go towards careful and intelligent planning of bulk storage of flour are mentioned. These will help in providing guidelines for building bulk storage facilities.

Chapter X is on Airtight Storage by M. B. Hyde. In recent years airtight storage has been developed both to control insects in dry grain and to prevent mold growth in high moisture grain. Dry grain storage in pits, provides a simple form of storage. Lower temperature are found in pits than in above ground storages; an in advantage in tropics.

Roof should be waterproofed and painted to reflect light. Pits should be filled to capacity to reduce free air and thus amount of oxygen available to the insects in infested grain.

Airtight storage is a useful method of preserving dry grain but not for malting and for seed. At very low moisture contents (1-2 per cent lower than that normally recorded for safe storage) seed grain can be stored safely.

In high moisture grain, requirements of air-tightness are more stringent. Cold weather is more suitable for air-tight storage of moist grain. Several types of airtight storage structures including flexible butyl rubber silos are discussed. Artificial lowering of oxygen concentration is possible. Of nitrogen or carbon-dioxide used as lethal atmosphere for control of insects, carbon dioxide proved better. For tropical climates airtight storage will not be safe for grain of 13-14 per cent moisture content. In drier grains air-tight storage can be used. At 11-12 per cent moisture content the grain can still be used for seed. Air-tight storage is a simple method without the spectre of pollution following in its wake.

Chapter XI by N. J. Burrell is on Chilling. "Many of the biological and physical factors described in this chapter lead to the conclusion that the risks involved with grain chilling are appreciable and the benefit gained is doubtful".

Chapter XII is on Aeration by the same author. In cool and temperate climates proper aeration system can greatly reduce the danger of damage by insects, mites, and fungi.

Grain drying is covered by W. V. Hukill in Chapter XIII. The importance of the contact period of the drying environments in reducing the moisture content of grain. The time factor in farm drying is the real problem, which depends upon method, machinery, man-hours and the system of the entire farm operation into which drying has to fit-in.

The hazards of weather and climate can be removed with a properly thought-out plan of farm drying.

The last (XIV) chapter by C. A. Soutnick Jr. is on Packaging of Cereal Products. Packaging is now accepted as a vital and necessary part of storage, handling, display and preservation of goods in our present economy.

"Storage of Cereal Grains and their Products" is a most valuable compendium on the subject and the American Association of Cereal Chemists should be congratulated for bringing out this comprehensive volume at a time when it is most needed.

M. MUTHU

Energy Balance and Obesity in Man: by J. S. Garrow. Publishers: North Holland/American Elsevier. 1974. pp. 335 Price \$ 30.80.

The "battle of the bulge" has always been a losing battle for mankind and obesity takes its toll through the many coronary, renal, neural and other complications that eventuate from the main syndrome. The disbalance between the intake and output of energy appears to be the main basic cause of all forms of obesity. Simple obesity arises from an excessive intake of food calories in relation to output or requirement. Metabolic obesity is the result of reduced output of energy arising from genetic/hormonal factors. These basic causes are simple to enunciate, but it is difficult to achieve the energy balance by simple regulation of diet as the causative factors involved—genetic, hormonal and other regulatory factors—are not accessible for easy control.

The book under review by an experienced member of the Medical Research Council of Great Britain discusses in a refreshingly critical manner the various aspects of the problem and some of the possible solutions in 7 chapters dealing with the definitions and criteria of obesity, methods of measuring the intake and output of energy, factors affecting intake and output, energy stores in the body, their composition and measurement, and finally, the physiology of obesity. The most valuable parts of the book are those dealing with the methods of measuring intake and output of energy and assessing the degree of obesity. All the chapters of the book critically review the areas of work concerned and indicate the lacunae in our knowledge regarding the subject. The same applies to chapters dealing with the treatment and the results obtained so far using anorexic drugs and reducing diet formulations.

The book merits a high place in the libraries of institutions and individuals who are concerned with the biochemical, metabolic and nutritional aspects of obesity.

M. V. L. RAO

The Use of Mercury and Alternative Compounds as Seed Dressings: (Joint Report of a FAO/WHO Meeting); World Health Organization, Geneva, 27 Switzerland, 1974, pp. 29, Price: Sw. fr. 5. Available through WHO Regional Office, Indraprastha Estate, Ring Road, New Delhi-1.

The technical report contains the collective views of an international group of experts on the use of mercury or alternative chemical treatments to control the seed borne pathogens. The meeting was convened especially after a serious outbreak of organo-mercury poisoning due to human consumption of treated grains occurred in Iraq in late 1971 and early 1972 and also due to hexachlorobenzene which caused death and permanent disability in Turkey during 1955-59.

