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A Small Scale Process for Milling of Wheat. Part I. Development of the Process

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For the first time, a simple small scale wheat milling process, using indigenously available low cost machinery has been developed, to help the small or cottage scale and rural bakeries for meeting their bakery flour requirement. The process consists of cleaning of wheat mechanically or manually, conditioning with addition of water, removal of bran (5-10%) by polishing in a huller and grinding of polished wheat in a *chakki* (disc mill) under standardised conditions, sieving on a mechanical sifter having 40, 60, 80 and 100 mesh sieves to separate bread flour (30-35%) or biscuit flour (30-40%), passing through 100 mesh and regrinding of the remaining portion for obtaining *roti/chapati atta* (50-65%). The process has also been employed for durum semolina milling with some variation in the degree of grinding in *chakki* and using sieves of 18, 30, 50 and 80 mesh, whereby semolina (55-60%) of different fineness and *roti/chapati atta* (30-40%) could be obtained.

Of the total wheat produced in the country, the roller flour milling industry processes hardly 10 per cent, though its capacity is about 6 million tonnes per annum[1,2]. Setting aside nearly 10 per cent for seed purposes and other uses, about 80 per cent of the wheat produced is processed in *chakkis* (disc mills) for obtaining *atta*, which is used mainly for the preparation of *chapati* or *roti*. It is estimated that about 3 lakh *chakkis* are operating in rural regions covering 2.76 lakhs of electrified villages and also in towns and cities. Many of these *chakkis* are functioning under the same roof, along with hullers (about 80,000), mainly used for dehusking of paddy or polishing of brown rice. Majority of these hullers are being rendered out of use, due to the ban imposed by many of the State Governments under the programme for modernisation of rice mills. As such, there exists considerable scope for harnessing the hullers fruitfully for other uses.

The baking industry today, consisting of both commercial and small scale units is entirely dependent for its requirements of *maida* (refined wheat flour) on the roller flour mills situated mainly in urban areas. The consumption of bakery products in recent years has shown considerable increase-about 13 per cent per annum in case of bread and 9 per cent in case of biscuits. According to the recent policy of the Government, the future growth of the bakery industry is restricted only to the small scale sector. As such, in the coming years, there is considerable scope for setting up of small and cottage scale bakery units in the country. However, the smaller bakery units are confronted with the problem of non-availability of *maida* of desired quality at controlled price.

The minimum capacity of an economically viable roller flour mill is about 50 tonnes per day which needs a capital investment of more than Rs. 50 lakhs. As such, the need for a small scale simple wheat milling process was never felt so acutely than in the present situation for meeting the daily flour requirements of a few quintals of the small and cottage scale bakery units, especially of those situated in the remote rural regions. This paper presents the salient features of a simple small scale wheat milling process developed for obtaining simultaneously bread/biscuit flour and *roti/chapati atta* or durum semolina and *atta*.

Materials and Methods

Raw materials: For different experimental trials, *aestivum* wheats generally considered suitable for the preparation of bread, biscuits, cakes, wafers and chapati and *durum* wheats suitable for semolina milling were procured from local market.

Development of the simple milling process: The milling process developed and standardised for *aestivum* wheat is mainly based on three main operations; debranning of the cleaned grain in a huller by polishing, grinding of the polished wheat in a *chakki* under controlled conditions and sieving the ground material on a mechanical sifter.

The process developed, has also been successfully

used for processing of durum wheat into semolina suitable for the preparation of vermicelli, sweet and savory snack items and *atta* for *chapati* or *roti*.

Polishing of wheat: McGill Polisher: Samples weighing 700 g of cleaned wheat were conditioned to different moisture levels of 10-20 per cent and polished after resting for 5-15 min in a laboratory McGill polisher using loads ranging from 2 to 10 lb. (Table 1). The polishing time required in relation to degree of polish obtained were recorded in a series of laboratory experiments.

Corcoran barley pearler: 200 g samples of different cleaned wheats were conditioned to moisture levels of 10-20 per cent, rested for periods ranging from 5-15 min, pearled for 1-5 min in a laboratory barley pearler for obtaining wheat grain polished to varying degrees (Table 2).

Huller No. 3: Since hullers are used for polishing rice, their suitability for polishing of wheat grain was explored. Preliminary trials using 5-10 kg wheat were carried out in huller No 3, using different conditions, such as addition of water (3-6 per cent), period of rest (5-15 min.), huller screens of different types and sizes of apertures and chemical treatments by using separately salt and chalk at 0.1-1.0 per cent levels.

Huller No. 1: Huller No. 1 was tried to increase the capacity of polishing of wheat upto one quintal (100 kg) per hour. The wheat used for different trials was cleaned manually or mechanically[3]. After several trials, optimum conditions were arrived at by trying different levels of water addition (2-6 per cent), resting periods (5-15 min), for removing 5-10 per cent bran in a single pass and about 10-12 per cent in double pass.

Grinding in chakki: Debranned (5-10 per cent) wheat grain was fed to a *chakki* consisting of two corrugated metallic discs-one stationary and the other revolving. The grain was ground to varying particle sizes by a simple standardised sieving test. Semolina of desired particle size was obtained in a similar way.

Sieving on a mechanical sifter: A mechanical sifter accommodating suitable meshes with 5 separate outlets was employed for getting flours or semolina. The optimum mesh sizes of different sieves for flour or semolina were arrived at after preliminary trials. *Atta* was obtained after regrinding of overtailings of all the sieves. In case of semolina milling, the coarse fraction (overtailings of 18 and 30 mesh) collected after separation of semolina was reground and mixed with flour passing through 80 mesh to obtain *atta*.

TABLE 1. EFFECT OF LOAD* AND MOISTURE** ON THE DEGREE OF POLISHING OF WHEAT IN MCGILL POLISHER

Trial No.	Load (lb)	Time (min)	Degree of Polish (%)	Polishing efficacy	Moisture (%)	Degree of polish (%)
1	2	3.0	4.6	Poor	10	10.0
2	2	3.0	4.3		12	10.8
3	5	3.5	15.7		14	12.1
4	5	4.0	16.3	consistent	16	13.0
5	5	4.0	16.0	but incomplete	18	14.0
6	7	2.0	12.1	Unsatisfactory	20***	14.1
7	7	3.0	21.3	due to grain		
8	10	1.5	8.1	damage		
9	10	2.0	28.0			

* Grain moisture—18%

** 700 g sample, 5 lb load, 5 min rest period, 3 min running time

*** No further increase in degree of polishing was observed.

Results and Discussion

Polishing of Wheat: McGill Polisher: Based on different laboratory trials conducted for polishing of wheat with minimum loss of endosperm, a maximum of 14 per cent bran could be removed under the standardised conditions: moisture level used for grain conditioning- 18 per cent, rest period-15 min, load

TABLE 2. EFFECT OF MOISTURE AND TIME ON THE POLISHING OF WHEAT* IN CORCORAN BARLEY PEARLER

Trial No.	Grain moisture** (%)	Degree of polish (%)	Polishing efficacy	Time*** (min)	Degree of polish (%)	Polishing efficacy
1	10	4.1	Underpolishing	1.0	5.0	Underpolishing
2	12	6.8	Incomplete and unsatisfactory	2.5	7.5	-do-
3	14	9.3	-do-	3.0	12.5	Good
4	16	12.7	Good	4.0	15.5	Grain damaged
5	18	12.6	Good but tendency for caking	5.5	19.0	-do-
6	20	12.8	-do-	—	—	

*Batch size: 200 g and resting time; 10 min; **Polishing time: 3 min; ***Grain moisture 16%

TABLE 3. EFFECT OF CONDITIONING WHEAT* ON THE DEGREE OF POLISH OBTAINED IN HULLER NO. 3

Trial No.	Water added (%)	Degree of polish: First pass (%)	Second pass (%)	Total (%)	Polishing efficacy
1	3	3.5	2.0	5.5	Under polishing grain damaged
2	3	3.0	2.2	5.2	–do–
3	4	5.0	3.0	8.0	Good and consistent
4	4	5.2	3.0	8.2	–do–
5	5	7.0	5.0	12.0	–do–
6	5	7.1	5.0	12.1	–do–
7	6**	—	—	—	

* Batch size; 10 kg; rest period 10 min.
** Polishing not possible due to cake formation and jamming of the machine.

TABLE 4. EFFECT OF CONDITIONING ON DIFFERENT TYPES OF WHEAT ON THE DEGREE OF POLISH OBTAINED IN HULLER NO. 1.

Type of wheat	Quantity (kg)	Water+ added (%)	No. of passess	Degree of polish (%)
Aestivum				
Punjab*	100	4	Single	6.8
,,	100	4	Double	10.0
,,	100	5	Single	9.2
,,	100	5	Double	10.8
,,	500	5	Single	9.6
Sharbati*	100	5	Single	9.5
Punjab**	100	5	Single	7.0
,,	200	5	Double	9.8
Durum				
Bansi++	100	5	Single	10.6
,,	100	5	Double	12.4

+Rest period 10 min.
*Medium hard/hard wheats suitable for bread/*chapati*
**Soft wheat suitable for biscuits
++Extra hard wheat suitable for soji/macaroni products

used-5 lb, no of passes-3, and duration of each pass-1 min (Table 1). However, the removal of bran from the grain was not uniform and the bran polishings contained endosperm and brokens.

Corcoran barley pearler: It was inferred from several trials that when wheat was conditioned to 16 per cent moisture for a period of 10 min, about 12.5 per cent of bran could be removed during a pearling time of 3 min (Table 2). The polishings were, however, free from endosperm.

Huller No. 3: The trials carried out to arrive at optimum conditions indicated that addition of 4-5 per cent water for conditioning and a rest period of 5-10 min were found to be optimum for polishing of wheat to 5-7 per cent in the first run without any loss of endosperm. By passing the polished wheat again through the huller, a total of 8-12 per cent polish could be achieved with a negligible loss of endosperm (not exceeding 0.5 per cent) into the bran (Table 3).

*Huller No.*1: The different pilot scale trials of 100 kg batch size carried out indicated that upto 10 per cent of the bran could be removed in a single pass from the wheat grain moistened with 4-5 per cent water and rested for 5-10 min (Table 4). The desirable feed rate was about 2 kg per min. When desired, a second run of the polished wheat at a feed rate of 5 kg per min could achieve 10-12 per cent polish. The polished grain and the bran separated could be collected at different outlets. The traces of bran coming out along with the polished grain are sucked into a cloth bag attached to the huller. The bran separated was found to be free from starch (not exceeding 0.5 per cent as confirmed by starch estimation test). It is interesting to note that in case of durum wheat, 1-2 per cent higher degree of polishing could be achieved, because of the extra hard nature of the grain.

Grinding in chakki: It was evident from the different trials that desired quality flours suitable for bread or biscuits could be obtained by controlling the degree of grinding. Also on regrinding coarse fractions, *atta* was of acceptable quality for the preparation of *roti* or *chapati*.

Sieving on a mechanical sifter: From the different combinations tried on the mechanical sifter, a set of 40, 60, 80 and 100 mesh sieves was found suitable for obtaining flours of different particle sizes. The flour passing through 100 mesh could be used as bread/biscuit flour. The overtailings of 100 mesh along with other fractions collected on regrinding in the *chakki* yielded *atta* of desired quality for use in preparation of *chapati* or *roti*. Alternatively, the overtailings of 100 mesh passing through 80 mesh could be excluded for the regrinding operation and mixed with the reground portion to form *atta*.

The desired combination for durum semolina milling consisted of 18, 30, 50 and 80 mesh sieves. After collecting the semolina of different particle size, the throughs of 80 mesh could be mixed with *atta* obtained after regrinding the overtailings of 18 and 30 mesh.

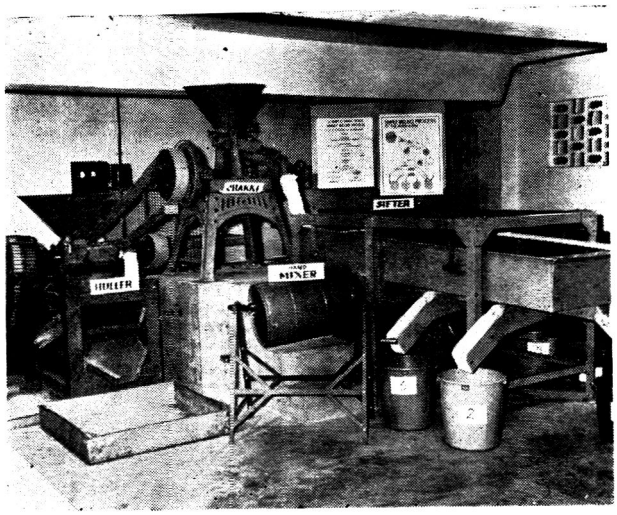

Fig. 1. A set up of the wheat milling process

TABLE 5. YIELD* OF MILLED PRODUCTS FROM DIFFERENT TYPES OF WHEAT

Type of wheat	Bran (%)	Bakery flour (%)	Soji (%)	*Atta* (%)
Aestivum				
Punjab-medium hard/ (*Chapati*/bread)	7.5	32.2	—	60.3
Punjab-soft (Biscuit)	7.0	36.5	—	56.5
Durum				
Bansi-extra hard (Soji/macaroni)	10.2	—	58.4	31.4

*Mean values for 5 trial runs of 100 kg batches.

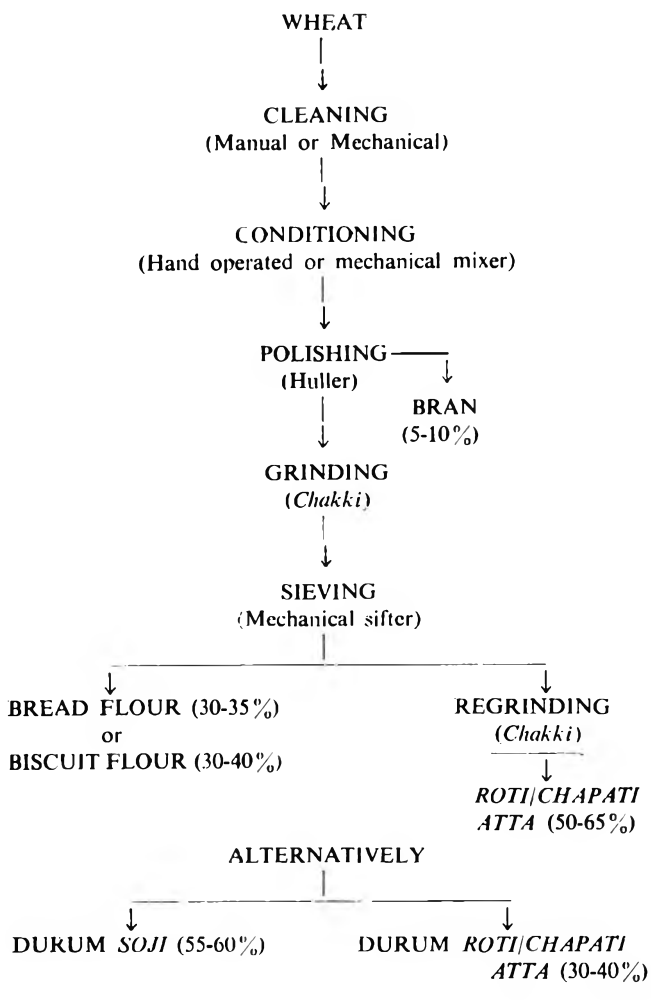

FIG. 2. Small scale wheat milling process (scale of operation: 100 kg per hour)

Flow diagram for the milling process: The different machinery and operations in the simple wheat milling process discussed above are presented in Fig 1 and as flow diagram (Fig 2) which gives the yields of flours/ semolina for different end uses. The yield of flour for bakery products ranged from 30 to 40 per cent, while that of *roti/chapati atta* was 50-65 per cent (Table 5). For durum milling, yields of semolina of different fineness was 55-60 per cent, while the *atta* yield of 30-40 per cent was comparatively lower.

According to preliminary estimates, the processing cost was about Rs 35 per 100 kg of wheat. The flour space required for a set up of simple milling process is about 60 sq meters. The machinery is estimated to cost about Rs. 60,000 for a new set up.

Advantages of the simple milling process developed: The most significant feature of the wheat milling process developed, is the use of indigenously available low cost machinery (so far used only for grinding of wheat into *atta* or for processing of paddy or polishing of brown rice) for obtaining milled products with different end uses, especially in bakery products and also *atta* for preparation of *roti* or *chapati*. Further, while commercial roller flour mills have a minimum capacity of 50 tonnes or more and are dependent on wheat supplies of widely varying quality, by Food Corporation of India, the process developed has a capacity of 100 kg per hr and hence the flexibility to mill even a few bags of wheat of desired quality available in the region. Also, keeping in view the situation of food *vs* population, the maximum utilisation of the wheat grain for human consumption is possible by this process to the extent of upto 95 per cent in comparison with only about 80 per cent in case of roller flour mills.

The easy adaptability of the process to operate on 'service charge' basis makes it a versatile proposition for making available the desired quality flour for the benefit of small and rural bakeries. Hence, the process offers an avenue for self-employed enterpreneurs to process wheat of their choice.

For the first time, the development of a simple small scale process for milling of wheat simultaneously into flours suitable for bakery products and *roti/chapati* making has been demonstrated successfully. Characterisation of flours and semolina obtained by the simple wheat milling process and their suitability for various end uses will be communicated separately.

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Effect of Germination and Cooking on Mineral Composition of Pulses

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Effect of dehusking to *dhal* (decuticled split bean), soaking followed by germination for 24 and 48 hr, and cooking, on ash, phosphorus, phytin phosphorus, magnesium, iron, zinc and copper content of the grain was studied in pigeon pea, green gram and black gram. Dehusking did not result in significant nutrient losses. Overnight soaking of green gram and black gram resulted in 20-48% loss of ash, iron and copper. Germination *per se* did not affect the mineral composition of the pulses except for a slight reduction in phytin phosphorus in 48 hr germinated green gram and black gram. Nutrient losses during cooking of both raw and germinated grains of green gram and black gram were significant, while in pigeon pea the losses were relatively low, because seed-coat remained intact during soaking, germination and cooking.

The beneficial effects of germination and cooking of legumes on some nutrients have been reported[1,2]. Information on the effects of these methods of processing on the mineral composition of Indian pulses is scanty and this has prompted us to undertake the present investigation.

Materials and Methods

Samples of six varieties of pigeonpea (*Cajanus cajan*), five of green gram (*Phaseolus aureus*) and six of black gram (*P. Mungo*), were obtained from the Pulse Breeder, Andhra Pradesh Agricultural University, Hyderabad. The samples were cleaned, washed with glass distilled water to remove contamination and dried at room temperature to constant weight.

Dhal: Whole grain was manually decorticated and split to obtain *dhal.*

Germination: Three g samples of seeds were soaked overnight in 15 ml glass distilled water at toom temperature. Soaked grains were separated and allowed to germinate for 24 or 48 hr. A sample prior to germination was also taken up for analysis. The germinated seeds were dried in an air oven at 50°C overnight.

Cooking: Raw or germinated grains (2-3g) were

cooked in 10 ml glass distilled water for 15 min at 1.1 kg/cm^2 pressure in an autoclave. Cooked seeds were drained of water, dried and analysed.

Analytical: All the samples-raw and processed-were ground in a cyclone mill and analysed for total ash and total phosphorus content by AOAC procedure[3]. Phytin phosphorus was estimated as phosphorus by the method of Makower[4]. Iron, magnesium, zinc and copper were estimated using atomic absorption spectrometer (Varian Techtron Model AAS 1000). All the data are on dry weight basis.

Results and Discussions

Total ash, phosphorus, phytin phosphorus, magnesium, iron, zinc and copper content of the whole grain of pigeon pea, green gram and black gram were in the reported range[5,6] (Table 1). Green gram had relatively more ash and phosphorus content than the other two pulses. Phytin phosphorus in black gram was significantly lower. Pigeon pea had the lowest magnesium content. The composition of the *dhals* was almost similar to that of the corresponding whole seeds.

Germination: Germinated seeds of all the three pulses were relatively poor in the mineral content as compared to ungerminated dry seeds. Nutrient losses occurred during overnight soaking in water (Table 1). The loss in ash content was 48 per cent in black gram and 34 per cent in green gram, the corresponding losses in copper content in these two legumes were 44 per cent and 27 per cent and that of iron about 20 per cent in both. Seed coat of black gram and green gram gets disrupted during soaking and the cotyledons of the grains are exposed, while the seed coat of pigeon pea remains intact even after 48 hr of germination. The loss of nutrients, in pigeon pea are relatively less. Loss in total phosphorus content of the three pulses occured during overnight soaking. On the other hand, phytin phosphorus content of the three pulses remained constant during soaking and germination upto 24 hr. In green gram and black gram, there was a slight decrease

TABLE 1. MINERAL COMPOSITION OF THE WHOLE GRAIN DHAL AND THE GERMINATED GRAINS OF THE PULSES[1]

Germination period (hr)	Ash (g%)	Total-P (mg/100g)	Phytin-P (as % T.P.)	Mineral content (mg/100g)			
				Mg	Fe	Zn	Cu
Pigeonpea							
Raw seed	4.2±0.06	390±32	68±5	154±5	3.0±0.12	3.9±0.24	1.3±0.06
Dhal	4.3±0.10	411±29	65±4	142±4	3.4±0.17	3.8±0.27	1.1±0.06
0	3.0±0.36	300±5	63±5	147±12	2.9±0.13	3.3±0.03	1.2±0.14
24	2.6±0.39	318±14	53±4	137±15	3.2±0.15	3.0±0.03	1.3±0.11
48	2.5±0.40	314±7	59±7	150±11	3.7±0.20	2.9±0.08	1.1±0.06
Green gram							
Raw seed	4.7±0.05	464±5	67±4	212±12	4.0±0.17	3.0±0.13	1.5±0.12
Dhal	4.5±0.05	489±8	71±1	191±5	3.8±0.13	3.1±0.16	1.2±0.04
0	3.1±0.12	403±11	66±2	176±5	3.2±0.12	2.5±0.08	1.1±0.05
24	2.8±0.11	386±3	67±3	163±8	3.2±0.09	2.4±0.07	1.1±0.07
48	2.7±0.09	353±10	61±3	153±4	3.0±0.12	2.3±0.05	1.1±0.05
Black gram							
Raw seed	4.4±0.03	415±27	49±2	242±4	4.0±0.07	3.1±0.22	1.3±0.12
Dhal	4.3±0.05	455±11	50±1	224±3	3.1±0.13	3.0±0.21	0.8±0.04
0	2.3±0.07	354±17	57±3	195±10	3.3±0.14	2.8±0.17	0.7±0.03
24	2.5±0.19	339±16	55±2	187±7	3.5±0.09	2.5±0.13	0.8±0.11
48	2.4±0.16	338±16	53±3	180±11	3.3±0.13	2.6±0.12	0.6±0.05

1. Values are Mean±S.E.

TABLE 2. MINERAL CONTENT OF THE WHOLE GRAIN OF PULSES AFTER COOKING[1]

Germination period (hr)	Ash (g%)	Total-P (mg/100g)	Phytin-P (as % T.P.)	Mineral content (mg/100g)			
				Mg	Fe	Zn	Cu
Pigeonpea							
Dry seed	3.6±0.14	314±16	54±7	166±4	3.3±0.16	3.3±0.17	1.2±0.04
0	3.1±0.39	303±14	60±8	145±8	3.0±0.05	3.3±0.23	1.1±0.05
24	2.6±0.50	323±7	53±6	152±10	3.3±0.08	3.1±0.12	1.3±0.11
48	2.4±0.45	306±10	56±5	142±15	3.9±0.18	3.0±0.14	1.1±0.08
Green gram							
Dry seed	3.6±0.16	403±9	66±2	180±6	3.1±0.16	2.5±0.12	1.0±0.02
0	2.2±0.07	386±13	62±1	143±7	2.7±0.55	2.1±0.04	0.8±0.06
24	2.3±0.06	333±6	68±1	144±5	2.8±0.12	2.2±0.15	0.9±0.08
48	2.1±0.09	307±12	63±4	127±5	2.8±0.13	2.0±0.07	0.9±0.08
Black gram							
Dry seed	2.3±0.08	323±8	63±3	187±6	2.9±0.10	2.9±0.17	0.7±0.06
0	1.8±0.14	260±16	70±2	151±8	2.7±0.09	2.3±0.11	0.5±0.05
24	1.7±0.12	262±12	73±4	157±5	2.9±0.13	2.3±0.11	0.5±0.03
48	1.8±0.12	268±15	64±2	161±8	3.0±0.10	2.4±0.08	0.5±0.04

1. Values are Mean±S.E. on cooked grains.

in phytin phosphorus after 48 hr of germination. Belavady and Banerjee[7] have observed significant reduction in phytin phosphorus content of pulses only after 72 hr germination. These observations thus indicate that the enzyme phytase which hydrolyses phytate was probably induced in appreciable amounts in pulses after 48-72 hr of germination.

Cooking: Nutrient losses on cooking of raw as well as germinated grains of pigeon pea were negligible (Table 2). However, in case of black gram, cooking of raw ungerminated grains resulted in significant loss of ash (47 per cent), copper (44 per cent), iron (29 per cent) and magnesium (23 per cent), while in green gram, losses in ash and iron were 23 per cent and in copper 30 per cent. Cooking losses of nutrients of germinated green gram and black gram were 5-15 per cent over and above the losses observed during the germination process. Meiners and co-workers[8] have also observed significant losses of mineral nutrients on cooking of several dry legumes which they attributed to the leaching of the nutrients into the cooking broth.

The results of the present studies suggest that the losses of mineral nutrients on soaking and cooking would be of significant magnitude especially in legumes like green gram and black gram, the seed coat of which is weak and vulnerable.

Acknowledgement

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An Appraisal of the Cooking Quality Enhancement of Cowpea by *Kaun (Akanwu)*

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Cowpea was cooked with different quantities of *Kaun* (*Akanwu*) to determine its effect on cooking and organoleptic qualities. It was found that, increase in the concentration of *Kaun*, decreases cooking time, alters the texture (calculated as percentage strain) and increases the nonenzymic browning reaction. If the ratio of Kaun and Cowpea seeds is lower than 1:250 (w/w) the product becomes salty and develops offensive odour. Similar effect is noticed with alkalies like NaOH and KOH, the effect more pronounced with NaOH. Concentration of the reducing sugar (glucose) in the cooked samples decreases as the concentration of *Akanwu* increases. Because of the reduction in cooking time, a considerable amount of energy can be saved.

Kaun (a salt mixture), is being used since many decades in Nigeria not only to facilitate cooking food materials such as cereals, pulses, meat and vegetables, but also as an essential ingredient in the formulation of feeds for camels and horses. It is also used in the manufacture of snuff from tobacco. *Kaun* an admixture of over 98 per cent Trona (Na_2CO_3, $NaHCO_3$. $2H_2O$) and traces of silica, sodium and magnesium chloride, aluminium oxide and calcium carbonate[1] has been long recognised as cooking aid and is being used by Nigerian families. Scientists are now engaged in a comprehensive study of the interactions of this salt with foodstuffs. It has been reported that an aqueous solution of the salt is alkaline, containing equimolar mixture of Na_2CO_3 and $NaHCO_3$ with a pH of about 11. Besides softening the food material, it also imparts salty taste, thus reducing the quantity of common salt generally required for acceptable taste.

The study is aimed at monitoring the softening activity of the salt as a function of its concentration with a view to determine the *Kaun* to cowpea ratio necessary to produce an acceptable product in terms of organoleptic and nutritional quality in addition to energy inputs required for cooking.