The Committee is of the view that the hazard is due to the misuse of the treated grain inspite of special warnings given on it such as labelling and coloring of the grain. It was observed that alkylmercury compounds, as seed dressings for grain has resulted in intoxication of seed eating birds and birds of prey, while this consequence has not been noted with alkoxyalkyl and aryl compounds. The meeting concluded that the use of mercury compounds as seed dressings has not played a significant part in the recycling of mercury in the environment as the agricultural use of mercury is about 3-5 per cent of total mercury used for all purposes in industrial countries.

The subject matter is covered in 8 Chapters: outbreaks of poisoning, agricultural needs, toxicological aspects, evaluation of the risks associated with the ingestion of fungicides on seed and recommendations for minimizing this risk, recommended safety measures, nonchemical methods of plant disease control and summary of recommendations.

The technical committee recommends that the alkyl mercury compounds should be used only for seed dressing purpose and never be permitted for the treatment of cereal seed to be exported for the production of food. All dressed seed to be exported for the production of food should be distinctly dyed, labelled and a bitter, nauseous substance may be added to make it unacceptable for use as food.

The book furnishes a good deal of information on the environmental pollution due to mercury compounds used for seed dressing purpose, toxicological hazards and the possible ways to minimize the risks. It will be of great value to agriculture departments, public health departments and other research organizations.

K. VISWESVARIAH

Interaction of Agriculture with Food Science: Proceedings of an Interdisciplinary Symposium, Singapore, 22-24 February 1974. Ed. by Reginald MacIntyre, Published by International Development Research Centre, Box 8500, Ottawa, Canada, pp. 166.

The publication embodies the proceedings of an interdisciplinary Symposium held under the joint auspices of the International Union of Food Science and Technology and the International Development Research Centre in Singapore, February 22-24, 1975. The Symposium arranged in 4 Sessions covers mainly contributions by various agricultural scientists and technologists from different regions of Asia.

The first two papers emphasise the critical food scarcity that has arisen in the world and in Asia in particular since 1972-73 and the causes for the same. The exhausting of reserves, onset of widespread drought conditions, meagre irrigation facilities, low soil fertility and unproductive land tenure systems, have been traced as the causes which both individually and jointly led to the difficult food situation. Denuded foreign exchange reserves led to difficulties in purchasing the required food from surplus regions. Even the stocks in the affluent countries reached the lowest levels. The strategy to meet the situation suggests increased production in each country through improved agricultural measures, wise land reform policies and building up regional buffer stocks in each country. Building up an internationally managed central reserve pool contributed by the surplus producing countries is also suggested.

The second Session dealing with crop improvement discusses in detail the programmes and achievements of the International Institutes on rice at the Philippines and the dry farm crops in Hyderabad. The unearthing of the high yielding IR. 8 parent variety which has been utilized for developing varieties coupling high yield and also grain quality is the practical achievement of the International Rice Research Institute. The details of the cultural practices, fertilizer use and plant protection measures have also been worked out. The major constraints to production in actual farmers' fields have been studied and developing improved genetic lines possessing high disease resistance or tolerance have been discussed. The objectives of the ICRISAT institute solely devoted to work on dry farm crops have been defined and the development of varieties with high grain yield, high lysine, high protein and good drought tolerance has been described.

Although it is generally known that the root crops produce much more food per acre than the cereal or legume crops, the paper by Dr. Wilson emphasises with

facts and figures the phenomenally high yields of food which can be expected from tuber crops particularly cassava. The difficult food problem posed for the future world may necessitate greater emphasis on producing the tuber crops and methods of improving their nutritional value by supplementation with other protein rich foods.

The third Session relates to nutritional improvement, quality control, processing and utilization of food crops. Breeding of high lysine maize, barley and Triticale having better essential amino acid balance which has been attempted at the International Centre at Mexico indicates an important approach towards improving overall nutritional quality of the food. Problems of harvesting, drying, storage, milling and processing have been dealt with by Dr. de Padua. What is true of rice applies equally to other food crops as some of these are more prone to deterioration and insect infestation than paddy. Nutritional supplementation of cassava diets and improvement of techniques for processing cassava have been well covered in a paper devoted entirely to this crop. A review on the important grain legume crops which serve as very important sources of protein in the developing countries is a welcome one. The details of production, amino acid balance, milling technology by wet and dry methods and cooking quality have been dealt with and the current status of research in different parts of the world summarised.

The last session reviews the status of the paddy and rice industry in Malaysia, development of dairy and cooperatives in India and the quality standards in food processing in Singapore. Experiences gained in one country could serve to help other neighbouring countries to learn from them.

As emphasised in the foreword, the participants are mostly from the different Asian countries who have knowledge of Asian problems and who have tried to apply modern science and technology for solving the problems of the region which are sometimes similar but may also be unique in certain situations. The experts from the developed countries emphasise that solutions to problems in the developing countries cannot be imposed by foreign experts but should come by devoted work of scientists and technologists from the region.

The book analyses the difficult food situation of the Asian continent and suggests both general and concrete approaches to face and solve these problems. It is only hoped that all concerned would rise to the occasion and produce more food available for the needy population.

Advances in Biochemical Engineering, Vol. 3: Edited by T. K. Ghose, A. Fiechter and N. Blakebrough. Springer Verlag, Berlin, Heidelberg, New York, Berlin, pp. 290, Price: \$ 31.90.

The book contains seven chapters by renowned scientists on various topics of Biochemical Engineering. Chapter One deals with genetic problems of Biosynthesis of tetracycline antibiotics. Systematic approach in selection of high production strains, isolation and characterization of mutants, genetic recombination and control of biosynthetic processes provides some of the basic guidelines for obtaining higher productivity of chemicals produced by microorganisms. Some aspects of basic genetic research on fungi and their practical implications are dealt with in chapter Two. Chapter Three deals with microbial oxidation of methane and methanol. Culture conditions, biochemistry of methane oxidation and biosynthesis of cell components are presented in this chapter. The potential applications of microbial oxidation of methane and methanol in single cell protein production, removal of methane from coal mines, petroleum prospecting and microbial fuel cell are briefly covered under this chapter. However, the cultivation of yeast cells on methanol having greater potential of acceptability as a SCP should have been included in this chapter. Chapter Four deals with modelling and simulations in biochemical engineering. Mathematical models on microbial growth, transient response of a plug-flow reactor, influence of mixing on the transient performance of continuous cultures, transient response of the turbidostat, enzymatic reaction with a porous support, tubular immobile enzyme reactor and control of activated sludge reactors with their computer simulations are nicely presented in this chapter. Understanding of transient and oscillatory behaviour in continuous culture systems provides a very important tool for design and efficient running of commercial systems as well as for the study of regulatory mechanisms in microorganisms. Extensive study on this vital subject of Biochemical Engineering including predictions from idealized models, transient responses of microbial cultures to perturbations of the steady state, and oscillatory phenomena in continuous culture of microorganisms are presented in chapter Five of this book. Chapter Six deals with the significance of microbial film in fermentor. Formation of microbial film, its general characteristics, control and kinetics are discussed in detail. Being relatively a new subject in the field of Biochemical Engineering, it offers a great future potential in persuing scale-up studies and design of large scale fermentors.

The last chapter of this book deals with the present state and perspectives of Biochemical Engineering and

its role in the development of potential fermentation processes.

Annual series on Advances in Biochemical Engineering have been covering important topics and are great assets to those who are involved in biological research and development work.

H. N. ASTHANA

Cooling and Ripening of Fruits in Relation to Quality: Refrigeration Science and Technology: Published by International Institute of Refrigeration, 177, Boulevard Male Sherbes, 75017, Paris, 1973, pp. 255; Price: £ 14.

This publication of the International Institute of Refrigeration is a useful compilation of twenty-nine papers read at the Meeting of Commission C 2, Food Science and Technology, held at Jerusalem between September 10–13, 1973. These papers were presented by various authors from 8 different countries on topics relating to (1) Control of ripening in fruits from the physiological and practical point of view, (2) new methods of controlled atmosphere storage and (3) influence of prestorage treatments and packaging on the quality of fruits. In the Plenary Session four papers were presented by four experts in different fields of specialization. The main points in the first four lectures in the Plenary Session were about controlling ripening of apples and pears from the physiological and practical point of view to enable the supplier to regulate the fruit supply to the consumer over wide time intervals. The necessity for expanding the capacity of cold chain of the world on a very large scale, cooling as well as freezing, was also emphasized. Controlled atmosphere storage of produce with its limitations was briefly discussed by giving reasons and requirements for successful controlled atmosphere storage. The last paper in this session on quality of produce as influenced by pre-storage treatments and packaging showed the need for cooperation from field to table among Biologists, Engineers, Economists and Businessmen.

Twenty five papers which were grouped into four sections dealt with the general theme of improvement of the quality of cold stored fruits. The first, second, third and fourth sections included 8, 5, 7 and 5 papers respectively.