Materials and Methods

The most common cowpea (*Vigna unquiculata*) popularly called the 'Black eye' bean was chosen for this study. A large quantity bought from the local market, was stored in air-tight cans and used in the experiments.

Sample preparation: Fifty gram samples of cowpea were cooked in 150 ml of water in an autoclave, preset at a chosen temperature, using varying quantities of crude samples of *Kaun* crystals. As a control, similar tests were conducted without *Kaun*. For comparison, NaOH and KOH were also utilized for similar treatment. To eliminate possible effects of the trace elements and compounds in the *Kaun* crystals, some cowpea samples were cooked with purified *Kaun*.

Sensory evaluation: Organoleptic quality of the processed samples was evaluated for colour, taste and odour by a selected panel of judges. Texture, expressed as a percentage strain (or per cent compression ratio) was measured in an INSTRON Machine, Model, 1140.

Chemical evaluation: Glucose solutions of 10-50 per cent were utilized for preparing the standard graph. Two ml of alcoholic copper tartarate reagent was mixed with 2 ml each of the above solutions and boiled for 6 min. After cooling for 3 min, 2 ml of phosphomolybdic acid reagent was added and solutions made up to 25 ml with distilled water. The absorbancy at 420 nm was measured using a spectrophotometer for each of the solutions of known concentrations which served as reference standard.

Samples of cooked cowpea were analysed for glucose content by mashing it and extracting the sugar with hot water. Protein, amino acids and starch from the filtrate, was removed by lead nitrate which was further treated with sodium oxalate to remove excess lead. The resulting solution was then treated with similar

TABLE 1. ORGANOLEPTIC PROPERTIES OF COWPEA COOKED WITH *KAUN*

	Quantity of *Kaun*, (g)					
	0.0	0.1	0.2	0.3	0.4	0.5
Colour	Whitish	Slightly brown	Brown	Brown	Dark brown	Dark brown
Flavour	Sweet	Sweet	Slightly salty	Salty	Very soft	Very salty
Odour	Pleasant	Pleasant	Acceptable	Slightly offensive	Offensive	Offensive
Texture K×0.5	0.48	0.19	0.19	0.18	0.17	0.14
% Strain K×1.0	1.30	0.60	0.58	0.56	0.53	0.48

reagent and the absorbance at 420 nm was dete mined.

Energy analysis and Kinetic study: Proximate analysis of gross input energy (based on the heater rating of 1850 Watts) to the autoclave was carried out to determine possible net energy savings resulting from decreased cooking times with increased *Kaun* concentrations.

The water absorption of cowpea at different time intervals during cooking was monitored and the activation energy of the process determined.

Results and Discussion

Table 1 shows the effect of *Kaun* concentration on the organoleptic quality of cooked cowpea samples. Non-enzymic browning probably catalysed by sodium ion (Na^+) was enhanced as concentration of *Kaun* increased while taste and odour quality of the samples was acceptable up to a *Kaun* to cowpea ratio of 1: 250 (i.e. 0.2 to 50 g of uncooked cowpea). Texture measured in terms of percentage compression or strain, decreased with the increase in *Kaun* concentration. It is also seen from Fig. 1 that there was decrease in cooking time with the increase in *Kaun* concentration. The catalytic nature of *Kaun* in increasing the rate of water absorption and gelatinisation, browning reaction and reducing the cooking time of cowpea was evident from the above.

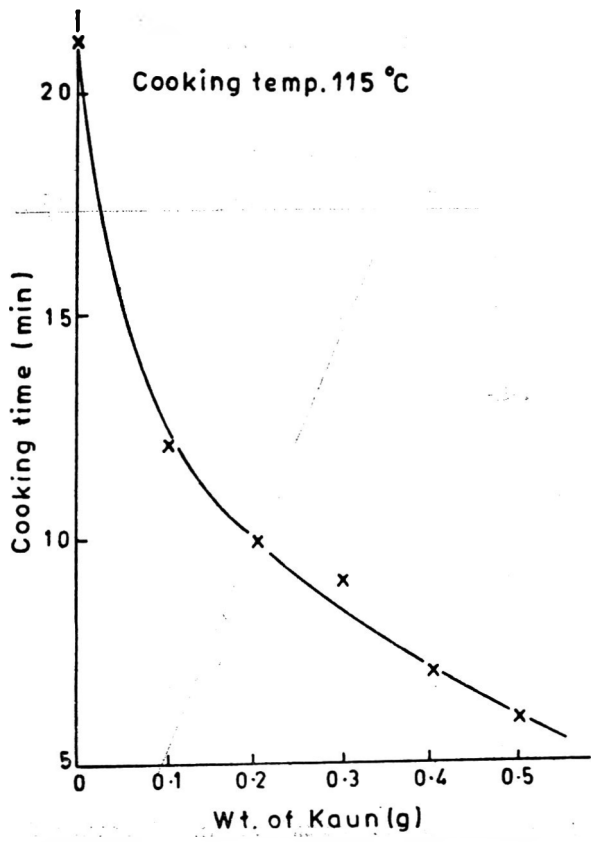

Fig. 1. Variation of cooking time with varying weights of *Kaun*

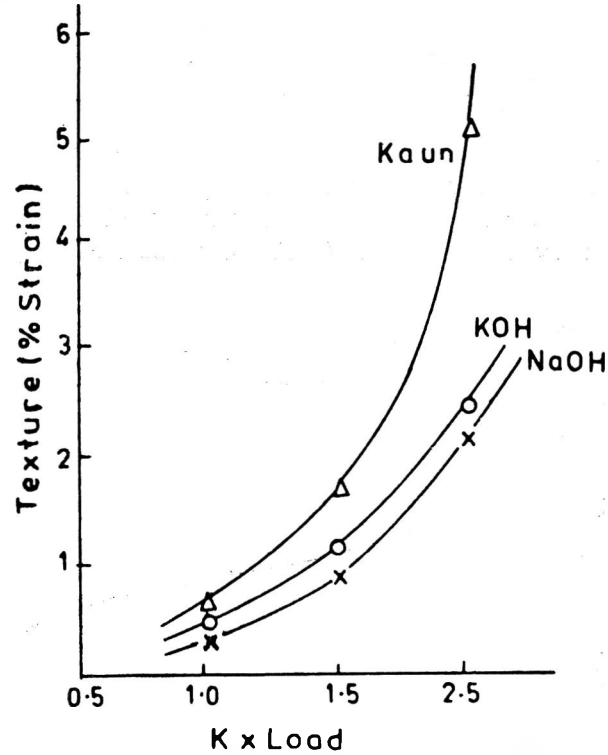

Fig. 2. Comparison of the softening effect of NaOH, KOH and *Kaun*

Duration of cooking, 9 min; cooking temp, 115°C; Wt of *Kaun*, 0.3g; Wt of cowpea, 50g.

TABLE 2. ORGANOLEPTIC PROPERTIES OF SAMPLES COOKED WITH NaOH, KOH AND *KAUN*

Alkali	Texture	Colour	Quantity of alkali	Cooking time (min)	Temp. (°C)
NaOH	Very soft	Dark brown	0.3	9	115
KOH	Moderately soft	Slightly brown	0.3	9	115
Crude *Kaun*	Moderately soft	Brown	0.3	9	115
Purified *Kaun*	Very soft	Slightly brown	0.3	9	115

A comparative study utilizing NaOH and KOH in place of *Kaun* shows that NaOH exerted a profound effect. Fig. 2 shows the texture variation with the alkalies for the three load factors employed. This is further emphasised in Table 2 for samples processed under similar conditions of temperature, time and alkali concentrations. It is also worth noting that the purified *Kaun* (Trona) showed both softening and non-enzymic browning properties. However, on purification its browning activity decreased slightly while the softening effect increased. It must be pointed out that the theory of texture and the softening effect of alkalies on food substances, by facilitating hydration leading to a drop in viscosity is well documented[2-4].

Possible chemical interaction of *Kaun* with cowpea during cooking is seen from the progressive reduction in glucose content with the increase in *Kaun* concentration (Fig. 3). It is presumed that the reaction between some amino acids and glucose may be catalysed by *Kaun*, thus decreasing the effective concentrations of both glucose and the amino acids present. Such interactions which lead to bond formation have been predicted by Davidson *et al.*[5] Fig. 4 shows the variation

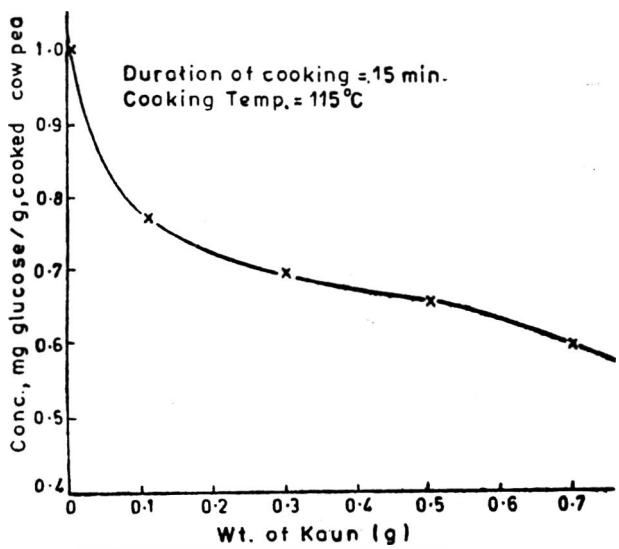

Fig. 3. Effect of *Kaun* on reducing sugar content of cooked cowpea

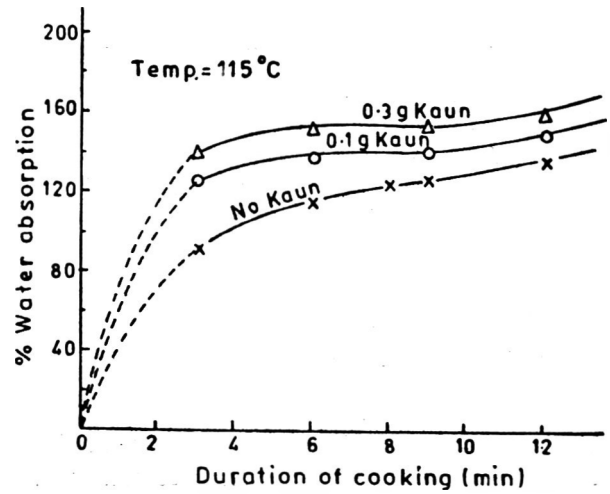

Fig. 4. Percentage water absorption as a function of duration of cooking

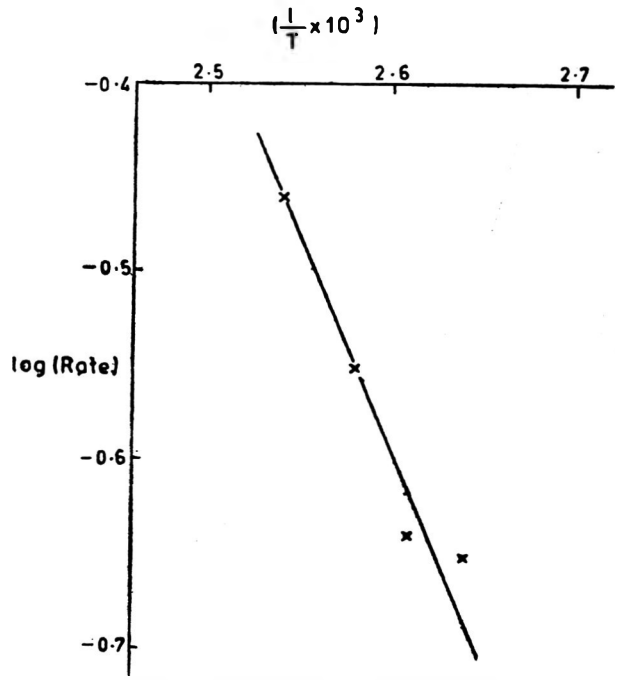

Fig. 5. Arrhenius plot for evaluation of activation energy of water absorption

in absorption rates with different quantities of *Kaun*. It is seen that higher the *Kaun* concentration, the greater the water uptake rate, until a maximum absorption was attained.

The activation energy of the absorption/cooking process can be evaluated from the plot of rate, R, against temperature (Fig 5) and has been found to be 9.55 to 10.4 Kcal/mole. This is within the range of values found for absorption processes.

In the plot of gross input energy and the additional cost of using *Kaun* for the cooking process, it is evident that the net energy saving compensates for the additional cost of *Kaun* and, therefore, seems to justify the use of the latter (Fig 6). Nutritional and consumer acceptability factors have been shown to limit the quantity of *Kaun* used to a value not greater than 1/250. [(w/w) Tables 1 and 2].

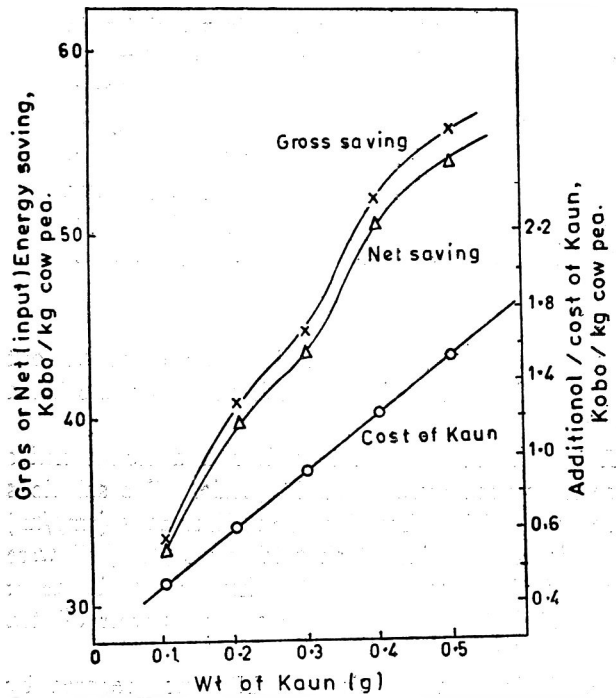

Fig. 6. Energy implication of using *Kaun* to facilitale cooking

Conclusion: *Kaun* reduces the cooking time of cowpea, effecting considerable savings in energy and also enhancing the colour of the cooked product. The optimum *Kaun*-cowpea ratio was found to be 1:250 to obtain an acceptable quality product. Reduction in glucose and possibly amino acid contents of the cooked samples demand further investigation.

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Non-aqueous Titrimetric Determination of Food Preservatives and Non-nutritive Sweeteners Using Internal Indicators

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Oracet blue B, Sudan blue GN, Sudan green 4B (amino anthraquinone dyes) and Resazurin have been used for the first time as internal indicators in the non-aqueous titrimetric determination of food preservatives such as sodium benzoate, sodium propionate, sodium salicylate and non-nutritive sweeteners such as sodium saccharin and sodium cyclamate employing acetous perchloric acid as titrant. The use of Azur A, Azur C, Toluidine blue O (thiazine dyes) and Bromo pyrogallol red is studied for their suitability in the titrations employing sodium methoxide as titrant for determination of acid forms of the above preservatives and non-nutritive sweeteners, sorbic acid and p-hydroxy benzoic acid. The results are accurate with a maximum percentage error of 0.1, 0.8 and 2.5 for macro, semi-micro and micro titrations respectively. The recoveries of added preservatives and non-nutritive sweeteners to food products are reported. Photometric titrations are also described.

Food preservatives (benzoic acid, p-hydroxy benzoic acid, propionic acid) and non-nutritive sweeteners (saccharin) are of great interest to food industry as processing aids. Certain unpermitted preservatives and non-nutritive sweeteners such as salicylic acid and cyclamate are used by some manufacturers. Only a few titrimetric methods[1-14] are available for their quantitative estimation. Most of these methods have inherent problem such as two phase solvent system and micro determinations are subjected to errors. Internal indicators such as oracet blue B (OB B; solvent blue 19), sudan blue GN (SB GN, solvent blue 63, C. I. No. 61520), sudan green 4B (SG 4B, solvent green 3, C. I. No. 61565) (amino anthraquinone dyes) and resazurin (7-OH, 3-H, phenoxazin-3one-10 oxide) were utilized in non-aqueous titrations employing acetous perchloric acid as titrant for the determination of sodium salts of the above preservatives and non-nutritive sweeteners and the results are presented here. The use of thiazine dyes such as azur A (C.I. No.52005), azur C (C.I. No. 52002), toluidine blue o (C. I. No. 52040) and bromo pyrogallol red (BPR) (dibromo pyrogallol sulphone phthalein) were also studied using sodium methoxide as titrant for the determination of the acid forms of the above preservatives and non-nutritive sweeteners

Materials and Methods

Instrumentation: Spectral, absorbance measurements and photometric titrations were carried out using Perkin Elmer, Coleman 575 model double beam digital spectrophotometer

Reagents: All the preservatives and non-nutritive sweeteners used were of A. R. grade. The solutions were prepared by dissolving the sodium salts (4mg/ml) in glacial acetic acid. Their acid forms (4mg/ml) were dissolved in methyl isobutyl ketone or benzene: methanol (3:1) separately. The solutions were diluted when required.

Perchloric acid solution (0.1N) was prepared by mixing 70-72 per cent perchloric acid (Riedel, Germany) with glacial acetic acid and adding 25 ml of acetic anhydride in a liter volume. The solution was standardised with potassium acid phthalate using crystal violet as indicator[15]. The solution was diluted to required strength when needed. Sodium methoxide was prepared by washing about 2.5 grams of sodium metal with methanol, dissolving in about 200ml of methanol and 800 ml of benzene was added and made upto one liter with benzene. It was standardised with benzoic acid using thymol blue as indicator[16].

Indicator solution (0.1 per cent) of OB B, SB GN, SG 4B (M/S Chroma-Gesellscaft, Stuttgart) and resazurin (M/S Gurr Co., England) were prepared in glacial acetic acid. Azur A, Azur C, Toluidine blue O and BPR (M/s Gurr Co., England) solutions (0.1 per cent) were prepared in methanol.

Method using acetous perchloric acid as titrant: To

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an aliquot portion (20-40 mg) of the sample soultion taken into a dry conical flask, 15 ml of glacial acetic acid, 0.2 ml of indicator solution were added and titrated against 0.05N perchloric acid solution dropwise till a sharp change in colour was observed. For macro determinations about 250 mg was taken in a dry conical flask, dissolved in acetic acid and titrated against 0.1N perchloric acid solution. For micro determinations an aliquot ranging from 250 to 1000 μg was taken and titrated against 0.005N perchloric acid.

Photometric titrations: The wavelength of the spectrophotometer was set at the absorption maxima of the acid form of the indicator (e.g. 639 nm for OB B). An aliquot of the sample solution ranging from 500 to 1000 μg was taken in a rectangular optical glass cell (2×4×8 cm) and enough acetic acid was added, 0.2 ml of indicator solution was added and the final volume in the titration cell was adjusted to 15 ml. The solution was slowly titrated in intervals against 0.005N perchloric acid. The absorbance readings were noted after each addition. A blank titration was also performed under identical conditions. The titration was carried out till the absorbance became constant even after adding excess of titrant. The observed absorbance values were corrected for dilution and plotted against the volume of titrant added. The actual volume of the titrant was calculated from the graph and the amount of the compound was calculated from the normality and the titre of the titrant.

Method using sodium methoxide as titrant: An aliquot sample solution (25-40 mg) was taken in a conical flask, 20 ml of methyl isobutyl ketone (20 ml of benzene: methanol, 3:1 in case of BPR indicator) and 0.2 ml of the indicator solution were added and titrated against sodium methoxide (0.05N) solution till a sharp end point was observed. For macro titrations, 250 mg of the compound was taken in a dry conical flask, dissolved in the solvent and titrated against 0.1N sodium methoxide. For micro titrations an aliquot ranging from 250 to 1000 μg was taken and titrated against 0.005N sodium methoxide.

Procedure for food products: The Official AOAC method was followed for the extraction of the added preservatives and non-nutritive sweeteners[17]. Benzoic acid, p-hydroxy benzoic acid, salicylic acid, saccharin and cyclamate were added separately (Table 5) to food products like synthetic ready to serve beverages, tomato ketchup, soya sauce, pineapple syrup and orange squash. After removal of the solvent the proposed method was followed for the estimation using 0.025N sodium methoxide as titrant. A blank experiment was also carried out.

Results and Discussion

The colour change at the end point was blue to pink for OB B, SB GN, Azur A, Azur C and toluidine blue O, bluish green to wine red for SG 4B, pink to orange red for resazurin and wine red to blue for BPR. The absorption maxima of acid and base forms of OB B, SB GN, SG 4B and resazurin were determined taking 0.2 ml of indicator solution in a 50 ml volume of 0.025N potassium acid phthalate solution and 0.025N perchloric acid solution (in glacial acetic acid) respectively. Benzoic acid (0.025N solution) and sodium methoxide in methyl isobutyl ketone (benzene: Methanol, 3:1 in case of BPR indicator) were used for determining the absorption maxima of acid and base forms of the

TABLE 1. TITRIMETRIC ASSAY OF PRESERVATIVES AND NON-NUTRITIVE SWEETENERS USING PERCHLORIC ACID AS TITRANT

Name of the compound	Maximum error (%)*					
	OB B**			SB GN	SG 4B	Resazurin
	Macro	Semi micro	Micro	——Semi micro——		
Sodium benzoate	0.06	0.64	1.80	0.54	0.80	0.78
Sodium salicylate	0.08	0.32	2.10	0.62	0.48	0.56
Sodium propionate	0.10	0.68	2.50	0.75	0.80	0.68
Sodium saccharin	0.07	0.51	2.20	0.58	0.78	0.64
Sodium cyclamate	0.04	0.32	1.20	0.38	0.54	0.63

*Values obtained from at least six determinations

**Similar results are obtained for other dyes in macro and micro scale

Amount taken for macro 250 mg, semi-micro 20-40 mg, micro 250-1000 μg.

TABLE 2. TITRIMETRIC ASSAY OF PRESERVATIVES AND NON-NUTRITIVE SWEETENERS USING SODIUM METHOXIDE AS TITRANT

Name of the compound	Maximum error (%)*					
	Azur A**			Azur C	Toluidine blue O	BPR
	Macro	Semi micro	Micro	——Semi micro——		
Salicylic acid	0.04	0.42	1.18	0.50	0.62	0.38
p-hydroxy benozic acid	0.08	0.56	2.00	0.60	0.52	0.42
Sorbic acid	0.09	0.80	2.48	0.72	0.79	0.62
Saccharin	0.08	0.62	1.86	0.59	0.49	0.48
Cyclamic acid	0.06	0.30	1.40	0.42	0.62	0.26

*Values obtained from at least six determinations

**Similar results are obtained for other dyes in macro and micro scale

Amount taken for macro 250 mg, semi-micro 25-40 mg, micro 250-1000 μg.

indicators (azur A, azur C, toluidine blue O and BPR) respectively. The λmax. (nm) for acid and base forms of the indicators were 639 & 525 for OB B, 642 & 510 for SB GN, 656 & no appreciable maximum for SG 4B, 528 & 508 for resazurin, 638 & 530 for azur A, 626 & 528 for azur C, 632 & 529 for toluidine blue O and 545 and 596 for BPR respectively. The percentage error in the photometric titration of micro quantities of sodium saccharin using 0.005N perchloric acid and OB B as indicator ranged from —3.2 to+1.8 which is similar to that of the visual titrimetric method.

The percentage errors in titrations employing these indicators using perchloric acid and sodium methoxide as titrants are reported in Table 1 and Table 2 respectively. The purity of some of the commercial samples of sodium salts of preservatives and non-nutritive sweeteners is given in Table 3. Table 4 gives the purity values obtained for the acid forms of some commercial samples of preservatives and non-nutritive sweeteners. As can be seen from the Tables 1& 2, the percentage error is low with all the indicators enabling the applicability of the method. The results obtained for the assay of some of the commercial samples by the proposed method, are very satisfactory and are in agreement with those of Official AOAC method (Tables 3 & 4). To check the recovery by the proposed method a known quantity of preservatives and non-nutritive sweeteners are added to food products. The per cent recovery of these added compounds is satisfactory and is in agreement with that of AOAC method (Table 5).

No indicator correction is needed for semi micro and macro determinations. However, for micro determinations using 0.005N titrant, indicator correction is necessary which is as follows: OB B, BPR and resazurin, 0.2 ml SB GB, SG 4B, azur A, azur C and toluidine blue O, 0.3 ml.

TABLE 3. PER CENT PURITY* OF SOME COMMERCIAL SAMPLES OF PRESERVATIVES AND NON-NUTRITIVE SWEETENERS DETERMINED USING PERCHLORIC ACID AS TITRANT

Name of the compound	Proposed method				AOAC method
	OB B	SB GN	SG 4B	Resazurin	
Sodium benzoate	98.7	98.6	98.4	98.6	98.5
Sodium salicylate	99.4	99.3	99.3	99.4	99.3
Sodium propionate	97.6	97.2	97.4	97.6	—
Sodium saccharin	98.1	98.0	97.9	98.0	98.0
Sodium cyclamate	99.8	99.6	99.6	99.8	99.8

*Average of five determinations.

TABLE 4. PER CENT PURITY* OF SOME COMMERCIAL SAMPLES OF PRESERVATIVES AND NON-NUTRITIVE SWEETENERS DETERMINED USING SODIUM METHOXIDE AS TITRANT

Name of the compound	Proposed method				AOAC method
	Azur A	Azur C	Toluidine blue O	BPR	
Benzoic acid	97.2	97.0	97.1	97.0	97.0
Salicylic acid	99.6	99.4	99.4	99.5	99.5
Sorbic acid	94.3	94.2	94.2	94.2	—
p-hydroxy benzoic acid	99.0	98.9	99.1	99.0	—
Saccharin	98.4	98.2	98.0	98.3	98.2
Cyclamic acid	99.6	99.5	99.5	99.5	—

*Average of five determinations.

TABLE 5. RECOVERY OF PRESERVATIVES AND NON-NUTRITIVE SWEETENERS ADDED TO FOOD PRODUCTS

Food product	Additive added	Concn. (ppm)	Recovery (ppm)*	
			Proposed method	AOAC method
Tomato ketchup	Benzoic acid	350	342	338
Pineapple syrup	–do–	500	494	512
Synthetic RTS beverage	–do–	120	118	126
–do–	Saccharin	100	102	101
–do–	Salicylic acid	100	98	99
–do–	Cyclamate	200	195	—
Orange squash	Saccharin	250	254	248
Tomato sauce (commercial)	Benzoic acid	—	1016	1052
Synthetic RTS beverage (commercial)	–do–	—	126	128
Saccharin tablets	Saccharin	12.00**	12.02 **	12.03**

*Average of four separate analysis;

**mg saccharin per tablet; the declared saccharin content in the tablet is 12.00 mg.

The normal food constituents such as glucose, sucrose and caffeine do not interfere in these estimations. However, vanillin is found to interfere in the titrations employing sodium methoxide as titrant. Oxidising agents such as nitrites and peroxides should be absent as these indicators readily undergo oxidation leading to the formation of N-nitroso or N-oxides which change the colour of the indicator.

All the proposed indicators are perfectly reversible. We believe that the colour change at the end point may be due to the formation of protonated secondary amine in case of amino anthraquinone dyes while the colour change is due to the formation of salt through the tetravalent sulphur (present in orthoquinoid form) leading to the formation of sulfonium base in case of thiazine dyes[18]. The colour change in case of BPR may be due to the breakage of the five membered ring and causing one of the benzene rings to take the quinonoid form.