In the first section, four papers dealt with pears and apples; one each with plum and avocado pear and the rest two with bananas. All these papers in general describe the effects of temperature, gaseous exchange, gas concentration, ethylene production and fruit maturity on the rate of ripening, quality and emanation of

volatile substances from fruits. In section two, the first paper described the extension of storage life of potatoes, apples, peaches, mushrooms, etc., by application of controlled atmosphere storage using gases other than CO₂ and O₂. The second paper described the disorder known as Brownheart in pears due to the influence of carbon dioxide content. The third and fourth papers reported the use of interferometers for CO₂ analysis and new types of exchange diffusers and diffusion wind bags for storage under controlled atmosphere. The fifth paper presented the studies on prolonging the storage life of strawberries by packaging in selected plastic films. In Section three, six papers reported the influence of different growth regulators, pre-cooling and stage of maturity on the ripening behaviour of peaches, melons, fragile fruits, persimmon and pears during storage and transport. The last paper reported the reduction of peel injury in citrus fruits due to ethylene dibromide by application of a fungicide, Thiabendazole. Section four included miscellaneous papers which reported the effect of coolers in precooling oranges (tight skinned) to 0-2.2°C for cold treatment against the Mediterranean fly; method to measure the electrical resistance of banana skin in the preclimacteric phase for detecting low temperature injury affecting ripening, and effects of different temperatures and relative humidity on the keeping quality of eggplants and grapefruits.

This publication entitled *Cooling and Ripening of Fruits in relation to quality* presents 25 interesting research papers which would serve as a guide and reference material to many research workers, fruit physiologists, growers, packers and traders in fruits and vegetables. The information contained therein is very useful for those who are connected with cold storage of fruits. This publication is worth possessing by every research worker engaged on storage of fruits and vegetables.

V. B. DALAL

Advances in Preconcentration and Dehydration of Foods:
edited by Arnold Spicer, Applied Science Publishers,
London-P/526: Price \$ 20.0

This book contains papers presented at an International Symposium held under the auspices of International Union of Food Science and Technology and sponsored by R.H.M. Ltd. The contents are classified under the following sections:

(1) Fundamentals; (2) non-membrane concentration; (3) membrane concentration; (4) spray drying; (5) freeze drying and other novel dehydration techniques.

The first section covers the history of food processing

industries and the basic mechanisms of concentration, dehydration process, recovery of essence in citrus evaporators and rheological aspects of fruit juice evaporation. In the second section, a novel method of freeze concentration and non-membrane concentration along with the concepts for the freeze concentration, processes and sucrose dehydration by the application of heat of crystallisation, has been discussed. The next section, membrane concentration—a recent innovation, a paper on tailored membrane by Alan S. Michaels, discusses at length the reverse osmosis membranes, polymers suitable for reverse osmosis membranes and ultra-filtration membranes, preparation and characteristics of asymmetric reverse osmosis and ultra-filtration membranes. Also discusses present status and future prospects of membrane preparation. Membrane concentration discusses in detail industrial application within the food and medical industries batch and continuous plants design, electricity consumption, cooling water requirements and cleaning solutions. Ultra-filtration for cheese production has also been discussed at length with various flow sheets and graphs. Industrial scale ultra-filtration and reverse osmosis plants in the food industries by Bernard S. Horton, discusses various large-scale industrial cheese and industrial plants in New Zealand and USA and confirms that ultra-filtration can be operated at 50°C with membrane life time of one year or more.

The section on spray drying deals with the effects of latest development on design and practice of spray drying with the relevant illustrations by courtesy of M/s Niro Automizers. Detailed discussions regarding aseptic and close cycle spray drying are also presented. Theoretical modelling of the drying behaviour of droplets in spray dryers deals with the motion of air and droplets in spray dryers, mass and heat transfer, transport properties of water and aroma in food, liquids, calculation of dry phenomena and drying behaviour of solutions. Novel and rather exciting developments regarding the manufacture of alcoholic powders like whisky powders, sake powder and wine powder and spray quoting in fluidized bed is dealt by T. Emada.

The last session deals with freeze drying and other novel dehydration techniques. The chapter micro-wave heating in vacuum drying discusses at length the latest concepts and summarises that products with much higher dry matter content than for freeze drying can be used.

The book has 55 tables and 125 illustrations. The book is a good addition to the libraries and research workers in the field of concentration and dehydration of foods.

M. M. KRISHNAIAH

Nestle Research News—1973: Published by Nestle Products Technical Assistance Co. Ltd., Technical Documentation Centre of Research & Development Department 1001, Lausanne—Switzerland.

“Modern pharmacology has demonstrated that minute amounts of certain drugs have the power to change completely the reaction patterns of animals and men and to adopt their behaviour to stress and strain. A new science has been born: Psychobiology—a challenging discipline.”

The Indian Ayurvedic system of medicine has recognised this affect for centuries. The foods have been classified as *satwik*, *thamasik* etc., The effects of these foods on mental activities have been described. It is indeed a very interesting development that the latest Nestle's Research News—1973 has thrown considerable light on this phenomena. This thin volume presented by Nestle Research contains a very interesting contribution by Daniel Bovet on the advent of psychobiology—a new approach to animal and human behavioural research. He has described the instinctive behaviour of several species and their ability to learn and develop intelligence. The studies on the critical periods of brain development in animals and in humans have been described. The next article by Prof. Jean Mauron, entitled “Food and the Mind”—is interesting from the point of view of the remarks made earlier. He has described research work which show that serotonin, a neuro transmitter in the brain, has tryptophan as a precursor. The effect of different types of food on the serotonin content has shown that it is not the high protein food like meat and fish relatively high in tryptophan, which induces a high serotonin content and consequently the alertness of the brain, but it is the cereal milk diet which does this. Though no general conclusion can be drawn from such an interesting observation, Prof. Mauron in passing mentions historically that the meat and fish diet was

always associated with the war loving nations and the cereal diet was associated with the thinkers.

Leathwood has presented interesting evidence regarding the much disputed question of early undernutrition and behaviour. He has described tests on animals and how they react to undernutrition in the early stages of life. Extrapolation from animal experiments to human situation are perilous. Still the experiments on animals are convincing enough for Dr. Leathwood to conclude that early nutritive deprivation in humans results in a slowly developed child which will be at a disadvantage both socially and educationally. ‘Education is linked to age rather than stage of development, Thus even a temporary delay in development would leave the child too far behind to catch up with his classmates.’

The Nestle Research Laboratories are doing excellent research work on many other problems besides what has been described. The rest of the volume gives summary of research papers published based on these investigations. Some of the papers are of very great interest to the Food Technologists. Finot and Mauron have investigated the damage to lysine by the Maillard reaction. Bauer has published a paper on the Milk Fat Globule Membranes and proposed a structural model of the Globule Envelope. Bracco and others have investigated the Composition of the Fat Globule Membrane of Human and Bovine Milk. There are two papers on Lactic Acid in Milk and one on the Microflora of Cheese. Papers are also included on flatulence factors in Legume seeds on the problems in the Detection and Determination of Aflatoxin, Determination of Copper by Atomic Absorption Spectroscopy.

The report is remarkable for the excellence of paper, printing, diagrams and presentation. It is a report which will stimulate interest in research workers in allied fields and a reference volume for every scientist in the field.

M. R. CHANDRASHEKHARA

NOTES AND NEWS

Neglected Food Sources

Under the auspicious of the *Karnataka Chemical Society*, Bangalore, Sri M.R. Chandrashekhara, Secretary, Association of Food Scientists and Technologists of India, Bangalore chapter gave a talk on 'Neglected Food Sources' of the country on 17th May 1975. The important aspects covered by him in his talk are as follows:

India is the largest producer of groundnut inspite of its lowest per hectare production. Mustard, sesame and rape seeds are other important oil seed crops of India. Oil seed cake is a very valuable food by-product for human consumption if it is properly handled, during crushing for oils. In spite of shortage in edible proteins this nutritive food is not properly utilised for human consumption. Much of it goes as cattle feed, manure and exported to other countries making it unavailable for human consumption. If properly and hygienically processed, the oil cake goes a long way in meeting national protein shortage.

CFTRI, Mysore, has developed technology required for production of protein rich foods, edible and palatable for human consumption, Aflatoxin from groundnut, gossypol from cottonseed and allylisothiocyanate from mustard could be freed and these oil cakes made edible and could be incorporated in multipurpose foods, high protein foods, infant foods and partially substituted milk beverages. The processed oilseed meals used in various food products will be readily acceptable by all age groups. The increased processing and use of oil cakes as human food, takes us a long way in overcoming nutritional deficiencies of vulnerable groups of our population.

Association of Foodscientists & Technologists. Revival of South Zonal Centre at Madras.

In response to the appeal of Honorary Executive Secretary Sri M. V. Sastry, Sri K. L. Radhakrishna, Chief Manager, Modern Bakeries India Limited, Adyar, Madras, Dr. R. N. Datta, Deputy Technical Advisor Department of Food, Government of India, Madras and Sri K. S. Krishnamurthy, Assistant Technical Advisor, Ministry of Food, Madras took the responsibility of reviving the South Zonal Centre at Madras. Dr. R. N. Datta, Convener, inaugurated the meeting on 27th March 1975 and Sri K. L. Radhakrishna presided.