Among the common solvents used in non-aqueous titrations, acetic acid and methyl isobutyl ketone(benzene:methanol, 3:1 in case of BPR indicator) are found suitable for titratious involving perchloric acid and sodium methoxide as titrants. The proposed non-aqueous titrimetric-methods have the added advantage for the reason that the acidity or basicity of these weak acids or bases is enhanced considerably paving way for an easy end point detection. These methods are very simple, rapid and can be used for the purity assay of commercial samples as well as their determination in foods.

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Polar Lipids of Coconut

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Coconut lipids obtained by extraction with chloroform-methanol (2:1 v/v) contained phospholipids in contrast to the oil obtained by pressing or by extraction of coconut with a non-polar solvent. Fractionation indicated that they were composed of 94.3 per cent neutral, 5.5 per cent glyco and 0.2 per cent phospholipids. Mono-galactosyl diglyceride was the predominant glycolipid and the major components of phospholipids were phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl inositol. Both glyco and phospholipids were rich in unsaturated fatty acids and had iodine values of 41.6 and 58.3 respectively. Linolenic acid was present in total (0.5 per cent) as well as in glycolipids (2.5 per cent).

Coconut oil as ordinarily marketed is considered to be resistant to oxidative deterioration owing to its low degree of unsaturation[1]. However, there are conflicting reports[2-5] with regard to the stability of this oil. Further, the low shelf-life of coconut products namely desiccated coconut and dry ready mixes containing desiccated coconut cannot be reconciled with the fact that coconut oil is highly saturated and therefore, not easily susceptible to oxidative rancidity. With a view to find an explanation for this, a study was made on the lipid composition of coconut and the data are presented in this paper.

Materials and Methods

Preparation of sample: Fully mature coconuts were procured from the local market. After removing the shells, the kernel was separated, cut into small bits and then shredded in a 'Fryma Mill'. The fine shreds obtained were freeze-dried in a Stokes freeze-drier. Five coconuts were used for preparing each sample.

Extraction of Lipids: Freeze dried coconut shreds were extracted with petroleum ether (40°-60°) using a Soxhlet fat extraction apparatus. Total lipids of coconut were obtained by: (1) the Bligh and Dyer method[6] using fresh coconuts or (2) by allowing 250 ml chloroform-methanol (2:1 v/v) to percolate at 3ml/min through 5 g freeze dried coconut of particle size 15-20 mesh packed in glass columns (12 cm×0.5 cm dia). After the extraction of lipids, the solvent was removed in a rotary flash evaporator. The non-lipid matter was removed by the Folch procedure[7] and true lipids estimated gravimetrically.

Cold pressed coconut oil was obtained by pressing about 500 g freeze dried coconut shreds in a laboratory 'Carver' hydraulic press at 350 kg/sq cm. The oil was allowed to stand overnight and then filtered under suction through a sintered glass funnel.

Refractive index of the oil samples was taken at 40°C in an oil refractometer (Advance Research Co., New Delhi, India). Iodine value (Wijs'), saponification value and Polenske value were determined according to AOCS procedures[8].

Separation of lipid classes: Countercurrent solvent partition method described by Galanos and Kapoulas[9] was used for the separation of neutral and polar lipids from the total lipids of coconut. Further fractionation into glyco and phospholipids was achieved by silicic acid column chromatography[10]. Quantitation of neutral lipids was carried out by gravimetry. Glyco- and phospholipids were quantitated by estimating hexose[11] and phosphorus[12] and multiplying with the appropriate factors[13].

The individual components of the phospholipids and glycolipids were separated by thin-layer chromatography[13] using the solvent systems: chloroform-methanol-acetic acid-water (65:15:10:4) and acetone-acetic acid-water (100:2:1) respectively. Identification of the spots was made by comparing with R_f values of reference compounds and spraying with specific reagents[14].

Preparation of fatty acid methyl esters: The sodium methoxide catalysed transesterification procedure as described by de Man[15] was followed. The separation of fatty acid methyl esters was carried out using a Varian Aerograph model 1400 series gas chromatograph equipped with a hydrogen flame ionisation detector under the following conditions:

SS column (6 ft×1/8 in) packed with 15 per cent

Diethylene glycol succinate on Chromosorb W (60-80 mesh); column temperature programmed from 75°C to 195°C at the rate of 10°C per min; injector and detector ports adjusted to 240°C; carrier gas, N_2, 15 ml/min; H_2, 20 ml/min; air, 300 ml/min. Identification of the separated fatty acid methyl esters was made by comparison with standards (Sigma). Further confirmation of unsaturated fatty acids was done by microhydrogenation[16] of the esters followed by gas chromatography. Quantitation of the peaks was by triangulation. Each analysis was carried out in triplicate and the average is reported.

Results and Discussion

Coconut lipids obtained by different methods namely petroleum ether or chloroform-methanol extraction or by cold pressing in a Carver press showed slightly different physico-chemical properties as indicated in Table 1. Whereas phosphorus was present in the chloroform-methanol extracted lipids (total lipids), it was absent in the coconut oil obtained by extraction with petroleum ether as well as in cold presssed coconut oil (Table 1). The lipid class composition of coconut lipids is shown in Table 2. Neutral lipids constituted 94.3 per cent, glycolipids 5.5 per cent and phospholipids 0.2 per cent. The major components of phospholipids were phosphatidyl choline (34.6 per cent), phosphatidyl ethanolamine (24.6 per cent) and phosphatidyl inositol (19.0 per cent) (Table 3). Lyso derivatives amounted to 8.0 per cent. Phosphatidyl serine which usually occurs in small amounts in plant lipids was present up to 4.8 per cent. The component which amounted to about 6.0 per cent and which remained at the origin on the chromatoplate could not be identified by comparing R_f values with those of reference compounds and by specific spray reagents[14].

TABLE 2. CLASS COMPOSITION OF COCONUT LIPIDS

Lipid class	% of total lipids*
Neutral lipids	94.34 ± 0.39
Glyco lipids	5.47 ± 0.37
Phospholipids	0.20 ± 0.06

*Mean ± SD of determination on 5 samples

TABLE 3. PHOSPHOLIPIDS OF COCONUT

Phospholipid	% of total phospholipids
Phosphatidyl choline	34.6
Phosphatidyl ethanolamine	24.6
Phosphatidyl inositols	19.0
Phosphatidyl serine	4.8
Lysophosphatidyl choline	4.6
Lysophosphatidyl ethanolamine	3.4
Phosphatidic acid	2.5
Unidentified (remained at the origin)	6.5

Values are averages of determinations on two samples.

TABLE 4. GLYCOLIPIDS OF COCONUT

Glycolipid	% of total glycolipids
Sterol glycosides	12.0
Monogalactosyl diglyceride	40.0
Digalactosyl diglyceride	30.0
Unknown (sulfolipid)	10.0
Unknown	8.0

Values are averages of determinations on two samples.

TABLE 1. EXTRACTION OF COCONUT LIPIDS BY DIFFERENT METHODS ANG PHYSICO-CHBMICAL CHARACTERISTICS OF THE EXTRACTED LIPIDS

Extraction method	% lipid (moist. free basis)*	Refractive index** at 40°C	Saponification value**	Iodine value** (Wijs')	Polenske value**	Phosphorus %
Petroleum ether (Soxhlet)	71.0 ± 1.2	1.4488	262	7.6	14.6	Nil
Cold pressed (Carver press)	65.0 ± 1.6	1.4488	262	7.4	14.5	Nil
Chloroform methanol 2:1 v/v (column procedure)	74.6 ± 0.9	1.4491	260	8.4	13.0	0.01
Bligh & Dyer method	74.4	1.4491	260	8.5	13.0	0.01
Coconut oil (market sample)	—	1.4488	262	7.6	14.2	Nil

*Mean ± SD of determination on 5 samples

**Values are means of determinations on 3 samples

As indicated in Table 4, the glycolipids consisted of mono and digalactosyl diglycerides of which monogalactosyl diglyceride was predominant.

The fatty acid composition of the petroleum ether extract of coconut presented in Table 5 is in agreement with the literature values for coconut oil[1]. However, no information is available on the coconut lipid class composition and their fatty acids. The presence of 0.4 to 0.5 per cent linolenic acid in total lipids and 2.5 per cent in glycolipids have been reported in this paper for the first time (Tables 5 and 6). Although Bezard *et al.*[17] had suspected the presence of arachidic and/or linolenic acid in coconut oil, they were not sure about their identity. This point has now been conclusively proved by microhydrogenation (Fig 1), silver-ion thin-layer chromatography and co-chromatography. As seen from Table 6, the glycolipids and phospholipids contain about 35 per cent and 64.0 per cent of unsaturated fatty acids respectively.

The present investigation has revealed that: (a) besides neutral lipids, coconut contains polar lipids which are not extracted by cold pressing or by extraction with a non-polar solvent and (b) polar lipids contain higher amounts of unsaturated fatty acids than the neutral lipids. These observations clearly bring out the differences between coconut oil obtained by

TABLE 5. FATTY ACID COMPOSITION OF COCONUT LIPIDS EXTRACTED BY PETROLEUM ETHER AND CHLOROFORM-METHANOL (2:1)

Fatty acid	% total fatty acids in extraction of Petroleum ether	Chloroform-methanol (2:1)
Caproic	0.5	0.4
Caprylic	8.1	7.6
Capric	6.4	6.2
Lauric	50.3	50.0
Myristic	19.1	18.5
Palmitic	7.5	7.4
Stearic	1.6	1.8
Oleic	4.7	5.2
Linoleic	1.8	2.4
Linolenic	Nil	0.5
Total saturated	93.5	91.9
Total unsaturated	6.5	8.1
Calculated iodine value (g I_2/100 g oil)	7.5	9.9

Values are means of determinations on three samples

TABLE 6. FATTY ACID COMPOSITION OF THE MAJOR LIPID CLASSES OF COCONUT LIPIDS

Fatty acid	% of total fatty acids in Neutral lipids	Glycolipids	Phospholipids
Caproic	0.6	Nil	Nil
Caprylic	7.6	0.9	Nil
Capric	6.2	2.9	Nil
Laurie	50.1	33.7	6.7
Myristic	18.0	16.0	7.4
Palmitic	7.6	7.7	19.2
Stearic	1.9	3.6	3.2
Oleic	5.2	24.8	59.5
Linoleic	2.4	7.9	4.0
Linolenic	0.4	2.5	Nil
Total saturated	92.0	64.8	36.5
Total unsaturated	8.0	35.2	63.5
Calculated Iodine value	9.8	41.6	58.3

Values are means of determinations on three samples

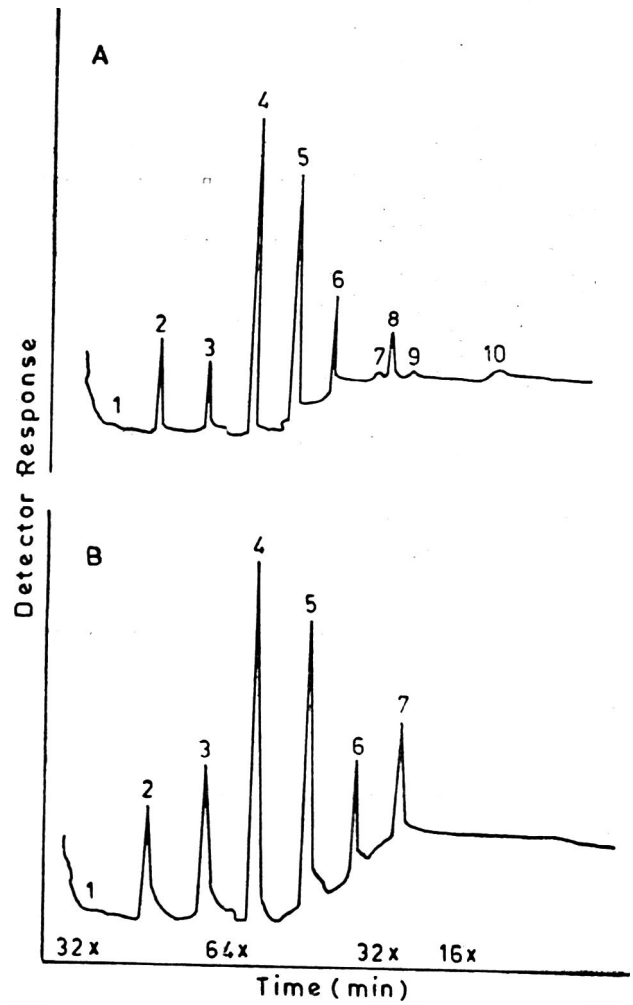

Fig. 1. GLC of Fatty acid Methyl Esters of Coconut Lipids (A) Before Micro-Hydrogenation; (B) After Micro-Hydrogenation

1 to 10 are methyl esters of 6:0, 8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 18:1, 18:2, and 18:3.

expression or by petroleum ether extraction and the lipids of coconuts and would probably help in explaining the poor stability of desiccated coconut.

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Studies on Packaging and Storage of *Sohan Papri*

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***Sohan papri*, an Indian sweetmeat based on Besan (Chickpea flour) has a short shelf-life of about 12 days. In order to extend the shelf-life, *Sohan papri* was treated with BHA and packed separately in flexible pouches (Aluminium foil, HDPE and Poly/LDPE) and rigid containers (Dingley and Tagger top) and stored at 27°C and 65% RH upto a period of 110 and 225 days respectively. The sorption-isotherm studies revealed that the product has a permissible moisture pick up of 1.7% with critical moisture content of 3% and ERH of 30%. Chemical parameters such as FFA, PV, Kries test, TBA and colour were periodically estimated. Sensory evaluation results indicated that the product could be stored well for 30 days in poly/LDPE, 110 days in HDPE and Al. foil, and 225 days in rigid containers.**

Sohan papri, one of the most popular Indian sweetmeats, is made from Besan (Chickpea flour), maida (refined wheat flour), sugar and vanaspati (hydrogenated edible oil) generally in the proportion of 1:3:2:4. The flours are mixed well roasted in fat and added to sugar syrup at 80°C and thoroughly mixed. The mass is hand kneaded and pulled to a thread-like structure in order to prevent crystallisation of sugar. It is spread on a tray to a uniform thickness and cut into rectangular bars. However, the method of preparation and

proportion of ingredients vary with individual sweet makers[1]. The product is conventionally stored in glass containers under ambient conditions. Date *et al*[2]. have stated that the shelf-life of sweets is not more than a few days. Afterwards the quality deteriorates showing fat bloom, rancidity and discolouration. Of late, sweetmeats are being increasingly exported mostly to the Middle East countries. Since *Sohan papri* has a short shelf life, investigations were carried out to study the effect of some antioxidants and packaging materials during storage to extend the shelf-life and the results are presented in this paper.

Materials and Methods

Sohan papri: Freshly prepared samples collected from a local sweetmaker was used in this study. At the time of preparation, antioxidants, 0.02 per cent of butylated hydroxy anisole (BHA) and 0.004 per cent citric acid on fat basis were added.

Sorption-isotherm studies: Effect of humidity-moisture relations on the product was studied at 27°C and at relative humidities ranging from 11-92 per cent maintained by salt solutions[3] of appropriate saturation. Weights of samples were recorded periodically till they attained constant weight or showed symptoms of fungal growth.

Packaging materials: Three flexible packaging materials, 300 G High Density Polyethylene (HDPE), Laminate of Polyester/150 G Low Density Polyethylene (Polyester/LDPE) and Laminate of 60 GSM paper/0.02 mm aluminium foil/150 G low density polyethylene (aluminium foil) were chosen based on their barrier properties and their water vapour transmission rates (WVTR) determined according to ISI method[4]. Using these packaging materials, pouches of 10 cm×5 cm size to hold about 100 g *Sohan papri* were made. The product cut into cubes was initially wrapped in PT-cellophane 300, put into these pouches and heat sealed. Only polyester/LDPE laminate was vacuum packed (25 in. vacuum).

Rigid containers-8 oz Tagger top cans and 3 oz Dingley cans-were also chosen and the product was canned under ambient conditions or nitrogen (only for Tagger top can). The samples packed in A-2½ cans under nitrogen and stored in refrigerator served as control for both flexible and rigid packages throughout the study.

Storage condition: Samples in flexible and rigid containers were stored at 27°C, 65 per cent relative humidity (RH), and analysed after a month and at intervals of 20 days there on upto 110 days, in the case of flexible packages and at 0, 105, 135, 165 and 225 days in the case of rigid containers.

Chemical analysis: Analyses for moisture (at 70°C for 24 hr at vacuum), peroxide value (PV) and free fatty acid content (FFA)[5], thiobarbuturic acid (TBA)[6], Kries test[7] and colour (using Lovinbond Tintometer) were carried out by standard procedures.

Sensory evaluation: Taste panel evaluations were carried out under ISI recommended laboratory set up[8]. A discriminative communicative panel of 20 persons participated in the evaluation. A quality description for desirable and undesirable qualities was developed during the panel orientation sessions evaluating samples varying in individual quality attributes-colour, appearance, texture (mouthfeel) and, flavour (aroma and taste)-and ranging from very poor to excellent (Table 1). In the regular storage study evaluation sessions, the panelists ranked the samples for individual quality attributes assigning Rank 1 for the best sample, Rank 2 for the next best sample and so on. Equal ranking was allowed. The panelists were instructed to comment on the quality deterioration, if any, picking words from the quality description.

TABLE 1. QUALITY DESCRIPTION OF *SOHAN PAPRI*

Quality attributes	Desirable	Undesirable
Colour	Shades of yellow/pale brown; Fresh, bright Uniform	Darker shades/patches Dull Not uniform
Appearance	Fairly clean cut long edge Fairly smooth surface except for added nuts/fruits	Frayed/broken long edge Dry/fat bloom surface
	Added nuts/fruits-fairly uniform size and optimum quantity	Added nuts/fruits odd size and sparse addition
	Separting into flacky fibrous layers when a piece is broken transversely.	Presence of extraneous matter. Hard/compact, poorly layered when broken transversely.
Texture (mouthfeel)	Crisp (sound), crumbly Fine crystalline texture Easily melting layers	Soggy/gummy/sticky Residual chewy matter Hard/coarse feel Residual chewy matter
Flavour (aroma & taste)	Fresh balanced Characteristic fried flavour Light sweet caramel aroma; sweet and pleasantly fatty taste	Unbalanced Bland/stale/rancid burnt Off aroma/raw pulse Off taste/waxy/rancid taste of added nuts/fruits
	pleasant after taste	Bitter/unpleasant after taste.

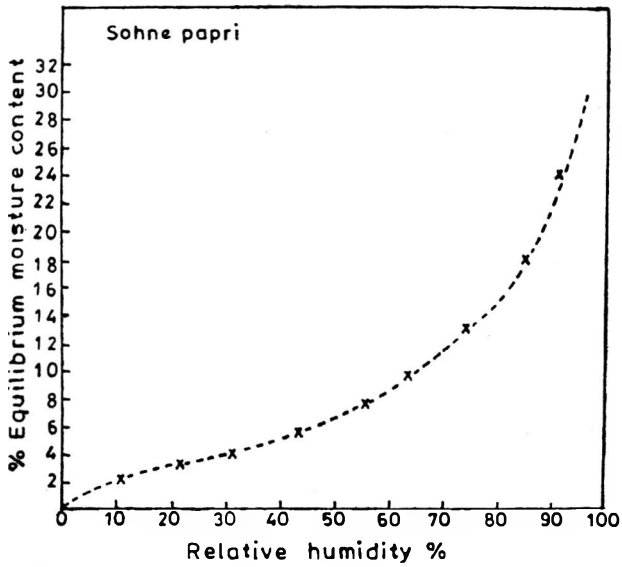

Fig. 1. Moisture-humidity relationship of *Sohan Papri*

The panelists also judged each samples whether acceptable or not for overall quality.

Results and Discussion

Sorption-isotherm studies: The critical moisture level for *Sohan papri* is shown in Fig 1. Equilibrium relative humidities at 27°C indicated that *Sohan papri* with an initial moisture content of 1.3 per cent equilibrated to 11 per cent RH. The sorption results indicated that the product when equilibrated to 32 per cent RH corresponding to a moisture level of 4.4 per cent, lost the desirable texture quality. The product with a moisture content of 3.2 per cent corresponding to an RH of 22 per cent showed just desirable texture. Hence a moisture content of 3 per cent which equilibrates to 20 per cent RH was fixed as the critical moisture level. The product had shown fairly low moisture tolerance with a permissible uptake of about 1.7 g per 100 g from the initial value. Above 60 per cent RH, the product picked up moisture rapidly indicating the capillery condensation.

The WVTR g/m²d at 38°C and 90 per cent RH for HDPE was 1.4, for polyester/LDPE 6.9 and for aluminium foil nil.

The rigid containers exposed to 27°C, 65 per cent RH examined for the conditions of the can showed that, the can interior was normal and free from feathering, detinned spots and rusting.

Chemical analysis: Tables 2 and 3 give the results of the Chemical analysis for flexible and rigid containers.

Moisture: From Table 2, it can be seen that even after 110 days of storage, the samples stored in different flexible packages did not attain the permissible moisture

TABLE 2. CHEMICAL ANALYSIS OF *SOHAN PAPRI* STORED IN FLEXIBLE POUCHES

Packaging material	Storage period (days)	FFA (%oleic acid)	TBA (μ g/100g)	Moisture (%)
	Initial	0.12	20	1.30
Al.foil	30	0.12	33	1.30
HDPE	30	0.20	33	1.47
Poly/LDPE	30	0.20	33	1.54
Al.foil	50	0.18	33	1.30
HDPE	50	0.28	25	1.59
Poly/LDPE	50	0.20	25	1.70
Al.foil	70	0.20	25	1.30
HDPE	70	0.21	25	1.71
Poly/LDPE	70	0.31	25	1.84
Al.foil	90	0.35	10	1.30
HDPE	90	0.50	10	1.84
Poly/LDPE	90	0.50	10	1.95
Al.foil	110	0.38	10	1.30
HDPE	110	0.60	10	1.96
Poly/LDPE	110	0.50	10	2.04

Kries test was –ve for samples stored in all the packaging materials throughout the storage period.

PV of samples stored in all packages is nil throughout the storage period.

TABLE 3. CHEMICAL ANALYSIS OF *SOHAN PAPRI* STORED IN RIGID CONTAINERS

Packaging material	Storage period (days)	PV (meq/kg fat)	FFA (%oleic acid)	TBA (μ g/100g)
	Initial	Nil	0.12	20
Dingley	105	Nil	0.10	15
Tagger top	105	0.5	0.13	20
Tagger top (N_2)	105	Nil	0.12	16
Dingley	135	Nil	0.13	18
Tagger top	135	0.8	0.15	25
Tagger top (N_2)	135	Nil	0.13	18
Dingley	165	Nil	0.14	17
Tagger top	165	1.0	0.15	26
Tagger top (N_2)	165	0.2	0.13	18
Dingley	225	Nil	0.20	20
Tagger top	225	1.2	0.18	30
Tagger top (N_2)	225	0.5	0.15	22

Kries test was –ve for samples sorted in all the packaging, materials through the storage period.

pickup of 1.7 per cent. However, aluminium foil picked no moisture, HDPE 0.66 per cent and polyester/LDPE 0.74 per cent after 110 days.

Peroxide value: PV of the samples packed in all the flexible packages was nill till the end of the storage period of 110 days showing no peroxide formation. Among the samples in rigid containers, samples in Dingley can (plain) did not show peroxide formation even at the end of 225 days. Peroxide values of samples packed in Tagger top cans (nitrogen pack and plain) towards the end of 225 days of storage period were 0.5 and 1.2 mcq per kg of fat. But the peroxide values of many freshly collected commercial samples[1] obtained from local market varied from 1.0 to 9.6 indicating that the values obtained for those packed in Tagger top cans were insignificant and did not affect the quality. Since the Tagger top cans (plain) had more head space, a slight increase in PV is noticed, whereas PV of sample packed under N_2 is significantly less.

Free fatty acid value: Although increase in FFA of samples packed in flexible pouches is slightly high compared to that in rigid containers, the change is not significant. The FFA values of freshly collected commercial samples[1] were in the range of 0.1-0.7. Compared to the range, the values obtained for the samples packed in different packaging materials are low throughout the storage period. Also, the sample stored under N_2 has low FFA compared to other samples.

Thiobarbituric acid value: The change in TBA values of samples packed in flexible pouches as well as rigid containers is very insignificant, compared to the initial values.

Kries test: The test was not answered by all the samples packed in both type of packaging materials even at the end of their respective storage periods.

Colour: The Lovibond colour units for samples stored in flexible and rigid containers for different storage periods are given in Tables 4 and 5. There was practically no change observed in the yellow, red and blue units.

Sensory evaluation: The results of the sensory evaluation data are given in Table 6 as rank sum analysis[9] and the percentage of acceptability of samples for different storage periods. The ranks were reranked to assess the significance between any two treatments.

Polyester/LDPE package did not store the sample well even for 30 days and was inferior in appearance, texture and flavour. This sample showed significantly identifiable inferior quality in all the attributes throughout the subsequent storage period and the acceptability

TABLE 4. LOVIBOND COLOUR UNITS FOR *SOHAN PAPRI* STORED IN FLEXIBLE POUCHES FOR DIFFERENT DAYS

Type of package	Storage period (days)	Lovibond units Yellow	Red	Blue
	Initial	5.0	0.2	0.1
Al.foil	30	5.0	0.3	0.1
HDPE	30	5.0	0.3	0.1
Poly/LDPE	30	5.6	0.2	0.1
Al.foil	50	5.0	0.3	0.1
HDPE	50	5.0	0.2	0.1
Poly/LDPE	50	5.3	0.3	0.1
Al.foil	70	5.3	0.3	0.1
HDPE	70	5.5	0.5	0.1
Poly/LDPE	70	5.1	0.5	0.1
Al.foil	90	5.2	0.4	0.3
HDPE	90	5.3	0.4	0.3
Poly/LDPE	90	5.3	0.4	0.1
Al.foil	110	5.1	0.3	0.1
HDPE	110	5.2	0.3	0.1
Poly/LDPE	110	5.3	0.4	0.1

TABLE 5. LOVBOND COLOUR UNITS FOR *SOHAN PARI* STORED IN RIGID CONTAINERS FOR DIFFERENT DAYS

Type of container	Storage period (days)	Lovibond units Yellow	Red	Blue
	Initial	5.0	0.2	0.1
Dingley	105	5.1	0.3	0.1
Tagger top	105	5.0	0.3	0.1
Tagger top (N_2)	105	5.1	0.2	0.1
Dingley	135	5.0	0.3	0.1
Tagger top	135	5.2	0.3	0.2
Tagger top (N_2)	135	5.1	0.2	0.1
Dingley	165	5.0	0.3	0.1
Tagger top	165	5.2	0.4	0.1
Tagger top (N_2)	165	5.1	0.3	0.1
Dingley	225	5.1	0.4	0.1
Tagger top	225	5.3	0.4	0.1
Tagger top (N_2)	225	5.0	0.4	0.1

TABLE 6. RANK SUM ANALYSIS OF INDIVIDUAL QUALITY AND PER CENT ACCEPTABILITY OF *SOHAN PAPRI* SAMPLES

Storage period (days)	Flexible pouches A	B	C	D	Storage period (days)	Rigid containers E	F	G	D
	Colour					*Colour*			
30	60^a	50^a	48^a	42^a	105	58	52	49	41
50	76^b	48^a	38^a	38^a	135	58	50	49	43
70	80^b	48^a	37^a	35^a	165	57	54	47	42
90	75^b	47^a	41^a	37^a	225	56	53	50	41
110	70^b	46^a	42^a	42^a					
	Appearance					*Appearance*			
30	70^b	45^a	42^a	43^a	105	56	54	48	42
50	70^b	50^a	45^a	35^a	135	58	51	48	43
70	79^b	46^a	42^a	33^a	165	57	53	48	42
90	69^b	50^a	47^a	34^a	225	56	52	50	42
110	64^c	57^b	40^a	39^a					
	Texture					*Texture*			
30	63^b	56^b	43^a	38^a	105	60	52	46	42
50	72^b	50^a	47^a	31^a	135	59	50	49	42
70	76^c	49^b	43^{ab}	32^a	165	58	54	46	42
90	69^c	52^b	49^{ab}	30^a	225	60	49	48	43
110	68^c	52^b	47^{ab}	33^a					
	Flavour					*Flavour*			
30	65^b	49^a	48^a	38^a	105	59	55	45	41
50	74^b	49^a	45^a	32^a	135	57	52	49	42
70	78^c	49^b	44^{ab}	29^a	165	59	48	48	45
90	66^c	56^b	47^{ab}	31^a	225	61	50	45	44
110	71^c	49^b	46^{ab}	34^a					
	% Acceptability					*% Acceptability*			
30	75	90	100	100	105	100	100	100	100
50	65	90	100	100	135	85	90	100	100
70	60	90	95	100	165	80	80	100	100
90	60	85	95	100	225	75	90	90	100
110	50	80	90	100					

A=Poly/LDPE B=HDPE C=Al.foil D=Control
E=Tagger top F=Dingley G=Tagger top N_2

Rank sums carrying different superscripts in the same row differ significantly ($P \leq 0.05$)

Rank sums in each row do not differ significantly ($P \leq 0.05$)

for overall quality fell down significantly after 30 days. The change in flavour aspect may have influenced the ranking for other attributes also. The HDPE package stored the sample well as equally comparable to aluminium foil throughout 110 days of storage period with an acceptability of over 80 per cent.