Dr. Datta, in his introductory remark, stressed the need for providing a forum for the Food Scientists and Technologists, to discuss various problems in the fields of Food Science and Technology and Nutrition. He stressed the need for a multidisciplinary approach to

take the problem of malnutrition. Sri K. L. Radhakrishna expressed his satisfaction for the impressive turn-out of Food Scientists and Technologists for inaugural function. He appreciated the role of Dr. Datta, Sri K.S. Krishnamurthy and their colleagues to make the Session a good success. He traced the achievements of Central Food Technological Research Institute, Mysore in the various fields of Food Science and Technology. The help rendered by CFTRI in the dissemination of knowledge and support it is giving for our Association in playing its role at the national level was appreciated. An appeal was made to intensify the efforts to rally new members from the city belonging to Institutes, Government departments, Food Processing Industries, Public and Private sector undertakings dealing in food and nutrition. He referred to the communication from Honorary Executive Secretary AFST headquarters Mysore, indicating that Madras shall be the Regional Headquarters for the Southern Zone and the progress will be watched with great interest. He requested all assembled, to strive hard to sustain the growth of the Association.

Sri Radhakrishna read out the Rules, regulations and constitutional bye-laws of the Association.

An appeal was made by Sri Radhakrishna and Sri K. S. Krishnamurthy for liberal contribution to run the day to day business. There was a very good response from the members assembled for an appeal for funds to enable the organisation to function till such time that the official funds of 40 per cent of the membership is received from the Headquarters.

To start with a seminar was planned for 9th June 1975 by Sri M. Sreekrishna, Tamilnadu Dairy Development Corporation, on Some Aspects of Dairy Development. The meeting ended with a cordial note and vote of thanks from Secretary, Sri K. S. Krishnamurthy.

The following members were elected to the working committee.

1. Sri K. L. Radhakrishna, *President*
2. Sri S. Subramanyam, Director, Indian Standards Institution Madras, *Vice-President*
3. Sri K. S. Krishnamurthy, *Secretary*
4. Sri O. P. Gera, Deputy Director, Fruits Products Order Madras, *Treasurer*
5. Sri M. Sreekrishna/Manager (Planning) Tamilnadu Development Corporation Madras, *Member*
6. Sri Kannan, Britannia Biscuits, Madras, *Member*
7. Sri S. R. Shetty, Regional Officer, Indian Institute of Packaging, Madras, *Member*
8. Sri A. V. Kuppaswamy, Principal Catering College, Madras, *Member*

9. Miss M. A. Nalini, Prof. & Head of S.I.E.T. Home Science College Madras, *Member*
10. Sri Radhakrishna, Assistant Director Central Warehousing Corpn. Madras, *Member*
11. Sri R. N. Ramani, Sri Ganesh Ram & Co. Madras *Member*

Membership forms were circulated with a request to fill in and forwarded along with the subscription and registration fees to Sri K. S. Krishnamurthy, Secretary of the Association.

Seminar on Dairy Development

The South Regional Branch, Madras, held a seminar on "Recent Trends in Dairy Development" on 9th May '75. The main speaker was Mr. M. Sreekrishna, Manager (Planning) Tamilnadu Dairy Development Corporation, Madras. The salient points highlighted in the symposium are as follows:

Although India marks fourth, in respect of cattle population of the world, its milk production is as low as 5 per cent. Recently the situation is undergoing a metamorphosis for the better and a bright future is slowly emerging. However, some important hurdles of meeting the inadequacies of feed and fodder position, implementation of modern scientific developments to improve the milk production, better management of farms and attention to health of the animals, and eradication of apathy from rural breeding to the modern technological and scientific advancements are few to mention.

Dairy development is of top priority for developing nations in order to meet the chronic shortages in the per capita availability of protein from animal origin. It requires a concerted national effort to develop our own sources rather than looking to the so called surpluses of flourishing countries for their aid. A ten year programme has been launched to increase the milk yield by 50 percent by adopting better breeding and feeding the animals with nutritious fodders.

With the assistance of UNICEF, FAO and Colombo plan, sophisticated dairies and milk handling plants sprang up in many parts of the country. Already there seems to be a danger of these plants remaining idle unless the production centres are geared up. The ideal situation of production, handling and processing is well organised, for the first time, at Kaira District Co-operative Milk Producers Union in Gujarat State.

In Tamilnadu, emulating Kaira District Co-op. further improvements in collection, individual evaluation and on the spot payments for the suppliers are effected. There is a better mobilisation of farmers into co-operatives to the extent, that middle men are eliminated and private creameries started tapering down.