Dingley and Tagger top (plain and under N_2) cans stored the sample well throughout the storage period of 225 days.

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A New Method for Curd Tension Measurement

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The curd tension of milk, measured by considering weight and time has been compared with the results obtained by considering weight alone. The mean curd tension of cow, buffalo and mixed milk by the two methods were 40.04, 54.16, and 44.98 g sec and 47.47, 86.38 and 74.22 g respectively, indicating significant difference in the values. The decrease in curd tension for cow's milk due to homogenisation when measured by the new method and old method was 85.58% and 31.82% respectively and the corresponding values for buffalo's milk were 37.25% and 54.87%.

The curd tension of milk measured by the standard ADSA method[1] is expressed as pressure in grams of force required to cut the coagulum of milk using a standard knife. The method is not commonly used in India due to the non-availability of the instrument. Another method developed by Chandrasekara *et al.*[2] reflects the points of maximum curd tension measured and expressed in grams (Method-A) and hence is not representative of the bulk. In this paper, a modified method (Method-B) has been discussed for the measurement of curd tension taking into consideration both time and force required to cut the coagulum of curd.

Materials and Methods

Milk: Samples of fresh milk were collected from the pooled bulk of buffaloes or crossbred cows. The average fat and total solids content of buffalo milk were 5.8 and 9.1 per cent respectively and the corresponding values for cow milk were 4.4 and 8.7 per cent.

Starter: A mixed cheese culture obtained from the experimental dairy of the Institute was used to coagulate milk.

Glass cylinders: Forty millimeter internal diameter and 300 mm length with a central hole of 22 gauge at the bottom, were used.

Blade: Tinned mild steel H-shaped blade of $35 \times 40 \times 23$ mm size as shown in Figure 1 was used as the cutting device. The average weight of the blade was 10.5 g.

Wire: A tinned copper wire of 450 mm length and 20 gauge diameter was used to tie the blades.

Pan: A plastic pan with a hook was employed to drop the weight; the pan weighed 15.6 g.

Analysis: The fat and total solids content of the milk were determined by the ISI procedures[3,4].

Homogenisation: Homogenisation was carried out in a two stage Gaulin homogenizer.

pH: The pH of milk was measured by a double electrode, 'Elico' pH meter and it ranged between 4.55 and 4.60.

The glass cylinder was held vertically by fixing to a steel stand. The cutting blade was fixed at the upper end of the wire after passing it through the bottom hole of the cylinder. The lower end of the wire was made into a loop to enable hanging of the pan. The hole at the bottom of the tube was closed with wax (Fig. 1).

Three hundred ml. of boiled, cooled milk was mixed with 2 per cent starter culture and allowed to set at 30 ± 1°C. After 8 hr, the cutting blade hitherto supported on the wall of the cylinder was made to rest on the coagulum, and weights were added until the blade started

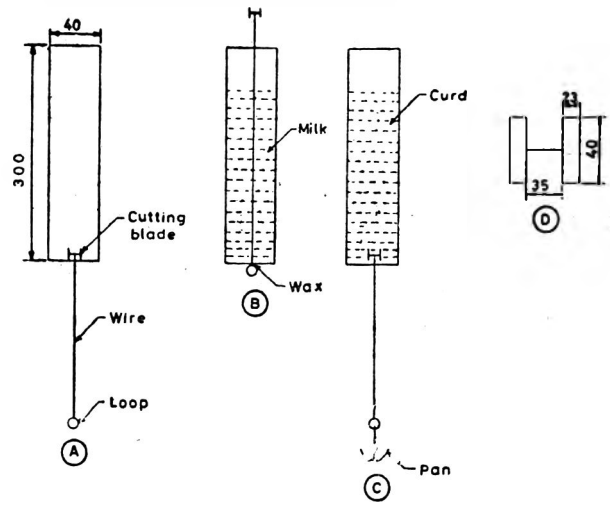

Fig. 1. Arrangement showing the measurement of curd tension

(A) Tube with cutting blade; (B) Milk setting; (C) After cudr tension measurement; (D) Cutting blade.

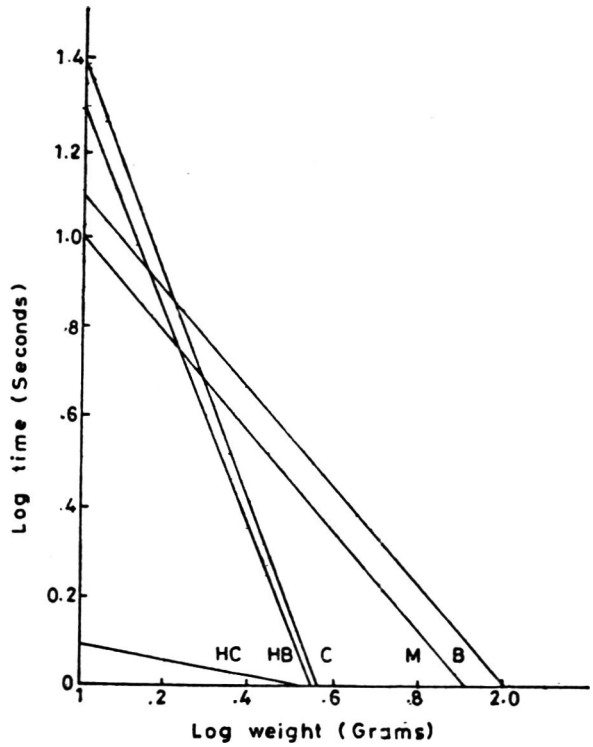

Fig. 2. Curd tension measurement graph.

HC—Homogenised cow milk 1000 lbs. HB—Homogenised buffalo milk 1000 lbs. M—Mixed milk. C—Cow milk. B—Buffalo milk.

cutting through the coagulum. The time taken for the blade to pass through the coagulum was recorded. Then the area under the plot of weights and time was taken as curd tension of the sample (Fig. 2).

Results and Discussion

The mean curd tension of cow and buffalo milk measured by methods A and B has been presented in Table 1. The values obtained by method A are in agreement with the values reported by Rao *et al.*[5] and Jairam *et al.*[6]. This however, reflects the maximum curd tension of milk at a particular point and hence not the uniform curd tension of the system, and since Method B took into consideration both time and weight the curd tension was significantly lower than that of Method A.

Cow milk: The curd tension measured by the Method B (mean, 40.4) is significantly ($P<0.01$) lower than that measured by Method A (mean, 47.57).

The values ranged from 35.9 to 47.2 g sec as measured by new Method B and from 41.0 to 52.2 g as measured by the old method.

Buffalo milk: The mean values by the new and old methods are 54.16 g sec and 86.38 g, respectively (Table 1). In this case also the values obtained by the two methods are found to differ greatly (t 91-76), the new method giving substantially lower values. The range of curd tension by the two methods is found to be 50.0-57.0 g sec and 80.6-92.0 g, respectively. The difference in the curd tension between cow and buffalo milk is observed to be 35.26 per cent (in terms of percentage increase) by the new method and 81.59 per cent by the old method indicating that the curd tension values obtained in buffalo milk is much higher than the value obtained by Method A.

Mixed milk: The mean values observed were 44.98 g sec and 74.22 g by the new and old methods respectively. The values were found to be significantly different (t 74.16). The range of curd tension was observed to be 42.1-47.1 g sec by Method B and 71.4-77.8 g by Method A. The difference in mean curd tension of mixed milk in comparison with cow milk was found to be 12.33 per cent (in terms of per cent difference) when measured by the new method (Method B) and 56.02 per cent when measured by the earlier method (Method A).

Homogenised milk: The mean curd tension values recorded by the two methods are listed in Table 1. As in the case of other milks, the significant 't' values of 94.47 for homogenised cow milk and 20.42 for homogenised buffalo milk indicate that there was an appreciable difference in the curd tension values obtained by two methods. The range of curd tension values by Method B and Method A were from 5.1 to 6.0 g sec and from 14.6 to 16.4 g respectively for cow milk and 32.1 to 36.3 g sec and 37.8 to 39.9 g respectively for buffalo milk. The reduction in curd tension due to homogenisation in cow milk was 85.58 per cent as measured by Method B and 31.82 per cent as measured by the Method A and the corresponding values for buffalo milk were 37.25 and 54.87 per cent respectively.

TABLE 1. CURD TENSION MEASUREMENT BY DIFFERENT METHODS

Method	Cow milk	Buffalo milk	Mixed milk	Homoginised (1000 lbs) Cow milk	Buffalo milk
Method-A (g)	41.0–52.2 (47.57)	80.6–92.0 (86.38)	71.4–77.8 (74.22)	14.60–16.40 (5.14)	37.8–39.9 (38.98)
Method-B (g sec.)	35.9–47.2 (40.04)	50.0–57.0 (54.16)	42.1–47.1 (44.98)	5.10–6.05 (5.77)	32.1 36.3 (33.98)
't' value	26.51*	91.76*	74.16*	94.47*	20.42*

Figures in parenthesis indicate mean. *Statistically significant

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Bacterial Quality of Fresh Mutton from Market

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The levels of bacterial contamination in market samples of fresh mutton examined during 1978 to 1981 ranged from 10^3 to $> 10^5$/g which could be categorised into low, middle and high. Contamination in the laboratory controlled samples was low. The incidence was not related to the meteorological conditions. There was 22 to 5 log increase in contamination levels on exposure to atmospheric conditions for 6-8 hr.

In India, animals are slaughtered in unhygienic abattoirs. Meat is retailed in small quantities from hung carcasses throughout the day. There is considerable variation in the microbiological quality as the carcass is exposed to ambient temperature, atmospheric and bacterial load due to handling practices.

Observations on the levels of contamination in meat over a period of $3\frac{1}{2}$ years are reported and discussed with reference to the available relevant meteorological data, in this paper. The range in which the contamination occurred has been categorised and the bacterial quality of the meat after 6-8 hr exposure to atmospheric conditions has also been ascertained.

Materials and Methods

Sample, source and size: Most of the samples were obtained from the same shop except on occasions when the purpose was to compare the meat quality from different sources. Postmortem age of the meat at the time of sampling was about 3-5 hr.

a) One to 2 kg mutton was purchased from the market in the mornings on sampling days and brought to the laboratory wrapped in clean cellophane sheets and analysed immediately. Sampling was carried out 3-4 times a month during 1978, 1979, 1980 and part of 1981 and 1982. In all, 200 samples were analysed.

b) On six separate occasions mutton from two meat stalls in the city was sampled at the same time. Simultaneously, samples were also obtained from selected animals ($1\frac{1}{2}$ years old, live weight 22 kg), slaughtered under supervision in the laboratory. In this experiment, two 200 g samples were obtained from each source and each sample was analysed in duplicate.

c) The bacterial quality of samples with increasing levels of initial contamination was monitored over a 6-8 hr period in 12 samples. Four laboratory samples (3 with TPC of 10^3/g and one with TPC of 10^4/g) and 8 market samples (TPC of 10^4/g and above) were exposed to ambient atmosphere conditions in the laboratory at a temperature of 25 ± 2°C and relative humidity of 70 ± 5 per cent.

Meteorological data: Monthly averages of temperature, relative humidity and rainfall for the sampling period were obtained from the Central Observatory of Government of India, Bangalore. Total moisture content of the air was calculated[1] from the relative humidity and temperature values.

Bacteriology: Ten gram samples of meat were aseptically macerated in 90 ml of sterile 0.1 per cent (W/V) peptone water. One ml aliquots from 10-fold serial dilutions were plated out on plate count agar[2] and incubated for 24-48 hr at 30°C for the enumeration of total aerobic mesophilic bacteria (TPC). Media were prepared from bactograde ingredients of Difco (Detroit, USA) origin. The counts of bacteria have

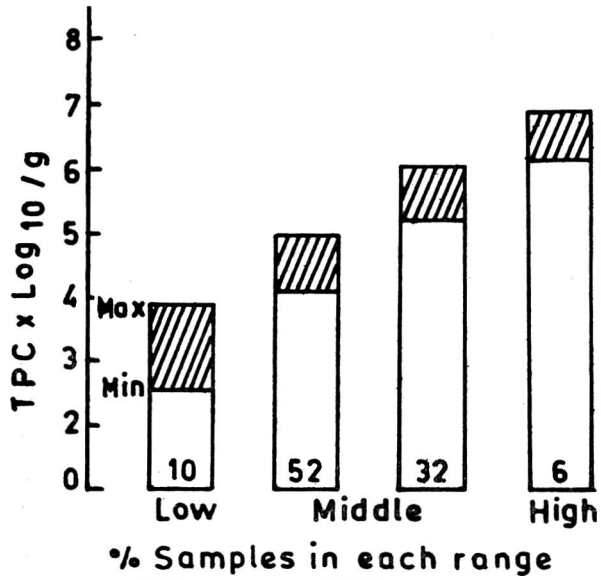

Fig. 1. Range of TPC in 200 fresh meat samples.

been converted to $\log_{10}$ values and the mean values are reported.

Results and Discussion

The incidence of bacterial contamination in 200 market samples of meat collected over a period of 43 months (1978-1981) could be grouped in 3 ranges (Fig. 1) depicted as low, middle and high. The majority of samples had bacterial load ranging from 10^4 to 10^5/g (middle level). A small percentage of samples however, had 10^3/g and less (low level) or $>10^6$/g (high level) of bacteria. Samples obtained at the same time from 3 sources on different days (Table 1), showed variation from low to early high, whereas the laboratory controlled samples on all occasions were found to be in the low range.

Bacterial load of fresh mutton samples, with initial low count, increased by 2-5 log upon exposure to the atmospheric conditions for several hours with a total post-mortem age of 10-12 hr (Fig. 2), While the initial 10^3/g or more counts increased to 10^8/g finally. In one case however, even higher counts (nearly 10^9/g) were observed in a sample with an initial level of 10^4/g. Slight off odour was detected when the TPC rose above 10^7/g. TPC of 10^8 to 10^9/g were associated with distinct off-odour, but samples were not slimy.

TABLE 1. COMPARISON OF BACTERIAL QUALITY OF MUTTON OBTAINED SIMULTANEOUSLY FROM THREL SOURCES

Sampling day	Bacterial counts $\times \log^{10}$/g		
	Market samples		Lab. samples
	Source A	Source B	
1	4.3	4.4	3.0
2	4.6	3.2	3.4
3	5.2	5.0	3.2
4	3.4	4.5	3.8
5	4.2	5.4	3.6
6	5.9	4.6	3.0

Two samples from each source were analysed in duplicate. The mean of the four values is reported.

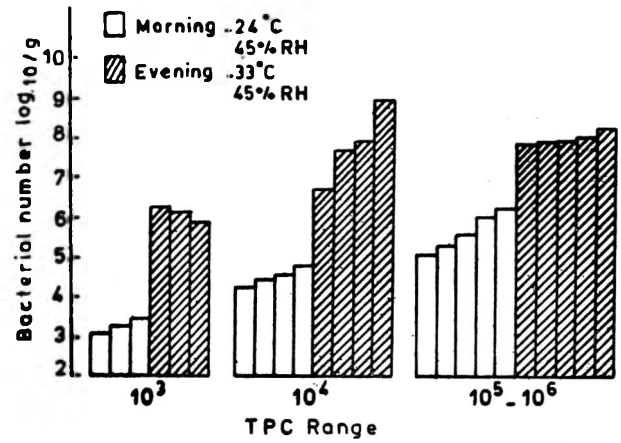

Fig. 2. Bacterial quality of raw mutton in April-May.

The monthly average total counts observed during 1978-81 were plotted against the corresponding weather data (Fig. 3).

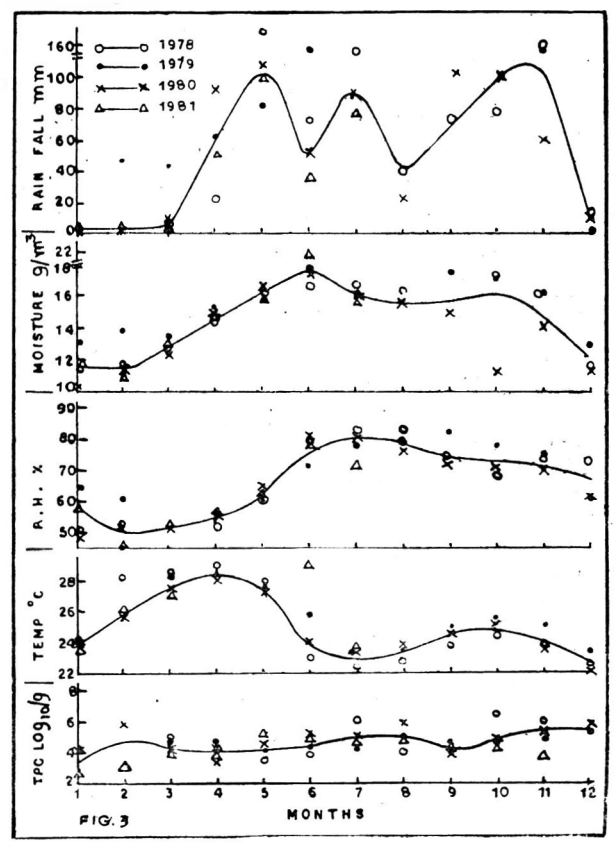

Fig. 3. Weather data and incident TPC/g in fresh raw mutton during 1978-1981

TABLE 2. LOWEST AND THE HIGHEST OBSERVED TPC VALUES (MEAN MONTHLY) AND THE CORRESPONDING METOROLOGICAL DATA FOR 1978–1981 IN MYSORE

TPC (x log ^{10}/g)		Date	Temperature (°C)					% Relative humidity			Moisture content	Total rainfall
Lowest	Highest		Average	Max.	Min.	at 0830 hr	at 1730 hr	Mean RH (%)	0830 hr	1730 hr	(g/m^3)	(mm)
3.2		May '78	28.0	33.6	21.6	24.7	31.2	61.2	78	45	16.06	168.5
4.2		Apr '79	28.4	34.3	21.9	23.5	33.2	58.5	81	36	15.25	128.9
3.4		Apr '80	28.1	33.5	21.1	23.8	32.4	57.5	79	36	15.51	92.8
3.4		Sep '80	24.6	29.7	19.3	21.3	27.4	72.0	86	59	14.89	100.2
2.4		Jan '81	23.4	28.9	16.6	19.0	27.7	58.5	79	38	11.90	5.4
	5.8	Jul '78	21.6	26.5	20.7	22.0	21.1	82.5	87	78	15.25	128.9
	6.4	Oct '78	25.2	29.1	19.7	22.9	27.4	69.0	81	58	15.53	70.5
	6.2	Nov '78	23.9	27.6	17.9	21.6	26.2	75.0	81	66	15.44	143.3
	5.2	Aug '79	23.4	26.9	18.3	21.2	25.5	77.5	85	70	15.77	NA
	5.6	Nov '79	23.8	27.5	19.5	22.0	25.6	75.5	85	66	15.85	128.6
	5.9	Feb '80	25.7	31.3	17.9	21.1	30.3	51.0	68	34	11.76	0.0
	5.9	Aug '80	23.8	27.0	19.3	21.9	25.6	76.5	86	67	16.06	23.8
	5.2	May '81	27.3	33.0	20.5	24.1	30.4	62.0	79	45	15.70	100.8

NA: Not available.

While the weather data curves for Mysore city followed a seasonal pattern from year to year (the same general trend has been recorded over 30 years), the same could not be said of the levels of TPC (Fig. 3). No definite pattern of TPC was observed. In relation to weather, the high TPC recorded in 1978 and 1980 occurred at different seasons of the year. The data for 1979 and 1981 showed no clear demarcation between high and low values, the TPC being similar after July.

When the variation in TPC was examined in relation to meteorological data, no correlation could be observed (Table 2). For example, under comparable weather situations, widely different TPC were recorded on October '78 and September '80. Again, the same high TPC of 5.9 was recorded when the weather conditions varied (February and August '80).

It is evident from the data that the variation in the TPC levels on fresh meat cannot be correlated to weather. Micozzi and Palarchi[3] of Italy reported similar conclusions eventhough definite correlation existed between the psychrotrophic spoilage bacteria in the chill room and abattoir contamination during different seasons[4]. Microbiological status of the laboratory slaughtered sheep carcasses was found to be better, while the meat from the butcher shops showed great variation in quality. Hygiene and sanitation[5-10] being one of the factors responsible for this variation the probable existence of inoculum pockets in the immediate environs of the shops cannot be ruled out. The weather conditions also favour survival and proliferation, of mesophilic organisms at all times. The spoilage of meat in India, should be studied in relation to the mesophiles which proliferate in warm temperatures and survive even at low temperatures. The studies prove conclusively that (*a*) there is considerable variation in the bacterial status of fresh mutton independent of weather conditions; (*b*) exposure of fresh mutton for 6-8 hr to atmosphere in the butchers' shops leads to rapid proliferation of the contaminants, and that (c) personnel and stall are important contributory factors for proliferation in Mysore.

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Quality Changes and Shelf-life of Pearl Spot, Mullet and Tilapia During Storage at Ambient Temperature and in Ice

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Data on changes occurring in the quality and shelf-life of Pearl spot (*Etroplus suratensis*), Mullet (*Liza corsula*) and Tilapia (*Tilapia mossambica*) during storage at ambient temperature (28-30°C) and in ice (0°C) are presented. The shelf life of Tilapia was 10 hr and 11 days at ambient temperature and in ice respectively, of Pearl spot 10 hr and 12 days and of Mullet 8 hr and 6 days. The corresponding changes in total bacterial count, total volatile nitrogen and Intelectron Fish Tester VI readings are also given.

Pearl spot (*Etroplus suratensis*), Mullet (*Liza corsula*) and Tilapia (*Tilapia mossambica*) are important edible fishes of Kerala, the first two being excellent table species. These are marketed fresh, either iced or uniced, and small quantities are frozen and marketed. In domestic trade, fish and fish products are not subjected to inspection or quality control due to lack of information on quality characteristics and methods of assessment. Though there are divergent views about the usefulness of individual tests for quality assessment, a group of tests will be more valid than any single test. Fishes of Pearl spot, Mullet and Tilapia stored at ambient temperature or in ice at 0°C were assessed for the changes in their physical, biochemical, bacteriological and sensory characteristics and the results are presented in this communication.

Materials and Methods

The fishes were procured from fresh/brakish water situated 10-25 km around Cochin. A portion of these fishes was kept without icing and the second portion put under crushed ice and brought to the laboratory. The uniced fish was kept at room temperature (28-30°C) and the iced fish was re-iced everyday and kept in an ice box. Samples were drawn from both the lots periodically and analysed for total volatile nitrogen (TVN) and total plate count (TPC). The readings on Intelectron Fish Tester VI and changes in the sensory characteristics of the fishes were recorded. Taste panel scoring of the fish was conducted after boiling the dressed fish in 2 per cent brine for 10 min. TVN was estimated from the trichloroacetic acid extract by the method of Conway[1] and TPC by ISI method[2]. The colonies were counted after incubating the plates at 37°C for 48 hr, at room temperature for 72 hr and at 5-10°C for 15 days.

Results and Discussion

It is seen from the Tables 1 and 2 that Tilapia kept for 10 hr at ambient temperature and 11 days in ice was acceptable. The tables also give the corresponding changes in Intelectron Fish Tester readings and TVN Values. Storage studies kept at ambient temperature indicated, that no significant change was observed in the Intelectron Fish Tester readings, while those stored in ice showed significant changes; organoleptically also, the fish was unacceptable. The TVN values increased proportionately and

TABLE 1. QUALITY CHANGES IN TILAPIA DURING STORAGE AT AMBIENT TEMPERATURE

Storage period (hr)	Intelectron reading	TVN (mg %)	Sensory characteristics Eyes	Gills	Texture	Appearance	Organoleptic score Max. 10
1	72	1.25	Convex clear black	Bright red	Firm	Black	9
3	66	3.2	Flat, clear black	Red slimy	Firm	White patches on surface	8
6	68	10.3	Sunken, slightly turbid	Darker, slight spoiled odour	Firm	Almost white	6
8	60	18.5	Sunken, more turbid	Darker, moderate spoiled odour	Firm	Slight reddish tinge	5
11	48	30.1	Sunken, more turbid	Black, highly spoiled	Firm	50% vent open gas on stomach	2

the values of TVN in iced Tilapia was less than the TVN value of Tilapia stored at room temperature. This may be due to the leaching effect in ice. While TPC at 5-10°C was found to be the highest, at 37°C, it was not of significance indicating that it is inapplicable as a quality index for iced fish. Considering the longer incubation time required at 5-10°C, incubation at RT can be taken as the most suitable. One significant observation noticed in Tilapia during storage at ambient temperature is the change in the colour of the fish. The fish stored at ambient temperature which was originally black in colour changed to white during storage and finally attained a prominent reddish tinge when spoiled. This important observation was not noticed when the fish was stored in ice.

As noticed in the case of Tilapia, the changes in

TABLE 2. QUALITY CHANGES IN TILAPIA DURING ICE STORAGE

Period of storage (days)	Intelectron readings	TVN (mg%)	Total plate count/g 37°C	28-30°C	5-10°C	Sensory characteristics Eyes	Gills	Texture	Organoleptic score Max. 10
0	72	1.25	50	3.0×10^3	3.1×10^4	Convex, bright, clear	Bright and characteristic odour	Firm	9
3	40	7.0	—	—	7.8×10^3	Flat slightly milky	Pink to fleshy slime no odour	Firm Finger impression disappears	7
6	34	8.5	156	3.1×10^4	1.1×10^5	Sunken milky	Pink to fleshy slime no odour	Impression disappears slowly. Scales slightly loose	5
9	26	15.4	78	7.0×10^4	6.1×10^5	Sunken milky red cornea	Bleached slightly spoiled odour	Permanent impression. Scale easily removable	4
11	22	18.2	165	6.9×10^5	3.2×10^6	Sunken milky red cornea	Bleached spoiled odour slime exudes through mouth	Permanent impression. Scales and flesh easily removable	3

All samples possessed the characteristic black colour.

TABLE 3. QUALITY CHANGES IN PEARL SPOT DURING STORAGE AT AMBIENT TEMPERATURE

Storage period (hr)	Intelectron reading	TVN (mg %)	Sensory characteristics				Organoleptic score Max. 10
			Eyes	Gills	Texture	Appearance	
0	66	2.5	Convex clear	Bright red characteristic muddy odour	Firm	Bright characteristic colour	9
2	66	6.5	Convex clear	Pale red; characteristic odour	Firm	Bright; slight sliminess; appearance slight yellowish tinge	9
4	64	18.2	Slightly flattened clear	Pale red; neutral odour	Firm; thumb impression disappears	Slight sliminess; yellowish tinge all throughout	7
8	52	30.2	More flattened opalescent	Pale, slimy, moderate spoiled odour	Impression disappears slowly	Same as above; bulging of belly portion	4
10	50	45.6	Flat	Pale, slimy moderate spoiled odour	Impression completely does not disappear	Same as above; bulging of belly portion, more intense	3

the Intelectron Fish Tester reading of stored Pearl spot was not significant during storage at ambient temperature (Tables 3 and 4), while significant changes were noticed in the ice stored sample thus indicating the usefulness of the instrument to measure the quality of iced fish. The shelf-life of the fish was 10 hr and 12 days at ambient temperature and in ice respectively. TPC at 37°C was found to be the lowest and remained almost constant throughout the storage, whereas at room temperature and at 5-10°C it increased. The colour of Pearl spot stored at ambient temperature changed from black to yellow gradually, whereas fish stored in ice did not.

Tables 5 and 6 give the changes in the quality of Mullet during storage at ambient temperature and in ice respectively. Similar findings were made in the

TABLE 4. QUALITY CHANGES IN PEARL SPOT DURING ICE STORAGE

Period of storage (days)	Intelectron readings	TVN (mg%)	Total plate count/g			Sensory characteristics				Organoleptic score Max. 10
			37°C	28-30°C	5-10°C	Eyes	Gills	Texture	Appearance	
0	62	2.5	6×10^3	2×10^3	2.5×10^3	Convex clear	Bright red, characteristic muddy odour	Very firm	Characteristic colour	9
3	46	15.0	2.2×10^3	2.7×10^3	1.3×10^3	Flat slightly milky	Red, slight muddy odour	Firm	Characteristic colour	7
7	32	24.0	7.0×10^3	8.8×10^4	9.6×10^4	Sunken milky	Slightly pale, slimy, slight fishy odour	Finger impression disappears very slowly	Characteristic colour	5
10	26	36.0	5.2×10^3	5.0×10^5	1.0×10^6	Sunken milky	Pale, slimy, fishy odour	Finger impression disappears very slowly	Characteristic colour	4
12	20	40.0	$4.0<10^2$	1.5×10^6	2.6×10^6	Sunken milky	Pale, slimy, slight spoiled odour	Soft impression retains	Dull	3

TABLE 5. QUALITY CHANGES IN MULLET DURING STORAGE AT AMBIENT TEMPERATURE

Storage period (hr)	Intelectron reading	TVN (mg %)	Sensory characteristics				Organoleptic score Max. 10
			Eyes	Gills	Texture	Appearance	
0	62	2.4	Convex clear	Bright red, characteristic odour	Firm	Characteristic bright colour	9
3.5	60	10.6	Convex clear	Red, slimy natural odour	Firm	Characteristic slight mucous	7
5.5	58	18.5	Slightly flat, clear	Slightly darkned, slimy slight spoiled odour	Firm	Surface slimy; increase in mucous; vent protruded	5
7.5	52	25.6	Almost flat, slight opalescent	Gray, slimy, moderate spoiled odour	Soft; thumb impression disappears slowly	Surface slimy; increase in mucous; vent protruded	4

TABLE 6. QUALITY CHANGES IN MULLET DURING ICE STORAGE

Period of storage (days)	Intelectron readings	TVN (mg%)	Total plate count/g			Sensory characteristics				Organoleptic score Max. 10
			37°C	28-30°C	5-10°C	Eyes	Gills	Texture	Appearance	
0	58	2.4	1.1×10^3	$2.0 \cdot 10^3$	4.1×10^3	Convex clear	Bright red; characteristic odour	Firm	Characteristic	9
3	22	6.2	5.6×10^3	7.6×10^4	9.8×10^4	Flat milky	Fleshy colour; slimy, neutral odour	Firm vent open; Swelling of belly	Characteristic	6
6	16	8.1	2.5×10^4	2.3×10^5	6.5×10^5	Sunken milky	Pale; slimy; slightly spoiled odour	Soft; thumb impression disappears slowly; vent open; belly very soft		4
8	12	19.2	2.3×10^4	3.5×10^5	2.7×10^6	Sunken milky red cornea	Pale; slimy putrid odour	Soft; impression retains; belly broken; completely	Slightly dull	2

case of Mullet on storage. Based on organoleptic evaluation, the shelf-life of Mullet was found to be 8 hr and 6 days at ambient temperature and in ice respectively. The important changes observed in the case of Mullet stored in Ice was the bursting of belly and the protrusion of the vent and the formation of gas in the belly of those kept at ambient temperature.

Acknowledgement

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Bacillus cereus Enterotoxin and Its Production in Different Foods

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Three test strains of *Bacillus cereus* synthesised enterotoxin in the early log phase; maximum activity was observed in late exponential phase. Culture filtrate from stationary phase did not reveal the presence of enterotoxin. The optimum pH range for production of enterotoxin was between 8.0 and 8.5. NaCl (1.5%) and $NaNO_2$ (1000 ppm) inhibited the growth as well as enterotoxin production in strains B-4ac and B-4ac-L. $NaNO_3$ above 2000 ppm inhibited enterotoxin production whereas the growth was not inhibited even at 3000 ppm. NaCl above 1.5% inhibited the growth of strain NCTC 2599 as well as production of enterotoxin while $NaNO_2$ (upto 2000 ppm) and $NaNO_3$ (upto 3000 ppm) had no effect on the growth as well as production of enterotoxin. Rice and bajra supported the growth of B-4ac-L as well as production of enterotoxin while bread, soybean and the baby foods, Banalona and Farex supported growth, but failed to support production of enterotoxin.

Bacillus cereus commonly considered to be a harmless saprophyte can be a significant source of both human and animal infections[1,2] and also a source of food poisoning[3,4].

The organism is reported to produce several extracellular products such as hemolysin, phospholipase and a lethal factor responsible for food poisoning outbreaks[5]. In recent years, an enterotoxin responsible for enteropathogenicity[6] has been associated with organism, which is distinguishable from hemolysin and phospholipase. Enterotoxin produced during growth of *B. cereus* is known to cause fluid accumulation in ligated ileal loops of rabbit[7], dermal reaction in guinea pigs[8] and alters vascular permeability in the skin of rabbits[9].

The present study was undertaken to understand the cultural conditions for enterotoxin production by this organism and the production of enterotoxin in some of the infected food materials under laboratory conditions.

Materials and Methods

Strains: Two strains of *Bacilius cereus* B-4ac and B-4ac-L were obtained from Dr. B. A. Glatz, Iowa State University of Science and Technology, Ames, Iowa. Strain B-4ac is a standard strain which elicits all enterotoxic activities. Strain B-4ac-L, a variant of strain B-4ac is known to produce higher toxin levels more consistently. *B. cereus* strain NCTC 2599 was obtained from National Collection of Type Cultures, Central Public Health Laboratory, Collindale Ave. London, England. These cultures were maintained on nutrient agar slants at 4°C after growth overnight and sub cultured at weekly intervals.

Inoculum: Inocula were prepared by transferring a loopful culture into 25 ml of nutrient broth in a 100 ml Erlenmeyer flask and incubating overnight (10-14hr) at 32°C on a roatary shaker.

Growth and enterotoxin production medium: Modified Casamino acid medium (CA)[10] with the following composition (g/l): was used, casamino acid (Difco), 20; yeast extract, 6; sodium chloride, 2 5; K_2HPO_4, 8.71; and trace element solution, 1.0 ml. (0.5 g of manganese chloride, 0.5 g of ferric chloride dissolved in 0.001 N H_2SO_4 and 5.0 g of magnesium sulphate in 100 ml of distilled water); the pH was adjusted to 8.0 with 5 N NaOH or 5 N HCl. Hundred ml of the medium was dispensed in 500 ml Erlenmeyer flasks and autoclaved at 121°C for 15 min.

Each flask was inoculated with 1 per cent inoculum of the particular strain of *B. cereus* and incubated on rotary shaker at 32°C for 8 hr. The culture filtrate was centrifuged at 15,000 rpm for 30 min at-5°C. Ten ml of the culture filtrate was dialysed against polyethylene glycol-6000 at 4°C to obtain a ten fold concentration in about 4 to 6 hr. These were stored at—20°C until use.

Enterotoxin production in various food materials by strain B-4ac-L: The food materials rice, bajra, soyabean, bread, Farex and Banalona were used for this study. Rice, bajra, soybean and dried bread were ground to fine powder. Ten grams of each food was added to 500 ml flask containing 100 ml distilled water and the flasks were autoclaved at 121°C for 15 min.

Each flask was inoculated with B-4ac-L strain at 10^6 cells/g of food material. One set of flasks was incubated on the rotary shaker at 32°C, while second set was kept under stationary condition at the same temperature. Flasks were removed at 8, 12 and 24 hr intervals, the contents centrifuged and the supernatant was concentrated as before. For enterotoxigenic activity, 0.1 ml of concentrated sample was injected into rabbit's skin. Enterotoxin assay was performed by altered vascular permeability technique[9]. A capillary permeability factor unit (CPFU) is defined as the amount of toxin which elicits an area of blueing of 5 mm diameter.

Results

Enterotoxin production during growth of B. cereus: To determine the time at which enterotoxin synthesis begins and the quantity produced, all the three strains were grown in the CA medium[10]. Samples for growth measurements were drawn at hourly interval, while for the enterotoxin assay, samples were drawn at 2, 5, 8, 11, 14, 17 and 24 hr.

With B-4ac-L strain, enterotoxin production started after 2 hr, reached maximum at 5 hr, and declined thereafter. At the end of 8 hr, the activity was 50 per cent, and remained at this level upto 14 hr. Very little activity was detected at the end of 17 hr and the activity was absent at the end of 24 hr. The strain B-4ac exhibited almost similar pattern. Measurable amount of enterotoxin was detected even at 2 hr of growth when NCTC 2599 strain was grown which reached a maximim at 11 hr, where after it declined to negligible amount at 17 hr and at 24 hr growth (Fig. 1).

Table 1. Effect of 24 hour culture filtrate on growth and enterotoxin production

Culture filtrate quantity (ml)	O.D. (560 nm)	CPFU/ml
1.0	1.525	16.0
2.0	1.165	13.0
3.0	1.040	12.0
5.0	0.825	4.0
10.0	0.699	Nil
0	1.740	16.0

Time of incubation with culture filtrate was 8 hr.
CPFU: Capillary permeability factor unit.

Absence of enterotoxin in 24 hr culture filitrate obtained from shake culture flask may be attributed to the degradation of enterotoxin or detoxification by proteases or inhibitors synthesised in early or late stationary phase. This was confirmed by adding a 24 hr culture filtrate to a 8 hr culture to study the effect on the growth and production of enterotoxin. Table 1 shows that addition of increasing amounts of 24 hr culture filtrate suppressed the growth as well as enterotoxigenic activity. Five ml culture filtrate inhibited

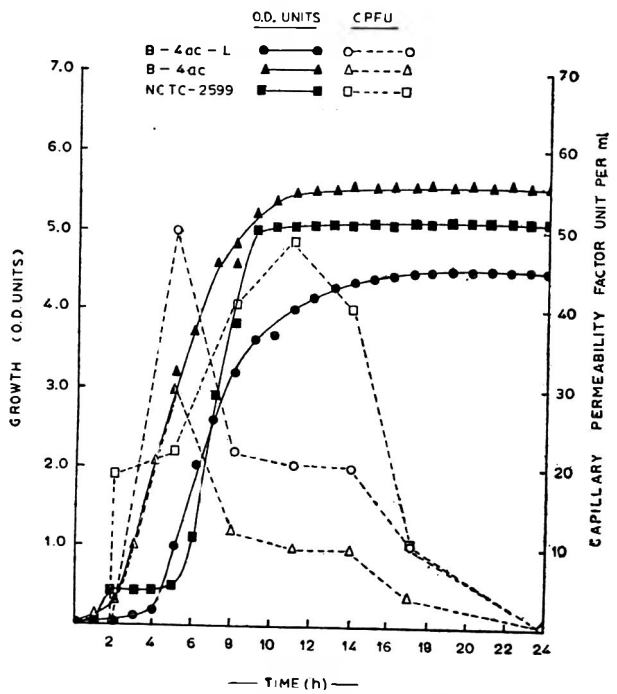

Fig. 1. Enterotoxin production during growth cycle of different strains of *Bacillus cereus*.

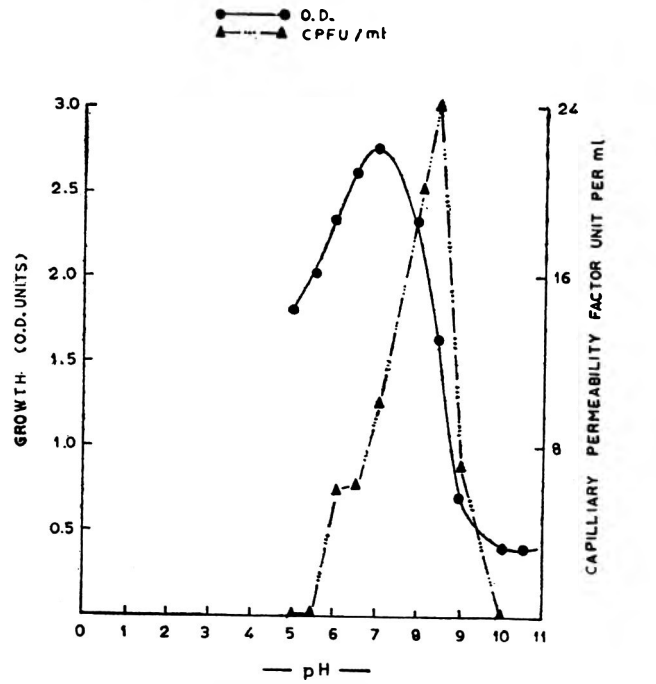

Fig. 2. Effect of pH on enterotoxin production in *Bacillus cereus* strain B-4ac-L.

about 75 per cent activity, while 10 ml culture filtrate eliminated the activity completely.

Effect of pH on enterotoxin production: The three strains were inoculated at 1 per cent level to CA medium of different pH ranging from 5.0 to 10.5. The flasks were kept under aerated condition at 32°C and after 8 hr, optical density of the filtrate was measured and enterotoxin determined.

Strain B-4ac-L showed good growth over a pH range of 5.0 to 9.0, however; the optimum range of pH for enterotoxin production was between 8.0 and 8.5. This strain produced enterotoxin between pH 6.0 and 9.0 (Fig. 3). The growth pattern as well as production of enterotoxin was almost similar in strain B-4ac and strain NCTC 2599 (Fig. 4). The optimum pH range for the production of enterotoxin with all the strains was between 8.0 and 8.5, although good growth was observed at other pH values.

Effect of salts on enterotoxin production: Different amounts of sodium chloride, sodium nitrite and sodium nitrate were added into CA medium to give a final concentration which varied between 0.25 and 10.0 per cent of sodium chloride; 100 and 2000 ppm of sodium nitrite and 500 and 3000 ppm of sodium nitrate. The effects of these variations on the growth and enterotoxin production were studied after 8 hr.

Sodium chloride above 1.0 per cent concentration strongly inhibited the growth as well as production of enterotoxin in B-4ac-L and above 1.5 per cent in strains B-4ac and NCTC 2599 (Table 2). Similarly, strains B-4ac-L and B-4ac showed good growth and enterotoxin production in the medium containing 1000 ppm of sodium nitrite. The growth was appreciably decreased at 2000 ppm, while enterotoxin production was completely inhibited. However, strain NCTC 2599 did not show appreciable change in growth as well as production of enterotoxin between 100 and 2000 ppm of sodium nitrite (Table 3). Data in Table 4 show that sodium nitrate at a concentration of 500 to 3000 ppm had no effect on the growth of strains B-

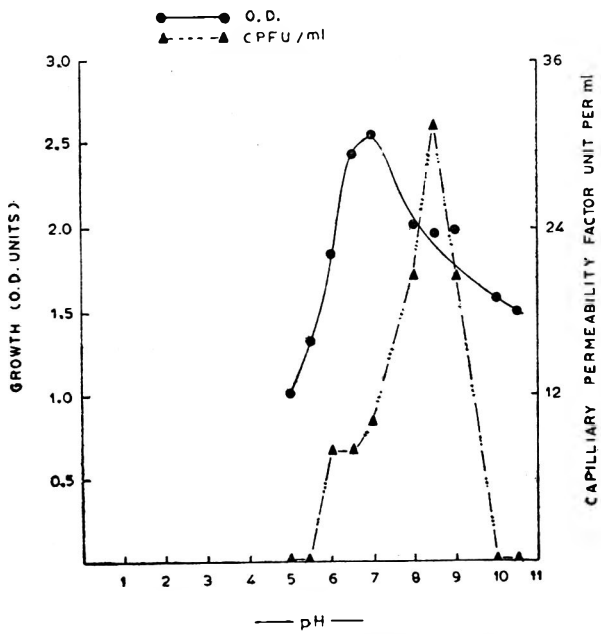

Fig. 3. Effect of pH on enterotoxin production in *Bacillus cereus* strain B-4-ac

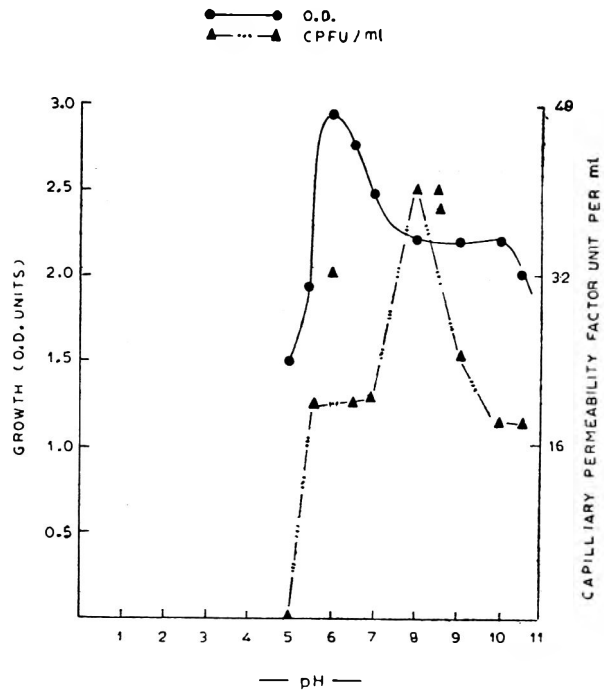

Fig. 4. Effect of pH on enterotoxin production in *Bacillus cereus* strain NC TC 2599

TABLE 2. EFFECT OF SODIUM CHLORIDE ON GROWTH AND ENTEROTOXIN PRODUCTION BY DIFFERENT STRAINS OF *BACILLUS CEREUS*

Concn of NaCl (%)	B-4ac-L		B-4ac		NCTC 2599	
	O.D. at 560 nm	CPFU/ml	O.D. at 560 nm	CPFU/ml	O.D. at 560 nm	CPFU/ml
0.25	3.280	20.0	3.010	24.0	3.870	18.0
0.5	3.190	16.4	3.100	13.2	4.380	12.0
1.0	1.250	8.4	1.340	7.2	3.100	12.0
1.5	0.704	—	0.870	4.8	2.330	6.0
2.0	0.362	—	0.734	—	0.276	—
3.0	0.036	—	0.125	—	0.046	—
5.0	0.024	—	0.041	—	0.020	—
10.0	0.000	—	0.022	—	0.000	—

Figures are average of two trials.
(–) Enterotoxigenic activity absent.

TABLE 3. EFFECT OF SODIUM NITRITE ON GROWTH AND ENTEROTOXIN PRODUCTION BY DIFFERENT STRAINS OF *BACILLUS CEREUS*

Concn of $NaNO_2$ (ppm)	Strain B-4ac-L		Strain B-4ac		Strain NCTC 2599	
	O.D. at 560 nm	CPFU/ml	O.D.at 560 nm	CPFU/ml	O.D. at 560 nm	CPFU/ml
100	5.090	20.0	4.320	17.0	3.670	14.0
200	4.260	20.0	3.370	16.0	3.570	13.6
400	4.090	20.0	2.840	16.0	3.280	13.0
600	3.670	18.0	2.010	17.6	3.280	12.0
800	3.190	18.0	1.740	12.4	3.190	12.0
1000	2.920	18.0	1.610	13.0	2.680	15.0
2000	0.805	—	0.469	—	2.620	13.0
Control	5.090	22.0	4.090	15.0	3.720	15.0

Figures are average of two trials.
(–) Enterotoxigenic activity absent.

4ac-L and B-4ac, while enterotoxin synthesis was completely inhibited above 2000 ppm. Strain NCTC 2599 did not show any change in the growth as well as enterotoxin production at all the concentrations of nitrate used.

Enterotoxin production in food materials by B. cereus *strain B-4ac-L:* Data on the production of enterotoxin in food materials by *B. cereus* strain B-4ac-L have been presented in Table 5. Rice and bajra were found to be excellent substrates for growth as well as enterotoxin production under aerated and stationary culture conditions. However, soybean, bread and the baby foods, Farex and Banalona supported the growth of the organism (from 10^6 to 10^9 cells/g), but failed to induce enterotoxin production.

TABLE 4. EFFECT OF SODIUM NITRATE ON GROWTH AND ENTEOTOXIN PRODUCTION BY DIFFERENT STRAIN OF *BACILLUS CEREUS*

Concn of $NaNO_3$ (ppm)	Strain B-4ac-L		Strain B-4ac		Strain NCTC 2599	
	O.D. at 560 nm	CPFU/ml	O.D. at 560 nm	CPFU/ml	O.D. at 560 nm	CPFU/ml
500	5.230	19.0	5.250	16.8	4.820	16.0
1000	5.240	18.0	5.237	19.0	4.740	16.8
1500	5.230	18.0	5.204	17.0	4.760	16.4
2000	5.040	17.0	5.010	18.0	4.740	18.0
2500	5.040	—	5.010	—	4.520	17.0
3000	5.240	—	5.020	—	4.820	14.4
Control	4.740	16.0	5.240	16.0	4.740	17.0

Figures are average of two trials.
(–) Enterotoxigenic activity absent.

TABLE 5. ENTEROTOXIN PRODUCTION BY *BACILLUS CEREUS* STRAIN B-4ac-L IN DIFFERENT FOODS UNDER AERATED AND STATIONARY CULTURE CONDITIONS

Foods	Enterotoxigenic activity at diff. periods (hr)					
	Stationary			Shaker		
	8	12	24	8	12	24
Soyabean	—	—	—	—	—	—
Bajra	—	+	+	+	+	+
Rice	+	+	+	+	+	+
Bread	—	—	—	—	—	—
Farex	—	—	—	—	—	—
Banalona	—	—	—	—	—	—

All used in 10% slurries in distilled water after autoclaving at 121°C for 15 min. Each flask was inoculated with 10^6 cells/g food material.

Discussion

The importance of *Bacillus cereus* in food poisoning outbreaks cannot be ignored. Since enterotoxin is not formed by cells growing within the intestine in the ileal loop model[7], the hypothesis that it is ingested in the preformed state during actual outbreaks is very attractive.

Detection of enterotoxin at an early log phase and late log phase and its absence at late stationary phase suggest that enterotoxin is unstable and may be either degraded by proteases or detoxified by inhibitors being synthesized during early stationary phase. The addition of culture filtrate obtained after 24 hr of growth to the growing culture of *B. cereus* in CA medium, inhibited growth as well as enterotoxigenic activity appreciably, thus indicating the presence of some unknown factors which had degraded or inactivated the enterotoxin. It was also shown that the crude culture filtrate when treated with trypsin and pronase lost enterotoxigenic activity[11]. In our laboratory, we have also found that partially purified enterotoxin when treated with trypsin and pronase completely lost its activity[12]. Recently, it has been shown that the proteolytic enzymes which destroy enterotoxin are membrane associated proteases and the inactivation of enterotoxin in growing culture (fermenter) can be prevented, if continuous supply of glucose and low tension of oxygen are maintained[13].

This organism has been shown[14] to grow under wide range of pH i.e., 4.9 to 9.3. The enterotoxigenic activity of crude culture filtrate of *B. cereus* has been reported[6] to be stable in the pH range of 5.0 to 10.0. It is therefore, clear that it is enterotoxin synthesis and not stability that is pH sensitive. The influence

of pH on enterotoxin production in fermenter grown culture has been studied[15]. The optimum pH for enterotoxin production was found to be 8.0 which coincides with our findings. It has been further shown that control of culture pH in a rather narrow range doubled the enterotoxin production.

Sodium chloride is commonly used in pickling and as canning brine whereas sodium nitrate and nitrite are used in meat curing. The effects of these salts on the growth and enterotoxin production by *B. cereus* show that this organism could tolerate sodium chloride upto 2 per cent level, whereas enterotoxin production was completely inhibited at 1.5 per cent or above. On the contrary, this organism could grow and produce enterotoxin at a fairly high concentration of sodium nitrite and sodium nitrate (1000 ppm and above) even though these are known to inhibit other pathogenic microflora like *Micrococcus*, *Achromobacter*, *Streptococcus* and *Escherichia coli*[16].

Large varieties of food materials such as rice, meat and meat products, vegetable sprouts, etc. have been implicated in food poisoning outbreaks, however, only rice and bajra could support enterotoxin production under laboratory conditions. The results suggest that growth of *B. cereus* has no correlation with enterotoxin production. Foods which did not show enterotoxin production may be lacking in some unknown specific growth factors which are essential to initiate the synthesis of enterotoxin.

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Role of Dietary Fiber from Pulses as Hypocholesterolemic Agent

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Hypocholesterolemic action of dietary fiber from the seed coat of common pulses, viz., green gram, black gram, Bengal gram, peas and lentils have been studied. The seed coat fiber along with the hypercholesterolemic diet was fed, at 5% level to albino rats and lipid levels of plasma, liver, heart and bile acid secretion of the animals determined. The results indicated considerable hypocholesterolemic action of these fibers. Bengal gram showed the maximum hypocholesterolemic effect followed by black gram, green gram, peas and lentils. Hypocholesterolemic action was found to be inversely related to the water holding capacity but no direct relation could be deduced with their bile acid adsorption capacity.