A scheme with Rs. 100 crores was afoot with FAO participation to bring four metropolitan cities of Bombay Delhi, Calcutta and Madras under its umbrella, finally to amalgamate them into a national milkgrid. A massive scheme to improve and distribute developed breeds is on the anvil. With the commissioning of Ambattur Dairy, 60 percent of the milk demand of Madras will be met soon. Bulk vending machines are going to be installed in order to overcome the high prices of bottles, containers and caps as well as to meet the water scarcity and electricity shutdown. The early teething troubles inherent in handling, processing, packing, marketing and distributing are to be solved by careful planning.

With an installation and introduction of sophisticated equipment as atomisers, Rail Tankers, Gaint Road Tankers, Pasturisation Plants India is in the forefront of dairy industry. This has been well recognised by the International Congress of Dairy Industry by choosing India as a venue for the 19th International Dairy Congress. Thus Indian Dairy Technologists, Scientists, Planners, Administrators and Farmers are straining every nerve to put India in a proud position of World Dairy Industry.

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x) *Editor—JFST*

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Sri S. T. Ramanujam, Plot No. 26, Ravi Colony, Palwells Road, Madras-600 016.

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Sri N. Sabapathy, Food & Nutrition Exten Officer, Community Canning and Preservation Centre, 11, Lady Doka College Rd., Madurai-625 002.

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Sri C. R. Seetharaman, Manager, (Marketing) 14 Mc. Nichols Road, Chetpur, Madras-600 031.

Sri N. Sithamurthy, Tamilnadu Industrial Investment Corporation, 26, Whites Road, Madras-600 014.

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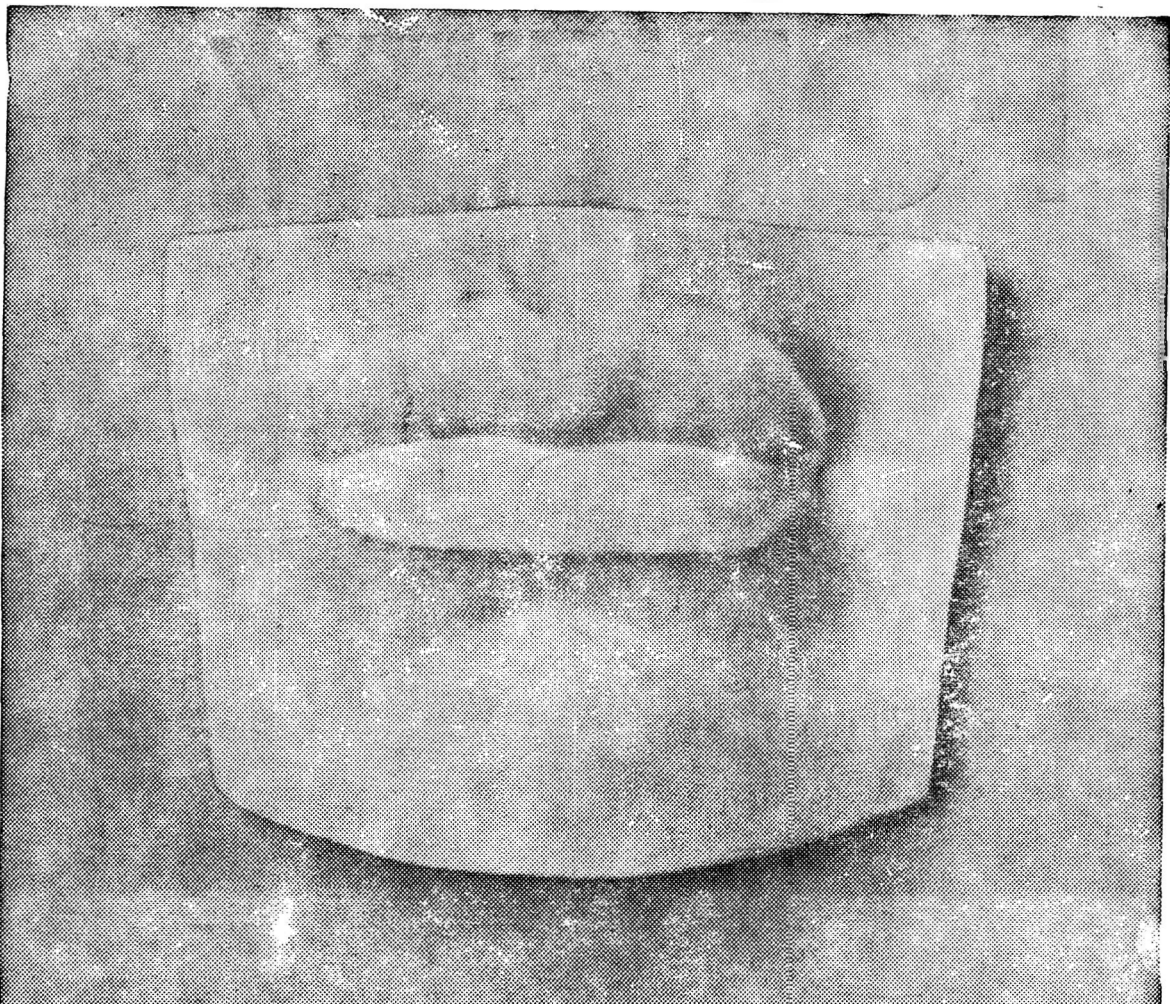