Atherosclerosis is characterized by massive accumulation of cholesterol in the arteries that form the heart of an atheromatous plaque. Epidemiological studies[1-4] have indicated a high level of plasma cholesterol as an important risk factor. There is continued search for agents which can lower the blood cholesterol levels. Pulses which form an important source of proteins in the diet of people from developing countries, are known to be hypocholesterolemic[5-7]. The hypocholesterolemic activity of pulses could be attributed to their carbohydrates[8], proteins[5,6,9] and their unsaturated fats[5]. Involvement of dietary fiber in lowering the blood cholesterol level have been reported[7,10]. Pulses are commonly consumed after decortication and as the seed provides significant quantity of fiber, studies were conducted to assess the potentiality of seed coat fiber of commonly used pulses as hypocholesterolemic agent. Relationship between the physicochemical properties of these fibers and their hypocholesterolemic action was also investigated.

Materials and Methods

The seed coat of the commercial samples of pulses, green gram (*Phaseolus aureus*), black gram (*Phaseolus mungo*), Bengal gram (*Cicer arietinum*), lentils (*Lens esculenta*) and peas (*Pisum sativum*) was removed after soaking in water overninght. Fiber from seed coat was separated by homogenizing, freezing and thawing and finally drying by using acetone, as described by Eastwood and Mitchel[11]. Disease free albino rats (Wistar strain obtained from Haryana Agricultural University, Hissar.) were divided into six groups of 6 animals each. After 10 days of acclimatization, the rats were fed on experimental diets consisting of casein (15 per cent), hydrogenated groundnut oil (20 per cent), cholesterol (1 per cent), Bernhart-Tomeralli salt mixture[12] (4 per cent), vitamin mixture[13] (1 per cent), cellulose or fiber from the seed coat of pulses (5 per cent), and starch (54 per cent). Water and the diet were supplied *ad libitum* to the rats. After four weeks, the rats were sacrificed and the blood was drawn by direct cardiac puncture. The liver and hearts were removed and stored for analyses.

The total lipids of the plasma and the tissues were determined by the method of Folch *et al.*[14] The cholesterol content was estimated by the method of Zak[15] after extraction of the samples with 1:1, v/v alcohol : acetone mixture. The phospholipids were determined by the method of Ames.[16] Glycerides+free fatty acids content was calculated by subtracting total cholesterol and phospholipid values from the total lipid values. The total bile acids in faeces were determined colorimetrically by the method of Boyd *et al.*[17] after their extraction with solvent ether.

The water holding capacity of the different fibers was measured using the method of McConell *et al.*[18] and the absorption of bile acids on the fiber was studied using the method described by Eastwood *et al.*[19].

Results and Discussion

Table 1 gives data on the effect of dietary fibers from pulses on the lipid levels of plasma. It is evident that the cholesterol levels vary with the dietary fiber used. The cholesterol levels (mg/100 ml plasma) in rats fed with fibers from green gram, black gram, Bengal gram, peas and lentils, were significantly ($p < 0.05$) lower than that of the control group. The total lipids and glycerides+free fatty acids were also signi-

TABLE 1. EFFECT OF DIETARY FIBER ON LIPID LEVELS OF PLASMA

Source of fiber	Total lipid (mg/100 ml)	Cholesterol (mg/100 ml)	Phospholipid (mg/100 ml)	Glycerides + FFA (mg/100 ml)
Cellulose (Control)	1029±105	215.4±24.5	265.0±35.4	548.6±56.2
Green gram	910±84*	165.5±21.2**	282.1±20.7	462.4±42.2**
Black gram	785±87**	136.2±16.4**	261.2±23.4	387.6±43.0**
Bengal gram	715±73**	100.8±6.2**	286.7±13.8	327.5±30.6**
Peas	1035±98	192.4±20.3*	290.8±20.1	551.8±48.7
Lentils	924±84*	186.9±18.6*	255.8±27.8	481.3±50.1

*$p<0.05$ Mean±S.D.
**$p<0.01$

TABLE 3. EFFECT OF DIETARY FIBER ON LIPID LEVELS OF HEART

Source of fiber	Total Lipid (mg/g tissue)	Cholesterol (mg/g tissue)	Phospholipid (mg/g tissue)	Glycerides + FFA (mg/g tissue)
Cellulose (Control)	49.1±4.4	2.21±0.24	13.6±1.5	33.29±3.61
Green gram	42.1±2.1**	1.58±0.19**	13.4±0.8	27.12±2.64**
Black gram	43.1±4.2**	1.29±0.11**	13.3±1.5	28.51±3.22*
Bengal gram	44.0±4.8*	1.10±0.08**	13.5±0.8	29.40±3.16*
Peas	49.5±6.2	1.87±0.19*	15.3±1.8	32.33±3.56
Lentils	48.5±5.7	1.70±0.21	13.8±1.6	33.0 ±3.82

*$p<0.05$ Mean±S.D.
**$p<0.01$

ficantly ($P<0.01$) lower in rats fed with fiber from green gram, black gram and Bengal gram, whereas peas and lentil fibers had only a marginal hypolipidemic effect. Effect of fiber on the phospholipid level was insignificant. Bengal gram fiber exhibited the maximum hypocholesterolemic and hypolipidemic effect, followed by black gram and green gram. Tables 2 and 3 give data on the effect of dietary fibers of pulses on the lipid levels of liver and heart. It is evident that feeding of fiber of green gram, black gram or Bengal gram lowered total lipid, cholesterol and glycerides+free fatty acid contents, but did not affect the phospholipid levels of the heart. Effect of peas and lentil fiber was only marginal ($p<0.05$). Effect of these fibers in lowering the lipid levels of liver and heart generally agrees with lipid levels of plasma.

TABLE 2. EFFECT OF DIETARY FIBER ON LIPID LEVELS OF LIVER

Source of fiber	Total lipid (mg/g tissue)	Cholesterol (mg/g tissue)	Phospholipid (mg/g tissue)	Glycerides + FFA (mg/g tissue)
Cellulose (Control)	116.5±12.0	25.6±2.3	13.4±1.5	77.5±8.8
Green gram	83.5±10.5**	18.9±2.1**	12.0±1.6	52.6±6.5**
Black gram	68.7± 7.5**	11.7±2.0**	16.7±1.8**	40.3±4.8**
Bengal gram	39.0± 7.2**	9.8±0.6**	15.5±1.9**	33.7±3.8**
Peas	105.4±11.4*	23.2±2.7	11.0±1.4*	71.2±9.4
Lentils	98.0±11.8*	21.6±2.5*	12.0±1.7	64.4±7.2*

*$p<0.05$ Mean±S.D.
**$p<0.01$

The effect of these fibers on bile acid excretion in faeces of rats is shown in Table 4. It is evident that the bile acid excretion in groups receiving dietary fiber from Bengal gram, black gram, and green gram is significantly higher ($P<0.01$) than that of the control, while the bile acid excretion in peas and lentil groups was not increased. The amount excreted is in agreement with the observed hypocholesterolemic and hypolipidemic trend of different fibers. Thus, it is evident that these fibers increase the catabolism of cholesterol. As fibers are known to absorb bile acids and check

TABLE 4. EFFECT OF DIETARY FIBER FROM PULSES ON BILE ACID EXCRETION

Source of fiber	Bile acids* (mg/24 hr) (mean±SD)
Cellulose (control)	1.82±0.42
Green gram	2.86±0.38[a]
Black gram	2.93±0.41[a]
Bengal gram	3.21±0.43[a]
Peas	2.24±0.32[b]
Lentils	2.33±0.28[b]

*Expressed in terms of cholic acid
a $p<0.01$ b $p<0.05$

TABLE 5. *IN VITRO* ADSORPTION OF BILE ACIDS BY FIBER AND WATER HOLDING CAPACITY OF FIBER FROM PULSES

Source of fiber	% bile acid adsorption*			Water holding capacity (g/g fiber)
	Cholic acid	Deoxycholic acid	Chenodeoxy cholic acid	
Green gram	28.6	51.0	42.5	5.4
Black gram	26.6	52.5	36.8	4.0
Bengal gram	24.5	48.0	29.0	2.6
Peas	22.0	55.0	28.5	5.7
Lentils	24.2	47.5	60.0	6.3
Cellulose (control)	8.4	6.2	6.0	7.8

*500 mg fiber, 100 μ moles of bile acid in 10 ml of 0.1 M, phosphate buffer, pH 7.6, incubated for 2 hr.

their enterohepatic circulation, their feeding will result in the shifting of equilibrium towards increased catabolism of cholesterol to produce bile acids.

In order to confirm whether this is the mechanism of action, of these fibers, the *in vitro* adsorption of bile acids was studied. It is evident (Table 5) that adsorption of deoxycholic acid was more than that of chenodeoxycholic acid and cholic acid. Similar observations have been reported with fibers of fresh carrots, celery, frozen peas and wheat bran[19]. The data also indicate that polarity is an important factor in adsorption, and that more polar a bile acid is, less would be its adsorption. These results indicate that *in vitro* adsorption of bile acids is not correlated with their hypocholesterolemic action. Peas and lentils showed almost equal adsorption of bile acids, but did not show comparable hypocholesterolemic effect. This indicates that there could be other factors involved in the action of fibers as hypocholesterolemic agents.

Tables 5 also presents the water holding capacity of these fibers. It is evident from the data that the most potent hypocholesterolemic agent has the least water holding capacity and *vice versa*, but no mathematical relation could be drawn to this phenomenon. The results indicate that adsorption of bile acids on the fiber of the pulses by itself is not related to its hypocholesterolemic property, but adsorption as well as the water holding capacity of the fibers can be useful in assessing the hypocholesterolemic effect of different fibers.

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REVIEW PAPER

The Present Status on the Use of Nisin in Processed Foods

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The heat process rendered to low acid and medium acid foods packed in hermetically sealed containers is targetted to destroy the spores of *Clostridium botulinum*—the causative organism for the botulinum toxin[1]. The food may, however, harbour spores of more heat resistant organisms such as *Bacillus stearothermophilus* and *Clostridium thermosaccharalyticum*. Considerable spoilage may occur when packs with few surviving spores are exported to countries with hot climate or stored in warm parts of the cargo ships. Damage and loss of canned foods as a result of flat sours and hard swells present a world wide problem[2-4]. The problem is of particular significance to the Armed Forces, in India as 40 per cent of the canned foods in the country is consumed by them.

Acid foods which are given a relatively milder heat treatment or processed at temperatures below 100°C are also likely to get spoiled by surviving bacteria. Spoilage of a wide variety of canned products like pears, pineapple, tomato juice, whole tomatoes, tomato puree, tomato sauce, apple sauce, tomato ketchup, worcester sauce, pickle, etc., as well as some non-acid foods such as evaporated milk, non-sterile meat items like canned ham, pre-cooked frozen foods, etc., have been reported[1,3,6,9]. Considering the magnitude of the problem, the canner normally applies a greater amount of heat which affects the organoleptic and nutritional quality[7] of the product. In products like soups, mushrooms, etc., which have delicate flavours and heat sensitive ingredients, are affected more adversely[8]. Therefore, milder heat treatments should be aimed at to retain much of the colour, flavour, texture and nutritional properties of the product. This will also bring about saving in thermal energy. A reduction in thermal processing is possible by incorporating a chemical additive like nisin for the following reasons.

(*a*) For reducing spoilage rates under a given processing condition.

(*b*) For reducing the processing conditions without increasing the spoilage rate.

(*c*) In many newer heat sensitive foods where minimal heating itself becomes too severe for getting a satisfactory product[9].

Nisin is an antimicrobial substance produced by *Streptococcus lactis* often naturally present in milk. The joint FAO/WHO Expert Committee on Food Additives defined nisin as the name given to several closely related polypeptide antimicrobial substances produced by strains of *S. lactis*[10].

Nisin was discovered accidently as the metabolite of certain species of *Streptococcus lactis*. Rogers[11] noted that certain species of *S. lactis* inhibited the growth of *Lactobacillus bulgaricus* during yoghurt preparation, while Rogers and Whitter[12] observed that certain strains of *S. lactis* controlled the blowing of cheese. They attributed it to the presence of an inhibitory substance produced by these strains. Later during an investigation of the behaviour of mixed strains of lactic *Streptococci* used in cheese starters, Whitehead and Riddel[13] in New Zealand also noted that a metabolite produced by some of the strains of lactic *Streptoccoci* inhibited other strains. Whitehead[14] subsequently was able to partially concentrate it. It was found to be proteinaceous in nature, possibly polypeptide, soluble in alcohol and hydrolysable by trypsin and alkali, but stable towards pepsin. In England, meanwhile, Meanwell[15] isolated similar lactic *Streptococci* from farm milk and dairy farm equipments. Shattock and Mattick[16] identified the strains producing this inhibitory substance as *S. lactis*. Mattick and Hirch[17] eventually isolated the inhibitory substance and gave the name nisin. More detailed investigations were made by Hirsch[18,19], Berridge[20-22], Berridge, Newton and Abrahm[23]. Its commercial production started in late 1950 by M/s Aplin and Barrett[9]. They marketted it under the brand name 'Nisaplin' which has the following composition[8]:

Nisin (i. u./mg)	1026
Sodium chloride (per cent)	74.7
Moisture (per cent)	1.7
Protein (per cent)	17.12
Fat	traces
Carbohydrate (per cent)	5.93
Fiber	Nil

Today, nisin is being used in a variety of products in many countries throughout the world.

Nisaplin brand nisin is freely soluble in water at the levels it is commonly used in foods. According

to Berridge[24], pure nisin was believed to contain approximately 50×10^6 Reading units (RU)/g, but according to M/s Aplin and Barrett Ltd, an activity of 40×10^6 RU/g is indicated. Nisaplin contains about 2.5 per cent nisin and is standardised to one million RU/g. In other words, one gram of Nisaplin contains 25,000 μg pure nisin. As regards the solubility, the values are conflicting as reported by many workers[25,26]. According to Hawley[27], the solubility in water at pH 7.0 is about 75 μg/ml and increases as the pH is lowered. At pH 5.6, it is 1000 μg/ml and at pH 4.2 it is 12,000 μg/ml. The presence of protein in the substrate increases the solubility without affecting nisin activity. The solubility of nisin is however, adequate to meet all applications in food technology[27], since the quantity of nisin required to inhibit spoilage organisms is 0.25 to 500 units (0.00625 to 12.5 μg) per gram of the substrate. It is reported that the stability of nisin depends on pH, temperature and nature of the substrate. At pH 2.0, it is remarkably stable and can withstand prolonged storage at 2-7°C or drastic heating at 121°C without loss of activity. On the other hand, the activity of nisin is rapidly lost under alkaline conditions, and at pH 11.0, the activity may be completely destroyed at 63°C in just 30 min. Some protection is rendered by many substrates with regard to loss of nisin activity[26,27]. e.g., an aqueous substrate containing 3 per cent protein and 50 μg/ml nisin when heated at 100°C required for complete inactivation of the nisin 2 hr at pH 8.0, 1 hr at pH 9.0 and 30 min at pH 10 and only 25 per cent reduction in activity in 60 min at pH 6.0 Holding a solution of nisin at 100°C for 30 min at pH between 2.0 and 6.0 produces no adverse effects. However, in most practical applications, it is preferable not to exceed pH 6.5[27]. It is apparent, therefore, that some loss of nisin activity is inevitable when Nisaplin is used as an aid in the heat sterilization of foods. Since the parameters of these activity losses are well recognised, compensating factors are built into Nisaplin treatment levels. As regards the stability of Nisaplin when stored in air tight containers with well secured lids, it is stable for a period of 2 years from the date of manufacture provided that it is stored dry, away from sunlight and at a temperature in the range of 4-25°C[8]. However, nisin in the dry crystalline state and such preparations in which the nisin content is high or near to 40×10^6 RU/g (high titre preparations) may be stored at room temperature for at least several years and probably indefinitely[28].

Structure

Nisin is a large polypeptide with a molecular weight of approximately 10,000 and consists of 5 polypeptide components with slightly different physical, chemical and biological properties[20,21,23]. Its structure has been studied by a number of workers[20-23,26,29]. Gross and Morrell[29] proposed the structure given in Fig. 1. It comprises of 34 amino acids and is in the form of a pentacyclic hetero-detic type of peptide containing sulphide bondages contributed by lanthionine and β-methyl lanthionine. The unique bicycle structure of the COOH terminal fragment consisting of rings with 13 members each is singular in the molecule. While there is present one additional 13-membered ring, none of its amino acids participate in a second cycle structure. The two remaining rings are larger in size. They are comprised of 5 and 7 amino acids respectively and thus give rise to 16 and 22 membered rings.

Mechanism of action

Nisin is known to inhibit the germination of all types of spores. The exact mechanism, however, is not clearly understood. Probably the outgrowth of germinating spores is inhibited. Lueck[34] considers the action to be directed against the cytoplasm membrane which is destroyed immediately after germination of spores. Consequently, the action of nisin against spores is greater than that against vegetative cells. Although nisin cannot be termed directly sporicidal, it appears to increase the heat sensitivity of spores. The quantity

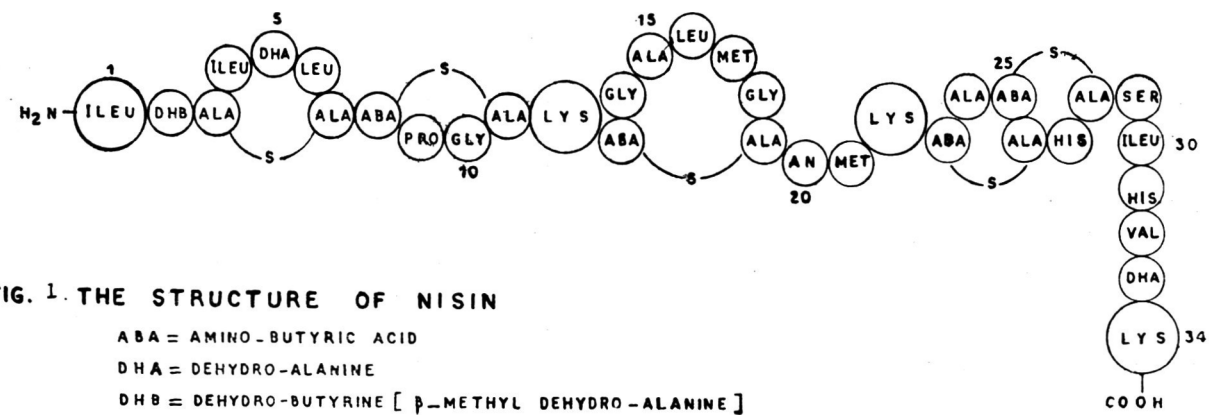

FIG. 1. THE STRUCTURE OF NISIN

ABA = AMINO-BUTYRIC ACID

DHA = DEHYDRO-ALANINE

DHB = DEHYDRO-BUTYRINE [β-METHYL DEHYDRO-ALANINE]

of nisin which acts is that which remains in active form in the mixture after heat processing. It is this quantity that inhibits the germination of bacterial spores. According to Hitchins *et al.*[35], nisin prevents the lysis of spore coats, but not the germination of spores of certain mesophilic aerobes. There has been reports that the effect of nisin is reversed by the presence of divalent cations like Cu^{++} and Mg^{++}[36]. Gupta *et al.*[37] found that the efficiency of nisin as a food preservative is significantly affected by the type of spores and the kind and nature of metabolites contained in the food.

Method of application

Maximum preservative effect of Nisaplin could be brought about by uniformly mixing into the food to bring it in contact with the surviving spores[8]. Some cheese manufacturers have favoured the addition of Nisaplin in the dry state; others prefer dissolving it in a small volume of water before mixing with the product. However, suspensions of Nisaplin in water should not be heated or made up for more than 30 min before use in order to avoid loss of nisin activity[38].

For soups and juices, this is achieved by thorough mixing at the most convenient stage prior to heat processing. It could be added to the liquid fraction whenever this is practicable. For the addition of Nisaplin to the hot brine used in canning of peas, mushrooms, broad beans, etc, precautions must be taken to avoid loss of Nisaplin activity by the combined effect of high temperature and high pH.

Use in foods

Nisin has been successfully used in a number of thermal processed foods without increasing the risk of bacteriological spoilage[39,40]. These include:

Cheese: Nisin was first used to control the blowing in cheese and cheese spreads by *Clostridia*[8,9,38]. The processed cheese often contains spores of anaerobic *Clostridia*, which eventually bring about spoilage. Besides, many of these *Clostridia* are potentially toxigenic. Attempts to control the *Clostridial* growth by other methods met with little success, whereas with nisin it was highly effective. At the same time, nisin does not enable the manufacturer to process decomposed cheese nor does it take care of unhygienic practices[41].

Pasteurised whole milk: Pasteurised whole milk has a very short life and needs refrigeration. Under the conditions prevailing in tropical countries where collection, transportation and distribution facilities are inadequate the milk curdles faster. Vanini and Moro[42] demonstrated that the shelf life of pasteurized milk could be extended by 2 days by the addition of nisin. Similar studies carried out in Argentina[43] showed that with the addition of 30 to 50 mg of nisin/l, the shelf life of pasterurised milk could be doubled.

Flavoured milk drinks: Nisin has proved effective in chocolate flavoured milk. Heinemann *et al.*[44] demonstrated that chocolate milk containing Nisaplin at 80 mg/l and heat processed did not spoil when incubated at 55°C for 3 weeks or at 35°C for 6 months.

Sterilized milk: In sterilized milk due to the survival of the thermophilic organisms the product is prone to spoilage under elevated storage temperatures. In a study conducted in France, it was demonstrated that Nisaplin at 20 mg/l completely checked the growth of the thermophilic organisms that survived heating at 115°C for 15 min[8].

Reconstituted and recombined milk products: Recombined and reconstituted milk have been used in the preparation of sterlized milk and flavoured milk drinks. The milk powder and butter oil used may contain heat resistant spores which eventually spoil the product on storage. Nisin has proved highly successful in controllling such spoilage[8].

Canned evaporated milk and other dairy products: Studies carried out in the Soviet Union showed that by employing nisin at 80-100 mg/l in evaporated milk, complete inhibition of the growth of spore forming bacteria is possible and a 10 min saving in the process time achieved when compared to nisin free samples. Similar results were obtained at other places also[8]. Nisin could be effectively used for cream as also low fat dairy products[8].

Canned milk puddings: Milk puddings contain cereals, sugar, whole milk or non-fat milk, cream, milk solid supplements, etc. Cereals such as sago, semolina, tapioca, etc., on heating gelatinise and thicken the product resulting in poor heat transfer. Naturally, the heating process has to be prolonged to have the desired keeping quality. Under such prolonged processing, the organoleptic properties of the product get impaired. The problem of thickening of the canned contents has also been experienced by us in the canning of *kheer* preparation containing milk, rice and sugar and also in canned pumpkin (*Cucurbita maxima*) *halwa* under the processing conditions normally followed (unpublished data). By the addition of nisin, gelatinization or thickening of the product could be reduced by reducing the processing conditions. Addition of 50-100 mg/kg was sufficient to retain the organoleptic properties and to prevent spoliage of puddings[8].

Other canned foods: Spoilage of canned vegetables and soups with pH above 4.5 by *Bacillus stearothermophilus*, *B. coagulans*, *Clostridium thermosaccharalyticum*, *C. nigrificans*, *C. putrificum*, *C. sporogenes*, etc. has been reported[45]. Foods with pH below 4.5 which are generally not subjected to processing above 100°C

are also likely to get spoiled. Spoilage of such foods by non-sporing and spore forming mesophiles and thermophiles of aerobic or anaerobic are acidric in nature such as *Bacillus thermoacidurans*, the *Lactobacilli* such as *Lactobacillus brevis*, *Leuconostoc pleofructi*, *L. mesenteriodes*, *Clostridia* such as *C. pasteurianum*, and other butyric anaerobes, etc. are not uncommon. The value of nisin in combating such spoilage of low acid and acid foods has been well established.

Canned peas and beans: Employing nisin between 100 and 150 mg/kg, control of spoilage for at least 2 years in hot climate has been reported.[46]. Poretta *et al.*[47] carried out some fundamental studies and established its use in canned peas. Similar studies were carried out in Belgium, Hungary, Sweden and the Soviet Union. Canned green peas in No. 83-1 cans inoculated with $3\text{-}7\times10^3$ spores of *B. stearothermophilus* per gram of the product and containing 100 units of nisin per gram of the product was giving a process $\frac{25\text{–}30\text{–}25}{116°C}$ and $\frac{25\text{–}10\text{–}25}{120°C}$ instead of $\frac{25\text{-}40\text{-}25}{116°C}$ and $\frac{25\text{-}25\text{-}25}{120°C}$. Addition of nisin to canned peas reduces the established process time for green peas by 15-20 min[30] and excludes flat sour spoilage of the product[48]. Besides, the food tasted superior to the one processed without nisin under normal processing condtions.

Canned mushrooms: Mushrooms being delicate in structure, do not permit a thorough cleaning as practised for other foods and hence may retain a heavy load of thermophilic bacterial spores. This would need a fairly long sterilization time and high temperature and a process value of $F_o=18$ is usually employed for 300×402 cans. Such a process would require 15 min for pre-heating the contents of the can to the required temperature, a holding time of 28 min, in a still retort and cooling the can for 30 min, which takes about 73 min for the entire process. Fundamental studies carried out by Heinemann *et al.*[49] on the control of thermophilic spoilage of mushrooms, showed that the sterilization of mushrooms without nisin processed for $F_o=3$ resulted in 100 per cent spoilage in three weeks at 130°F, while the same process with 40-80 RU of nisin per g resulted in 100 per cent sterility under similar conditions. Thus, by employing a shorter process, the holding time is reduced from 28 to 12 min. and a saving of 22 per cent in the total process time. Accordingly, under commercial condtions in a day of 8 hr, 8.4 cycles are possible with the use of nisin as against only 6.5 cycles without nisin.

Canned soups: The delicate flavour and aroma and the heat sensitive ingredients in soups often get impaired with high processing conditions[8] and therefore, such products need a minimum heat process. However, to control the growth of thermophilic spores present in them, a higher process is needed resulting in a product of poor sensory quality. Under such circumstances, the use of nisin at 100-200 mg/kg permits a shortening of the process[26]. This has been confirmed by studies in U.K., U.S.A. and Australia[8].

Canned tomato products: Tomato and tomato products are acidic in nature and are normally processed at temperatures below 100°C. However, the spoilage caused by mesophilic, aciduric organisms such as *Bacillus coagulans* and *Clostridium pasteurianum* cannot be precluded. Such spoilage problems could be effectively controlled by the use of nisin. Nisin remains fairly stable due to the low pH in such foods. Poretta *et al.*[50] reported that 50-100 mg/l was effective in the control of mesophilic spoilage by *Clostridia* in canned tomato juice. The effectiveness of Nisaplin in controlling spoilage of whole tomatoes by different bacteria was demonstrated by Masalennikova[51]. Campbell, *et al.*[39], in their experiment with canned tomato juice inoculated with 1.5 million spores of *B. coagulans* and processed at 206°F, showed a considerable decrease in D-value when nisin was used. Their studies revealed that 77 per cent spoilage occurred even though the cans were processed for 38 min without the use of nisin. But no spoilage was noted in those containing 14 ppm. of nisin in which the cans received just 6 min, processing at the above temperature[26,39]. Later, Campbell and Sniff[52], extended their studies to 31 strains of *B. coagulans* and observed that all 31 strains were inhibited by 5 ppm of nisin in tomato juice adjusted to pH 5.3. According to Bardsley[26], the addition of 200 RU of nisin per ml of sauce prevented spoilage by *C. thermosaccharolyticum* of beans in canned tomato sauce.