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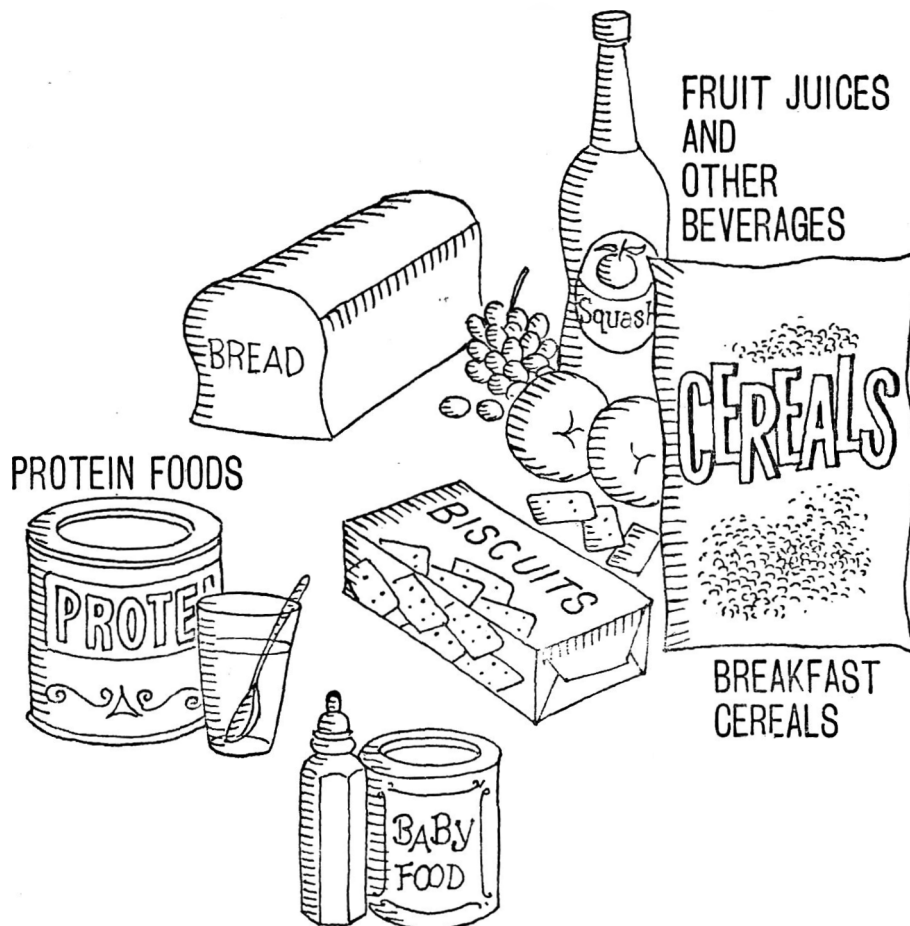
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2. Short communications in the nature of Research Notes should clearly indicate the scope of the investigation and the salient features of the results.
3. Names of chemical compounds and not their formulae should be used in the text. Superscript and subscripts should be legibly and carefully placed. Foot notes should be avoided as far as possible.
4. **Abstract:** The abstract should indicate the scope of the work and the principal findings of the paper. It should not normally exceed 200 words. It should be in such a form that abstracting periodicals can readily use it.
5. **Tables:** Graphs as well as tables, 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. Nil results should be indicated and distinguished clearly from absence of data.
6. **Illustrations:** Line drawings should be made with *Indian ink* on white drawing paper preferably art paper. The lettering should be in pencil. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size. Photographs must be on glossy paper and contrasty; *two 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 should be cited completely in each reference. Abbreviations such as *et al.*, should be avoided.

In the text, the references should be included at the end of the article in serial order.

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

- (a) *Research Paper:* Menon, G. and Das, R. P., J. sci. industr. Res., 1958, **18**, 561.
- (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:* As in (c).
- (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.

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