Canned asparagus: Heat resistant thermophilic bacteria are often found to be present in *asparagus*[1]. The problem of sterilization of *asparagus* has been of much concern especially when packed in large sized containers. Heating at temperatures higher than 100°C obviously impairs the quality attributes. Herrandez *et al.*[53] found that addition of 10 ppm of nisin to 3-kilo cans followed by sterilization at 115-116°C for 17 min prevented spoilage particularly by *Clostridium sporogenes*.

Canned hams: Although members of the *Clostridium* and *Bacillus* groups cause spoilage in canned cured hams, the most important species causing spoilage of pickled meat products including canned hams, spiced ham, bacon, etc. are *B. subtilis*, *B. cereus*, *B. megaterium* and *B. polymyxa*. Besides, spoilage is also caused by non-spore formers. *Streptococcus liquifaciens* may cause liquifaction of jelly in canned hams. Off-flavours and bad odours in canned hams may be caused by

faecal *Streptococci* and they are also responsible for souring the stored hams. Though *Streptococcus faecum* and *Streptococcus faecalis* are involved, the former is more important due to its greater heat resistance[45]. Canned hams are only pasteurized and require storage below 5°C. Pasteurization is carried out in hot water to get a temperature throughout the can contents of not lower than 150°F and usually not higher than 160°F. This degree of heat does not protect the canned hams against spoilage[54] which is often encountered when the product is shipped to outside countries when strict maintenance of refrigeration temperature is not possible. Addition of nisin at the rate of 1-2 g per can of 4 kg (0.2-0.5 g/kg) has proved effective especially to get a longer shelf life[25] at 38-40°C. Similar observations were also made in Australia[55].

Fruits: Yeasts and moulds are commonly encountered in fruit juices. Since they are not sensitive to nisin, it has a somewhat restricted application in fruit and fruit juice processing. However, canned peas and pineapple are susceptible to fermentation by butyric acid bacteria. *C. pasterurianum* will grow at pH of 3.5[5,6]. Similarly *Bacillus thermoacidurans* grows at pH 4 and *B. macerans* at 3.8-4. Such spoilage could be controlled by nisin.

International Unit

An international reference preparation of nisin has been established by the WHO Committee on Biological Standardisation[56]. The international unit is defined as 0.001 mg of this preparation. It is identical with the unit of nisin activity of Nisaplin. Thus, 1 million units of Nisaplin equals 1 million international units of nisin[8].

Estimation of nisin in foods

It is clear that it is the residual nisin in foods which causes the preservative action by inhibiting the germination of bacterial spores on storage[34]. More nisin is retained in high temperature short time heating than in low temperature long time treatments. More destruction of nisin takes place as the heat treatment is increased under each retort condition[9]. This could be well seen from Table 1 indicating the rate of destruction of nisin under various conditions of heating in various food products. Nisin is not sporicidal[26] and should be added in such quantities that a minimal residue of at least 5.0 i.u./g is retained in the product to guarantee the keeping quality[57,32].

Factors contributing to the loss of nisin potency

Initial spore types and their concentration: A high initial spore load necessitates the addition of a higher initial level of nisin. Effective Nisaplin treatment levels will also depend upon the types of spores present. The demand for nisin in foods is therefore, related to the types and number of organisms present[8,37].

pH of the product: Nisin is more stable to heat in acid solutions than in neutral solutions.[9] The stability of nisin is highest in the acid range and is maximum at pH 2.0 and much of its potency is lost at pH 11.0. The effect of pH on the stability of nisin in various foods, is indicated in Table 2. The loss of nisin activity

TABLE 2. PER CENT RETENTION OF NISIN AT 250°F AFTER HEATING IN VARIOUS FOODS

	pH	F=3	F=9	F=15
Cherries sour	3.28	69.5	52.6	37.7
Apples	3.42	55.0	37.5	25.3
Peaches	3.77	36.9	29.3	19.3
Tomato juice	4.35	57.0	49.0	29.0
Okra	4.77	68.0	53.3	23.3
Asparagus	5.32	56.0	33.4	28.6
Beets	5.80	55.5	45.4	35.4
Beans, lima	6.10	52.6	32.3	24.7
Mushroom soup	6.10	72.9	46.5	32.2
Peas	6.40	75.5	38.0	25.3
Skim milk	6.55	47.0	26.4	18.4
Chocolate milk	6.79	55.0	27.3	13.0
Cream style corn	6.88	47.9	19.7	7.6

Heinemann *et al.*[9]

TABLE 1. EFFECT OF HEAT TREATMENT ON PER CENT NISIN RETENTION IN THREE LOW-ACID FOOD PRODUCTS AT DIFFERENT TEMPERATURES

Product	pH	240°F			250°F			260°F			270°F		
		F=3	F=9	F=15	F=3	F=9	F=15	F=3	F=9	F=15	F=3	F=9	F=15
Cream of mushroom soup	6.1	81.8	20.9	10.7	73.0	41.5	32.2	32.7	41.1	27.2	66.2	37.2	26.1
Skim milk	6.6	47.6	16.0	9.4	47.5	24.9	17.2	52.3	29.8	20.9	41.1	36.1	29.3
Cream style corn	6.9	24.9	11.2	14.9	47.9	19.7	7.6	64.5	50.5	22.7	64.5	76.5	50.0

Heinemann *et al.*[9]

when used to preserve heat processed foods as a result of the various parameters are well recognised[8,9,28,32,37]. Such losses are inevitable. However, the recommended Nisaplin treatment levels in foods are made after taking into consideration all such losses.

Temperature and duration of storage: At elevated storage temperatures, spoilage is possible after a few months as a result of the slow degradation of nisin. Even when no appreciable amounts of nisin could be detected in the food, it remained in good condition without spoilage[26,32]. This is probably due to the nisin getting bound to the bacterial spore and thus not being available for extraction and quantitative determination[26] and under such circumstances the only reliable method available is a biological assay.

Biological assay of nisin

Several methods are available for the estimation of nisin[9,10,58]. The plate diffusion assay, was outlined by Tramer and Fowler[58] has been found to be suitable with certain modifications.

Amongst the ingredients used in the bio-assay medium, the quality of Tween 20 has been found to be very critical. Sub-standard grade of the surfactant adversely affects the zone of inhibition and thus the determinations become inconclusive. Tween 20 being a fatty acid derivative is prone to oxidation. This in turn gives abnormal inhibition zones when used for diffusion purposes. Therefore, it has to be stored in the cold and in the dark.

Another method employed is the reverse phase disc assay technique[58]. This is simpler than the plate diffusion assay technique, but is only semi-quantitative. By this method, solutions containing between 0.5 and 1.0 i.u./ml will produce a detectable zone of inhibition, and concentrations upto 5-10 units can be assessed semi-quantitatively. This method could be useful as a sorting test and may prove valuable as it will help the user in assessing the quantities of nisin to be incorporated into the raw material to ensure a satisfactory residual level. This method may be used prior to the quantitative assay by the plate diffusion assay technique.

Toxicological aspects

Nisin has been systematically and thoroughly investigated for its toxicological aspects by a number of workers. In Japan, Hara *et al.*[59] conducted acute toxicity tests on mice and rats by oral administration and concluded that there is no toxicity being caused by nisin. They reported the LD_{50}'s to be similar to that of common salt (6950 mg/kg) when administered orally. Frazer *et al.*[60] fed albino rats 40,000 times the standard dietary dose (taking it as 25 i.u./kg of body weight/day) over a period of 3 months and could not observe any deleterious effects. On the basis of the 'no effect' level observed in the toxicological evaluation in laboratory animals and with the extrapolation of such findings to man, the WHO recommednded the maximum acceptable daily intake (ADI) as 33,000 i.u./kg[61]. The Joint FAO/WHO Expert Committee on Food Additives recommended that the use of nisin should be considered acceptable, the unconditional ADI being 33,000 units/kg of body weight[61]. To quote from their report: 'The available evidence indicates that a level of 3,300,000 units of nisin/kg of body weight has no adverse effect'.

Work carried out in the Research Division of Aplin and Barrett Ltd., showed that nisin resistant bacteria examined are not cross resistant to medical antibiotics[27], since nisin resistant bacteria were sensitive to other related antibiotics such as polymyxin, bacitracin and subtilin[26]. Therefore, there is no danger that the use of nisin would militate against the therapeutic effectiveness of the medical antibiotics[26].

Nisin is sensitive to ptyalin and is digested by trypsin. It is destroyed by yeasts[28], *Lactobacilli*[62] and many lactic *Streptococci*[63]. These facts added to its narrow spectrum and the insensitivity of most gram negative bacteria and its instability in the vicinity of pH 7.0 make it unlikely that it could have any effect on intestinal flora. Experiments carried out on pigs[64] and poultry[65] have corroborated this.

There is also evidence that nisin is not foreign to the human alimentary tract since nisin producing strains of *S. lactis* have been isolated from the human throat and from stools[66].

International legislation on the use of nisin

The use of nisin in foods is absolutely safe since toxicological studies favour the use of this antibiotic. However, wide differences exist between nations in respect of the legal standards concerning its use[8]. Some nations permit its use in any food without restrictions in quantity, while others restrict it to certain foods with maximum permissible limits laid down. Even in the same food, like, cheese, it varies from 100 i.u./g in certain countries to 1000 i.u./g in some other countries.

Countries can be put under four categories with respect to the international legislation on the use of nisin.

(*a*) Countries where specified approval exists for the use of nisin—under this are included 28 countries including India, Australia and UK.

(*b*) Countries where authorisation to use nisin is currently being sought—under this we have about 9 countries including USA.

(*c*) Countries which generally accept foods prepared

in accordance with Codex recommendations or which follow the legislation of other permitting countries or those which have no published legislation but are known to use nisin—under this we have 9 countries including USSR.

(*d*) Countries which, while not permitting nisin in products for domestic consumption, will allow the use of nisin in foods for export to countries where nisin is permitted—under this we have 3 countries including Federal Republic of Germany.

Indian scene

Surprisingly, in spite of nisin being a very good heat sterilization aid and having good applicability in the Indian conditions, very little work has been done in India. Gupta *et al.*[37] and Gupta[67] studied the effect of monovalent ions like Na^+ and divalent ions like Cu^{++}, Fe^{++}, Mn^{++} Mg^{++} and Ca^{++} on the germination of spores of *B. stearothermophilus*, *B. cereus* and *B. megaterium* both in the absence and presence of nisin. The influence of ions in the germination of spores is in relation to the release of DPA. They found that the presence of Mg^{++} or Ca^{++} helps in the release of DPA and hence helps in the germination of spores. But in the presence of nisin only very little DPA is released and consequently spore germination is inhibited. The studies indicated that in the presence of 100 μg/ml of nisin both Ca^{++} and Mg^{++} increased the germination of *B. stearothermophilus* by 10.8 and 14.2 per cent, *B. cereus* by 30.4 and 19.9 per cent and *B. megaterium* by 6.6 and 30.9 per cent respectively at 1 M concentration. The increase in germination of spores treated with nisin in the presence of Ca and Mg ions indicate that these ions compete with nisin; thereby lessening the harmful effect of nisin on spore germination. The other ions increased inhibition of germination by nisin. Since these ions could be present in the food either as natural constituents or through ingredients added or additives and by way of contamination from equipment, the presence of such ions would be an additional factor for consideration while deciding the nisin level to be used in food processing.

Various sugars and their derivatives are known to influence germination of bacterial spores. Since some kind of sugars are always present in most foods, the use of nisin as a food preservative must be taken into account in the presence of various sugars and their derivatives. Gupta *et al.*[68] studied the effect of various sugars and their derivatives upon the germination of *Bacillus* spores in the presence of nisin. They concluded that the efficiency of nisin as a food preservative is significantly affected by two factors: the kind of *Bacillus* species spores present and the nature of metabolites contained in the food.

Wajid and Kalra[69] used nisin to reduce the heat resistance of *B. subtilis* and *B. stearothermophilus*. They found that 100 RU/ml of nisin was very effective in extending shelf life of sterilized milk. According to them the keeping quality of sterilized milk in the presence of nisin was extended upto 60 days, while the corresponding samples without nisin got spoiled within 3 to 7 days.

According to Aplin and Barrett[8], India has been listed under countries where specific approval exists for the use of nisin and is in accordance with the current food legislation. The maximum level of addition per kg is 1000 mg of Nisaplin per kg of food product and has been permitted for use in hard cheese and processed cheese. However, there is some reluctance on the part of cheese manufacturers to use nisin for the reason that normally cheese in India is sold in about 2 or 3 weeks after its preparation[70].

Use of nisin in Defence rations

With the acute shortage of tin plate and its prohibitive cost and the need for light weight food packages at reduced cost the use of nisin will be more in flexible packages.[9] At present the flexible packaging materials available in India do not withstand the high autoclaving temperatures normally given to canned foods. With the discovery of nisin, the potentiality of using such materials for heat processed foods appears to be very great, since less drastic processing conditions need be employed in the presence of nisin. The results obtained in studies carried out with beef-stew and chicken soup in flexible packages incorporating nisin greatly substantiate the validity of such a view[1]. Beef-stew and chicken soup containing 200 RU of nisin per ml were processed in 4×6 in. flexible packages in a still retort to $F_o=3$ and in the case of the samples containing no nisin to $F_o=9$ (a process necessary to achieve commercial sterility). The studies revealed that a large number of packages processed to $F_o=9$ were got distorted or started leaking soon after the process as against no such damages noticed in the case of samples which received a reduced process of $F_o=3$. Thus indigenously available flexible packaging materials which have insufficient thermal resistance could be used for thermal processed foods alongwith nisin without any adverse effect.

In the light of today's consumer concerns regarding preserved foods, factors such as flavour, odour, texture, appearance, nutritional status and above all its economics are of utmost consideration. The importance of nutrition has been well documented with reference to dietetics, disorders and diseases[71-73]. The effect

of heat on food is very complex depending on the food and on the condition of heating. The nutritional imbalance rendered by heat to carbohydrates, proteins, vitamins, minerals, etc.,[74] may be correlated to some extent and the organoleptic qualities to a considerable extent by judiciously altering the processing technology. Heating conditions that tend to conserve the desired organoleptic properties also tend to conserve nutrients. Reduced heat treatments therefore, by the use of a heat sterilization aid such as nisin would give amazing results. Nisin may therefore said to be a useful chemical in the hands of the canning technologist to meet his requirements.

Future prospects and research needs

From the foregoing it is clear that the use of nisin has tremendous applicability in giving better, more nutritious and safer foods and at the same time bring about considerable savings in energy in these days of energy crisis. The application of nisin to the bacteriological problems of meat and meat processing opens up many attractive possibilities, since normal heat treatment although adequate to ensure a botulinum kill, may not be sufficient to achieve sterility. In other words, the recommended process for all such products is not adequate enough to inactivate all the possible spoilage organisms. This is better explained by the data available on the comparative heat resistance of bacteria normally found in foods.

The problem of inadequacy of processing is often noticed in the case of products containing spices, condiments, various cereal fillers and soya[75,76] which may harbour large number of thermophilic spore bearing bacteria. Meat extracts and other ingredients used such as starches, milk powder, etc., also have often been reported as sources of thermophilic and mesophilic *Clostridia*[77,78] bringing about spoilage of the canned foods in which they are used. Most of these defects are amenable to effective control by nisin. The judicious use of nisin in these, products therefore will not only eliminate most of the bacterial defects, but will also diminish the spoilage and potential spoilage organisms in similar products. In other words, it may be said that the use of nisin considerably reduces the thermal processing schedule required by canned foods. The effectiveness of nisin in preventing spoilage of some processed foods is shown in Table 3. The data repre-

Comparative heat resistance of bacteria

Bacterial groups	Approximate heat resistance
Low-acid and semi-acid foods (pH > 4.5)	
Thermophiles	**D. 250**
Bacillus stearothermophilus (flat-sour group)	4.5 - 5.0
Clostridium thermosaccharolyticum (gaseous spoilage group)	3.0 - 4.0
C. nigrificans (sulphide stinkers)	2.0 - 3.0
Mesophiles	
C. botulinum types A & B	0.10 - 0.20
C. sporogenes group (including P.A. 3679)	0.10 - 1.5
Acid foods (pH 4.0-4.5)	
Thermophiles	
B. coagulans (facultative mesophile)	0.01 - 0.07
Mesophiles	**D. 212**
B. polymyxa and *B. macerans*	0.10 - 0.50
Cl. pasteurianum (butyric anaerobes)	0.10 - 0.50
High acid foods	
Mesophilic non-spore bearing bacteria	**D. 150**
Lactobacillus spp., *Leuconostoc* spp. Yeasts and moulds	0.50 - 1.00

Stumbo, C. R., *Thermobacteriology* in Food Processing, Academic Press, New York, 1965, 99.

TABLE 3. SUMMARY OF STUDIES OF USE OF NISIN AS AN AID TO THE HEAT STERILIZATION OF FOODS*

Product and processes	No of cans tested	Control (no nisin)	Nisin RU/ml	Can size
Beans in tomato sauce	0/24	12/12	200	No. I.T.
(F=3)	0/24	12/12		(301×409)
Chicken chow mein flat sour	0/64	14/14	40-100	208×006
*PA 3679 (F=3)	8/64	14/14	40-100	208×006
Cream style corn flat sour	0/63	13/13	40-100	208×006
*PA 3679 (F=4)	13/64	14/14	40-100	208×006
Mushrooms	0/42	21/21	40	300×402
(F=3)	0/42	21/21	80	300×402
Peas	0/39	9/42	100	A1 (211×300)
	0/47	11/47		A2 (307×408)
(F=3)	0/44	12/45		
	0/47	7/40		
Tomato juice	0/300	77/100	560	208×006
(F=0.018)		21/100		208×006
Chocolate flavoured milk	0/1968	24/24	80	300×402
(F=3)	21/2831	282/801		

*Inoculated at levels 1000-10000 times as great as occur naturally Heinemann *et al.*[9] (combined work of several investigations)

sents a total of 2,831 cans tested. The spoilage recorded is less than 1 per cent when nisin was used and 35 per cent when nisin was absent.

In order to obtain the maximum preservative effect, the addition of nisin to foods needs standardisation. Since nisin treatment levels will vary from food to food, the various parameters involving nisin loss and stability needs careful considerations. Factors such as food consistency, pH, processing cycle, storage time and temperature, initial bacteriological status, residual nisin level, etc., in respect of each food are some of the important considerations while standardising the nisin treatment level. An intensive study has therefore, to be taken up in this line in the case of foods suited to the Indian palate.

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RESEARCH NOTES

WELDED OTS CANS AS AN ALTERNATIVE TO SOLDERED CAN

Suitability of welded open-top sanitory can as an alternative to soldered can was investigated. Welded can retained good vacuum and the side seam strip lacquer in the can interior was intact.

Recent trend in the food canning industry is to use welded cans in place of soldered ones. Attributed advantages for this change are, rapid fabrication and prevention of lead contamination in canned products. In the developed countries, welded tin plates and tin free steel containers have already been introduced for packaging of soft drinks, beer and other beverages. In India, welded cans have been introduced for packing non food products. Efforts are being made to fabricate welded open top sanitary cans for food products also. Of late, the importing countries are insisting on lead free canned fruit products. With this in view, experiments were undertaken to find the suitability of indigenously fabricated welded cans for processed food products. The results are presented in this note.

One batch of welded OTS cans (interior side seam being coated with lacquer) was received from a fabricator and was tested for porosity by ferricyanide[1] and copper sulphate tests[2]. Resistance of strip lacquer was also evaluated by boiling in 2 per cent citric acid for 30 min, as specified by ISI[3]. Potatoes in brine, tomato paste (Brix 28°; pH 3.9), mango juice and mango pulp were packed in these cans. Shelf life with respect to the retention of vacuum and resistance of strip lacquering were determined and organoleptic quality of the products was evaluated.

Testing of strip lacquer: The ferricyanide and the copper sulphate tests did not reveal iron exposure throughout the welded portion. Tinplate with strip lacquering in the welded portion, did not show peeling of lacquer, when it was dipped in 2 per cent citric acid solution for 30 min.

Cutout analysis of canned products: Both soldered (control) and welded cans (experimental) packed with different products retained good vacuum. The quality of the products was satisfactory and acceptable (Table 1, 2, 3). However, potatoes showed slight metallic taste in both types of cans stored at 37°C. Strip lacquering in the side seam (can interior) of welded cans was intact without any sign of peeling. Light to heavy feathering with detinning was noticed in the can interior

TABLE 1. SHELF LIFE STUDIES OF CANNED POTATO

Storage period (months)	Storage temp. (°C)	Soldered can		Welded can	
		Vacuum (in.)	Can interior	Vacuum (in.)	Can interior
Initial	—	14	Normal	14	Normal
3	25-30	14	LPF	14	LPF, strip lacquer intact
	37	14	LPF	14	,,
6	25-30	14	MPF SD	13	MPF SD
	37	13	HPF SD	14	HPF, MD strip lacquer not affected.
9	25-30	14	HPF SD	14	HPF
	37	13	HPF SD	13	SD strip lacquer intact.
12	25-30	13	HPF SD	13	,,
	37	13	HPF MD	12	HPF MD

LPF–Light purple feather; HPF-Heavy purple feathering; SD-Slight detinning: MD-Medium detinning.

Note: The colour of canned potato was yellowish cream throughout the storage period in both types of cans. The taste was also good in both types of cans except after 12 months at 37°C, where it developed slight metallic taste, but was acceptable in both types of cans.

TABLE 2. SHELF LIFE STUDIES OF CANNED TOMATO PASTE

Storage period (months)	Storage temp. (°C)	Soldered can		Welded can	
		Vacuum (in.)	Can interior	Vacuum (in.)	Can interior
Initial	—	8	LF	10	LF strip lacquer intact
3	25-30	6	HF SD	9	HFSD, strip lacquer intact
	37	3	HF SD	5	HF SD strip lacquer intact
9	25-30	2	HF SD	2	HF MD strip lacquer intact
	37	+ ve	HF MD	+ ve	HF MD strip lacquer intact
12	25-30	1	HF MD	3	HF MD strip lacquer intact

LF: Light feathering; H.F.: Heavy feathering, SD: slight detinning.

Note: The colour of the product was brick red and tasted slightlty bitter both at RT and at 37°C after 12 months. The cans were showing swelling after 9 months at 37°C.

TABLE 3. SHELF LIFE STUDIES OF MANGO JUICE AND MANGO PULP

Storage period (months)	Storage temp. (°C)	Soldered can		Welded can	
		Vacuum (in.)	Can interior	Vacuum (in.)	Can interior
Mango juice					
Initial	—	15	VLF	14	VLF
3	25-30	17	LF	15	LF
3	37	17	MF SD	12	MF SD
Mango pulp					
Initial	—	16	VLF	16	VLF
3	25-30	15	LF	17	LF
3	37	15	MF SD	14	MF SD

VLF: Very light feathering; LF: Light feathering.

with all the products depending on the storage period and temperature. In the case of mango juice and mango pulp, only 3 months report is given and further storage studies are in progress.

The above observations are in agreement with the earlier findings of some fruit and vegetable products canned in welded Tin Free Steel cans[4] and mango pulp in welded German A12 cans [157×251 m]. (unpublished data).

The results of these studies indicate the possibility of introducing welded OTS cans as an alternative to soldered cans for processed food products. However, the results have to be confirmed by commercial trials.

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VOLATILE COMPONENTS OF DASHEHARI MANGO

Distillate of the ripe 'Dashehari' mango was found to be waxy in nature and imparts pleasing effervescence, characteristic of its maturity. Aroma of the 'Dashehari' mango appears to be a mixture of 30-40 volatile components and there exist the possibility of the presence of osimene, *cis*, *trans* allocimene, myrcene and lactone group in mango flavour.

Little is still known of the components which give characteristic flavour to mango. Although few studies[1-5] have been reported on the volatile odourous compounds of South Indian mangoes, no information is available on the aromatic principles of 'Dashehari' mango. Effort has been made here to find out the volatile constituents of 'Dashehari' mango.

Ripe mango fruits of bright orange yellow colour having pleasing flavour, total soluble solids content of around 18-20 per cent and acidity 0.3 to 0.35 per cent were selected for the study. Fruits were washed, peeled and pulp squeezed with hand. Macerated mango pulp (0.5 kg) was mixed with saturated brine solution and distilled in Cleavengers apparatus. The distillate collected on the diethyl ether was passed through anhydrous sodium sulphate to exclude moisture. Isolation and characterization of principles was made by GLC using varian aerogra series 1800 equipped with thermal conductivity detector and stainless steel column (5'×1/8") packed with S. E. 30 on acid washed chromosorb w. Injection port was kept at 280°C and carrier gas maintained at 20 psig. The column temperature was programmed from 75 to 275°C @ 2°C/min. The chart flow was 5 mm/min. Effort was made to tentatively identify the volatile components of 'Dashehari' mango by comparison of the emergence time of some standard components.

The study revealed that the steam distillate on removal of the solvent and water was found to be waxy in nature, which was found to deposit as a white solid material during winter and may be a high molecular hydrocarbon, as reported by Kunishi and Seale[6].

Gas liquid chromatogram (Fig 1) studies indicate that the aroma of the ripe 'Dashehari' mango comprises of 30-40 components and those emerging at Et 30 to 70 are the important volatile components, characterizing the aroma of ripe mango fruits. On comparison of the emergence time of these components with those of the standard volatile components (Table 1), there appears to exist the possibility of the presence of ocimene, *cis*-and *trans*-allocimene, myrcene and hexa and hepta lactones in the aroma of the ripe 'Dashehari' mango as reported by Gholap and Bandyopadhyay[5]

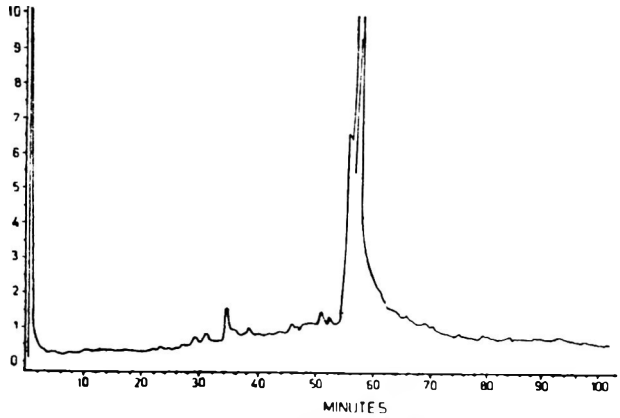

Fig. 1. GLC of 'Dashehari' fresh mango

TABLE 1. EMERGE TIME OF CERTAIN VOLATILE COMPOUNDS

Emergence time (min)	Volatile components
21	Furfural
24	Ocimene
47	Linalool
51-52	Cis-and trans
54-56	Allocimene
55	Myrcene
56	Hexalactone
62-63	Heptalactone

and Hunter *et al*[7] in unripe 'Alphanso' and 'Batali' mangoes and 'Alphanso' canned mangoes respectively.

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A STUDY OF NITROGENOUS CONSTITUENTS OF TEA CREAM

There is a cream formation in hot, strong infusion of black tea when it cools down to room temperature. The nitrogenous constituents of the tea cream are caffeine, proteins and humic acid-like substances. The present paper discusses the hydrogen bonded interactions between tea polyphenols and proteins. Theanine, the major amino acid of tea could not be identified in the cream by paper chromatographic methods.

When a strong infusion of black tea cools down, a coloured precipitate is formed known in the tea trade as "cream". While creaming is valued by professional tea tasters as a contributory indication of tea quality, it is considered as a negative factor in instant tea processing industry as it affects the appearance and dispersability of the iced tea beverage. The creaming characteristics of a tea infusion can be expressed as "creaming power"[1] or "cream index"[2]. Sanderson[3] has reviewed the work on the components of tea cream and the conditions that lead to the formation of the cream.

The origin[2] of the nitrogenous substances in cream can be traced primarily to caffeine which is present along with theaflavins and thearubigins in the ratio of 17:17:66. Vuataz and Brandenberger[4] have isolated thearubigin-like substances by chromatographic and solvent fractionation of black tea polyphenols.

These are reported to contain 0.55 per cent N which cannot be accounted for either by caffeine or amino acids. Hydrolysis of these fractions with 5N HCl at 120°C for 12 hr and analysis by paper chromatography led to the identification of 14 amino acids but not theanine. This is attributed to humic like-acid substances formed by the interaction of 0-quinones, derived from polyphenols, condensing with proteins and amino acids. This explains the lower solubility of this group of substances and their poor extractibility into the tea infusions.

Wickremasinghe *et al*[5,6] during their investigations on shot hole borer infestation of tea bushes, studied the formation of complexes between gelatin-theanine-catechin and gelatin-theanine-chebulagic acid. Paper chromatography of the individual mixtures showed

a new ninhydrin positive spot of lower Rf value in comparison to theanine in both the cases indicating a complex formation. Wickremasinghe[6] during his earlier investigations on thearubigins surmised that similar complexes are formed mainly between theanine, protein and polyphenols.

Theanine, the γ-ethylamide of glutamic acid is the major amino acid of tea. It is present to the extent of 625 mg/100 g in withered tea flush (on dry weight basis), 520 mg/100 g in made tea[7] and 37 mg in 100 ml of black tea infusion (calculated on the basis of 1.1 per cent on total soluble solids assuming an average soluble solids of 33.3 per cent)[8]. Since it is present in substantial concentration in black tea and in the infusion, it was expected to participate in complex formation with tea polyphenols. Earlier work has clearly substantiated the fact that it is a non-essential amino acid and hence it has not been identified in the protein hydrolysis derived from any part of the plant or from thearubigin fraction containing nitrogen (as referred earlier). Though Kito *et al.*[9] used ethyl amine-1-^{14}C, to demonstrate the possibility of N-ethyl carbon of theanine to be finally metabolized to ring A of catechins in tea leaves, no compound with theanine coupled with ring A of either theaflavins or thearubigins has so far been isolated.

In order to find out the role played by the nitrogenous substances especially theanine in cream formation, the tea cream was isolated from instant tea processing by the following procedure. Crushed green tea leaves were spread on trays and allowed to ferment for 90 min. After visual assessment for the completion of optimum fermentation, the fermented material was extracted with boiling water, filtered to squeeze out the extract and a similar process was repeated four times. All the extracts were pooled, concentrated in a rotary film evaporator and centrifuged for isolating cream designated as 'cream from hot soluble tea'. Another lot was chilled at 4°C for one hour and centrifuged at high speed and the isolated cream was designated as 'cream from cold soluble tea'. The two types of tea creams were analysed for total nitrogen[10], caffeine nitrogen[10] (Bailey Andrew's method) and protein [(per cent total nitrogen — per cent caffeine nitrogen) × 6.25]. The values from two batches are given in Table 1. The total soluble solids, ash, and tannins were estimated by standard methods[10,11].

The dried cream isolate was decaffeinated in Soxhlet extract or using chloroform. The tea cream was also digested with heavy magnesium oxide to remove the bound caffeine. These steps ensured the complete removal of free and bound caffeine. To destroy any charge transfer complexes that formed between polyphenols and caffeine due to hydrogen bonding, alkali

TABLE 1. PROXIMATE COMPOSITION OF CREAM ISOLATED FROM INSTANT TEA PROCESSING

Type of tea	Total soluble solids (%)	Ash (%)	N (%)	Soluble caffeine (%)	Tannins (%)	Protein (%)
Hot souble	12.37	7.32	6.32	2.60	12.63	27.72
Cold soluble	33.80	8.28	4.32	2.24	21.24	23.18

soluble samples were spotted on a Whatman No. 1 filter paper along with an authentic sample of theanine and the spot was wetted with one or two drops of IN HCl to neutralise the alkali. The chromatogram was developed with n-butanol-acetic acid-water (120:30:50) solvent system overnight, dried in an air oven and sprayed with ninhydrin reagent and heated again to develop the spots. No spot corresponding to theanine (R_f 0.25) nor any ninhydrin positive spot of lower R_f value could be detected. No free amino acid spot could be seen on the paper.

Though there is some difference in the levels of soluble caffeine in the two types of creams, the total nitrogen and hence the protein content is significantly higher in the hot cream than in the cold cream, indicating that cream yields increase with lowering of temperature. Proteins to a larger extent and soluble caffeine to a lesser extent seem to play a significant role in the composition of tea cream isolated at ambient temperature in comparison to that isolated at 4°C. The polymeric polyphenols expressed as tannins, do not contribute significantly to cream formation at ambient temperature, are major constituents of cream isolated at lower temperature. It has been surmised that the nitrogenous compounds especially caffeine complexes with the highly acidic phenolic groups of the tea polyphenols viz., theaflavins and thearubigin via the formation of hydrogen bonds[12]. These hydrogen bonds are stable at lower temperatures and increasingly become unstable at higher temperatures. The fact that the amounts of polyphenols participating in cream formation is higher at lower temperatures substantiates the hydrogen bonded interactions between tea polyphenols and the nitrogenous substances present in tea infusions.

These results also indicate that no free amino acid is involved in the cream formation in the tea infusions though proteins play a dominant role. The fact that theanine, the major amino acid constituent of the tea infusion does not take part in cream formation is significant.

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BACTERIOLOGICAL QUALITY OF CUCUMBER SLICES AND ITS PUBLIC HEALTH SIGNIFICANCE*

Sixty eight samples of cucumber (*Cucumis sativus*) slices, collected in Calcutta over a period of one year in two different seasons from itinerant vendors were analysed for bacteriological quality. Average standard plate count, psychrophilic and lipolytic bacteria, yeast, mould, enterococci, coliform and faecal coliform were 43.8×10^3, 5.4×10^3, 2.3×10^3, 1.8×10^3, 0.8×10^3, 1.3×10^3, 2.9 and 0.5 respectively per gram of sample. Coliform, faecal coliform and enterococci were detected in 94.2, 77.6 and 89.7% of the samples, respectively. In 19 samples, presence of *E. coli* was confirmed. Multiple resistance to commonly used antibiotics was observed in coliforms and also in the isolates of *E. coli*. A small number of coagulase positive *Staphylococcus aureus* was detected in a few samples. *Salmonellae, shigellae, Vibrio cholerae* and *V. parahaemolyticus* were not detected in any sample.

There is no report on the microbiological quality of sliced cucumbers (*Cucumis sativus*) sold on the road pavements. The product is consumed without any heat treatment. The present study relates to the microbiological quality of cut cucumber sold in the pavements of road sides in Calcutta.

Forty eight samples of cucumber slices were collected in summer (March-Oct.) and twenty in winter (Nov.-Feb.) from the itinerant vendors in Calcutta. They were immediately brought to the laboratory in sterile containers, homogenised and diluted with phosphate buffer (pH 7.2)[1]

Total plate count agar medium[2] was used for determining standard and psychrophilic plate count and the plates were incubated for 48 hr at 32 and 7°C for 10 days respectively. For yeast and mould counts acidified potato dextrose agar medium[2] plates incubated at 32°C for 5 days were used. *Enterococci* and lipolytic bacterial count were determined by the procedure described by Sharf[3] and Seely and Vandemark[4] respectively. Coliform and faecal coliform counts were determined by the MPN method using MacConkey broth[5] and the tubes were incubated for 48 hr at 37 and 44-45°C respectively. Confirmation was done on EMB plates and typical coliform colonies were isolated and identified on the basis of biochemical reactions.[6,7]

Presence of *Salmonellae* and *Shigellae* was detected in 25 g of sample according to the procedure described

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by Thatcher and Clark[8]. In practice, selenite cystine broth was used as enrichment medium and brilliant green agar and desoxycholate citrate agar as selective media. Vibrios were detected on thiosulphate citrate bile salts sucrose agar medium[9]. *Staphylococcus aureus* was detected by their formation of golden yellow colony on milk salt agar medium[8]. Culture (0.1 ml of 24 hr old) of *S. aureus* in brain heart infusion broth was added to 0.3 ml of rabbit plasma and incubated at 37°C. Clotting of plasma within 24 hr was regarded as coagulase positive.

The antibiotic resistance of the isolates of coliforms was studied by disc diffusion method against the commonly used antibiotics as per the procedure described earlier[10,11]. Reference grade antibiotics used in the study were obtained from Central Drug Laboratory, Calcutta.

Internal tissue of fresh cucumber is expected to be free from microorganisms. The incidence of microorganisms in this product will reflect the sanitary practice followed in the processing steps and microbiological condition of the substance at the time of processing.

Table 1 gives the microbial population at two different seasons with the minimum and maximum found in 68 samples of cut cucumber marketed by different itinerant vendors of Calcutta. The overall average of each of standard plate count, psychrophilic count, lipolytic bacterial count ($\times 10^3$) yeast and mould count was 43.8, 5.4, 2.3, 1.8 and 0.8 respectively. Standard plate count of 40 per cent of the samples in winter and 25 per cent in summer was above 50,000 per g. The psychrophilic bacterial count was determined because this group of bacteria may produce a variety of off-flavours as well as physical defects though they may not be pathogenic. In each sample, the psychrophilic bacterial count was lower than standard plate count. Average counts of these two groups of bacteria were highest in winter than in summer. The seasonal variations noted were statistically significant ($P<0.05$).

Count of lipolytic bacteria was very much similar to that of psychrophilic bacterial count. These may be due to psychrophilic nature of the lipolytic bacteria. The number of lipolytic bacteria was slightly higher in winter than in summer but the difference was statistically not significant ($P>0.05$). Yeast and mould counts of the product did not show any seasonal variation ($P>0.05$).

Average coliform, faecal coliform and enterococci counts noted were 2.9, 0.5 and 13,000 per g and detected in 94.2, 77.6 and 89.7 per cent of the samples respectively. In 19 samples, the presence of *E. coli* was confirmed. Types of coliforms isolated were *E. coli*, *Klebsiella aerogenes*, *Enterobacter cloacae* and *Citrobacter* spp. The seasonal differences in counts (Table 2) were not statistically significant ($P>0.05$).

One hundred and eighty isolates of *Staphylococcus aureus* from 68 samples were tested for coagulase activity and 10.3 per cent were found positive.

Table 3 presents the antibiotic sensitivity of coliforms. Number of strains of *Escherichia coli*, *Klebsiella aerogenes*, *Enterobacter cloacae* and *Citrobacter* spp tested for antibiotic sensitivity were 30, 28, 22 and 18 respectively. Most of the strains were resistant to penicillin, followed by erythromycin and ampicillin but sensitive to chloramphenicol and streptomycin. Tetracyclines

TABLE 1. MICROBIAL POPULATION OF CUT CUCUMBER

Season	Standard* plate count ($\times 10^3$/g)	Psychrophilic* plate count ($\times 10^3$/g)	Lipolytic bacterial count ($\times 10^3$/g)	Yeast count ($\times 10^3$/g)	Mould count ($\times 10^3$/g)
Winter	15.0-225.0 (81.80)	0.3-37.5 (10.60)	0.45-8.8 (2.55)	0.05-4.7 (1.80)	0.0-3.5 (0.68)
Summer	4.7-91.0 (36.50)	0.02-17.1 (3.50)	0.15-10.25 (2.20)	0.0-5.8 (1.84)	0.0-9.3 (0.93)

Figures in parentheses indicate average values.
*Seasonal difference is significant at $P<0.05$.

TABLE 2. INDICATOR ORGANISMS IN CUT CUCUMBER

Season	Coliform/g* ($\times 10^3$/g)	Faecal coliform/g ($\times 10^3$/g)	Enterococci ($\times 10^3$/g)
Winter	0.82-9.50 (4.41)	0.0-5.40 (0.74)	0.0-3.8 (0.94)
Summer	0.0-13.0 (2.58)	0.0-1.40 (0.45)	0.0-6.9 (1.41)

Figures in parentheses indicate average value.
*Seasonal difference is significant at $P<0.05$.

TABLE 3. ANTIBIOTIC SENSITIVITY OF COLIFORMS*

Organism	*E. coli*	*Kl. aerogenes*	*Ent. cloacae*	Citrobacter spp.
Penicillin	10	14	9	33
Ampicillin	43	56	27	50
Streptomycin	90	85	91	100
Erythromycin	20	14	18	16
Chloramphenicol	93	85	91	100
Tetracycline	50	70	55	66
Oxytetracycline	50	70	55	66

*Results are as % strains sensitive to antibiotic.

were less effective than chloramphenicol and streptomycin. Multiple resistance to different antibiotics combination was also noted. Thus out of 30 strains of *E. coli* isolated only two strains were sensitive to all the antibiotics used. Number of strains resistant to a combination of 2, 3, 4, 5, and 6 antibiotics were 2, 6, 5, 4, 9 and 2 respectively.

Antibiotics resistant coliforms in food causes great concern as R-factor can be transferred *in vivo*[12]. *E. coli* strains with R-factor plasmid are responsible for many human infections. It has also been reported that *Klebsiellae* were isolated from a variety of human infections[13], so it may be a source of potential danger for the health of man. *Klebsiellae* derived from natural environment are generally mere diverse genetically than those of clinical isolates. There are reports of *Klebsiellae* of faecal origin possessing transmissible R-factors[14]. Above findings reveal that there is a strong case for enforcing proper sanitary practices in the cut fruits sold by vendors.

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EXTRACTION OF TALLOW FROM MEAT OFFAL

Meat offal, a byproduct of meat-packing industry, contained 8.9 per cent moisture, 26.5 per cent fat and 49.0 per cent protein. The solvent-extracted fat from meat offal resembled animal tallow and was refined and bleached to a light colour.

The byproducts of the meat-packing industry are not processed scientifically in India. In rural areas, the bulk of meat remnants go waste. while even in most of the urban slaughter-houses or abattoirs, proper arrangements for collection and processing of these materials hardly exist.

Meat offal consists of finely-ground residue from animal tissues, exclusive of hair, hoof, horn, hide trimmings, blood meal and stomach contents. Rendering was not done as is practised in the case of adipose tissue. Alvarezg *et al.*[1] published rate extraction of fat from meat meal by suitable solvent.

The meat offal sample of bovine origin obtained from Brooke Bond India Limited, Aurangabad was analysed for various constituents following AOCS methods[2]. The sample was extracted a number of times with normal hexane in 1:4. ratio in a batch type extractor. From 5 kg of the material, 1.26 kg fat was recovered. The fat was further analysed for its constituents. Crude fat was refined and bleached by various methods. In one of these, fat was neutralized with 80 per cent excess of an alkali lye of 16°Be' strength. The high melting point of the fat resulted in refining losses (25 per cent) since fat was entrapped in soapstock. The neutralized fat was bleached with a combination of 3 per cent bleaching earth and 0.3 per cent activated carbon. In another variation of refining, the crude fat was pretreated with 15 per cent oxalic acid solution and then bleached with 3 per cent earth and 0.3 per cent carbon.

The colours of fat were measured in a Lovibond Tintometer using 0.635 cm cell; since the fat was solid at ambient temperature, solutions in carbon tetrachloride (24 times dilution) were read.

The meat offal analysed contained 8.9 per cent moisture, 26.5 per cent fat and 49.0 per cent protein and 9.8 per cent ash. The water content is low as the material was received in dried condition. Extraction with normal hexane yielded 25 per cent fat. Table 1 shows that the main characteristics of the meat offal fat compared with the ISI specifications[3]. Crude fat was dark green in colour. This may be due to the method of extraction with hexane which is non-selective in nature, while heat rendering would result in a yellow fat. Direct bleaching did not reduce the colour significantly. It is seen from Table 2 that when the crude fat was pretreated with oxalic acid and subsequently bleached with earth and carbon, a light-coloured fat was obtained.

TABLE 1. CHARACTERISTICS OF MEAT OFFAL FAT

Characteristics	Meat offal fat	Animal tallow*
Moisture (%)	0.1	1.0
Acid value	7.0	10.0
Saponification value	192	192–202
Iodine value (Wijs)	34.5	30–56
Unsaponifiable matter (%)	4.0	1.0
Titre, °C	46.5	40–51

*As per ISI specification of IS:887–1977. (Second revision).

TABLE 2. COLOURS OF MEAT OFFAL FAT

Type of fat	Lovibond Tintometer colours, (0.635 cm cell)		
	Y	R	
Crude fat	5.2	0.6	Dark green in solid state
Crude fat bleached directly with earth and carbon			No reduction in colour
Alkali-neutralized fat	2.2	0.1	Light green in solid state
Alkali-neutralized fat, bleached with earth and carbon	0.6	0.0	White with greenish tinge in solid state
Crude fat pretreated with oxalic acid and bleached with earth and carbon	1.2	0.1	Light yellow with greenish tinge in solid state

Colours were read in solutions of carbon tetrachloride (24 times dilution)

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CONTROLLED DEACIDIFICATION OF 'BANGALORE BLUE' GRAPE MUST WITH *SCHIZOSACCHAROMYCES POMBE*

To control deacidification and off-flavour production by *Schizosaccharomyces pombe*, use of both *Sch. pombe* and *Saccharomyces cerevisiae* cultures in wine making from 'Bangalore Blue' grape was investigated. Addition of *S. cerevisiae* after 2 days to *Sch. pombe* fermenting must or inoculation of *S. cerevisiae* and *Sch. pombe* in the ratio of 1:10 produced wine with optimum acidity. A process for the production of acceptable quality wine with optimum acidity from 'Bangalore Blue' grape has been described.

Deacidification of high acid grape musts for wine making with *Schizosaccharomyces pombe* has been studied by many workers[1-3]. Drawbacks observed in adopting this method are excess-deacidification and development of off-flavour in wines. To overcome these defects, use of both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* in fermentation has been suggested. Since growth of *Sch. pombe* is slow, *S. cerevisiae* can easily overgrow it. Therefore, the latter organism has to be added at a proper time during fermentation to attain optimum level of acidity in wines. 'Bangalore Blue', which is grown over a large area in Karnataka is unsuitable for wine making because of its high acidity. To produce wine with optimum acidity, various experiments on controlled deacidification were carried out and the results are reported in this communication.

'Bangalore Blue' grape harvested from Experimental Farm at Hesaraghatta, Bangalore during Feb.-May,

Contribution No. 12/82

TABLE 1. EFFECT OF REINOCULATION OF S. CEREVISIAE ON DIFFERENT DAYS TO SCH. POMBE FERMENTING MUST ON ACIDITY OF WINE

No. of days for reinoculation with *S. cerevisiae* to fermenting must	Titratable acidity (as g tartaric acid/100 ml) Must	Wine	%reduction in acidity
1	1.13	0.75	33.6
2	1.13	0.68	39.8
3	1.13	0.67	40.7
4	1.13	0.67	40.7

TABLE 2. EFFECT OF INOCULATION OF DIFFERENT PROPORTIONS OF S. CEREVISIAE AND SCH. POMBE YEASTS

Proportion of *S. cerevisiae* to *Sch. pombe*	Titratable acidity (as g tartaric acid/100 ml) Must	Wine	% reduction in acidity
10 : 90	1.13	0.79	30.1
20 : 80	1.13	0.82	27.4
30 : 70	1.13	0.86	23.9
40 : 60	1.13	0.86	23.9
50 : 50	1.13	0.87	23.0
S. cerevisiae (control)	1.13	1.04	7.9
Sch. pombe. (control)	1.13	0.64	43.4

1978 and 1979 were used. Twenty kg of grape was crushed, sulphited potassium metabisulphite was added to give 100 ppm SO_2 and the Brix was raised to 22° with cane sugar. The must was divided into 11 lots. Two control lots were inoculated separately with 2 per cent of 48 hr old *S. cerevisiae* and *Sch. pombe*. Four lots were initially inoculated with *Sch. pombe* and later on one lot each was inoculated with *S. cerevisiae* on first or second or third or fourth day (Table 1). The remaining five lots were inoculated with varying levels of both *S. cerevisiae* and *Sch. pombe* (Table 2). In another experiment, 10 kg of fruit was crushed, sulphited, ameliorated with cane sugar to 22° Brix and divided into five parts. One part each inoculated with *S. cerevisiae* or *Sch. pombe* was kept as control. The other three lots were inoculated with *Sch. pombe*. After third, fourth and fifth day, the must was pressed, centrifuged at 10,000 rpm for 10 min and the partially fermented juice was inoculated with *S. cerevisiae* and allowed to ferment at 20±1°C till completion. The vinification procedure followed has been described earlier[3]. The must and wine samples were analysed for different constituents according to the procedure of Amerine and Ough[4].

Mixed fermentation with S. cerevisiae and Sch. pombe on the acidity of wines: *Sch. pombe* fermented wine showed 43.4 per cent reduction in acidity as compared to 7.9 per cent by *S. cerevisiae* (Table 2). Experiments carried out in this laboratory by us with other high acid varieties revealed that acid reduction upto 73 per cent could be achieved (unpublished work). This could be attributed to the difference in malic acid content of the variety. Inoculation of *S. cerevisiae* into the must being fermented by *Sch. pombe* at different time intervals showed that optimum acidity in wines could be obtained by delaying the addition of *S. cerevisiae* for 2-3 days. (Table 1). Gallander[2] has also reported that *S. cerevisiae* inoculated into the *Sch. pombe* fermenting must 3-4 days after the start of fermentation reduced the level of acidity.

Mixed culture fermentation with different proportions of *S. cerevisiae* and *Sch. pombe* inoculum indicated (Table 2) that deacidification was dependent on the proportion of *S. cerevisiae* present in the mixture. A combination of 10 parts of *S. cerevisiae* and 90 parts of *Sch. pombe* reduced the wine acidity to near optimum level. However, in other combinations where the proportion of *S. cerevisiae* was high there was less reduction in acidity. This indicated that *S. cerevisiae* can easily over grow and suppress *Sch. pombe* which is a slow growing yeast.

The study made by Snow and Gallander[5] on white grape juice fermentation showed that the problem of excess-deacidification and development of off-flavour by *Sch. pombe* could be prevented by fermenting initially with *Sch. pombe*, removing *Sch. pombe* cells by storing at 4°C for 2 days, racking and reinoculating with *S. cerevisiae* to complete fermentation. They also suggested that the quality of wines can be imrproved by centrifugation as a better method for removing *Sch. pombe* cells. Based on this information and on the results obtained in this study, a method has been suggested for the production of acceptable quality wine from 'Bangalore Blue' grape (Fig. 1 and Table 3). From Table 3, it is clear that

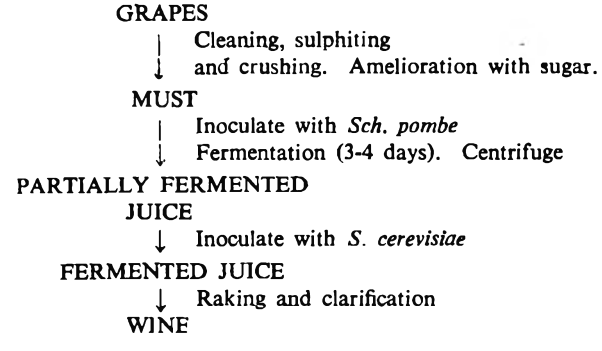

FIG. 1. A process for preparation of wine from high acid "Bangalore Blue" grapes.

TABLE 3. MUST AND WINE COMPOSITION OF 'BANGALORE BLUE' GRAPES PARTIALLY FERMENTED WITH SCH. POMBE AND REINOCULATED WITH S. CEREVISIAE

S. cerevisiae added on	Must			Wine							
	°Brix	Acidity (g. of tartaric acid/100 ml)	pH	Alcohol (%)	Acidity (g. of tartaric acid/100 ml)	pH	Volatile acidity (g of acetic acid)	Reducing sugar (%)	Tannins (mg/1)	Colour (1:10 dil)	
										+Brightness	Hue*
3rd day	18.6	0.99	3.25	10.5	0.64	3.40	0.030	0.136	1520	0.497	0.911
4th day	18.6	0.99	3.25	11.1	0.69	3.40	0.057	0.136	1460	0.605	0.795
5th day	18.6	0.99	3.25	10.5	0.67	3.40	0.060	0.136	1580	0.595	0.865
Sch. pombe control	18.6	0.99	3.25	11.5	0.62	3.40	0.057	0.268	1520	0.497	0.854
S. cerevisiae control	18.6	0.99	3.25	11.2	0.98	3.30	0.036	0.136	2020	0.648	0.815

+ Sum of absorbance at 420 and 520 nm

* Ratio of absorbance at 420 and 520 nm

the composition of wine did not show much variation due to reinoculation with *S. cerevisiae* after the third day. Sensory evaluation of one year old wine revealed that the *Sch. pombe* fermented wine had no off-flavour and was comparable to the wine made from mixed culture fermentation. However, *S. cerevisiae* fermented wine was highly acidic and obtained lowest ranking. Because of its slow growth *Sch. pombe* takes longer time to complete fermentation and remove more acid than necessary in high malic acid grapes, hence controlled deacidification with *S. cerevisiae* and *Sch. pombe* is suggested as a better method to obtain optimum acidity in wines.

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ANTIFUNGAL ACTIVITY OF ORANGE AND LIME OILS

Terpene fraction of orange oil at 250 mg %, non-terpene fraction of lime oil at 200 mg %, terpenyl formate and limonine oxide at 300 mg % concentration were inhibitory to the spores of *Aspergillus*, *Rhizopus* and *Fusarium*. Terpenyl acetate at 300 mg % level was inhibitory to *Rhizopus* and *Fusarium* but had little effect on *Aspergillus*. At sub-inhibitory levels of terpenyl acetate, terpenyl formate and limonine oxide, all the three test fungi showed poor growth without sporulation.

All essential oils are not bactericidal because of the variation in their composition[1]. Placentini[2] reported that aqueous solution of orange and lemon essences possessed disinfectant properties against spore forming organisms. Zukerman[3] reported that d-limonine, freshly distilled over sodium did not inhibit the growth of *Saccharomyces elliposoidieus*, *Willia hansenulla* and *Oidium lactis*, while vapours of the compound had fungistatic effect. Orange peel oil and ∝-limonine in water or orange juice were lethal to Zygosaccharomyces[4]. Subba Rao *et al.*[5] reported that yeasts, in general were more sensitive to orange oil than bacteria. Orange oil had more antifungal activity compared to lime oil. A method has been developed to fractionate

orange and lime peel oils at this Institute and utilise the desirable fractions as flavouring ingredient. The terpene fractions are undesirable in the beverages. Normally fruits and vegetables are contaminated with *Aspergillus*, *Rhizopus* and *Fusarium*. Studies were carried out to find out the antimicrobial property of different fractions and derivatives of the orange and lime peel oils against strains of *Aspergillus*, *Rhizopus* and *Fusarium*.

Terpene and non-terpene fractions of orange and lime oil, terpenyl formate, terpenyl acetate and limonine oxide used in our experiments were obtained from Plantation Products and Flavour Technology Discipline of the Central Food Technological Research Institute, Mysore. The fungal cultures (*Aspergillus* sp., *Rhizopus* sp. and *Fusarium* sp.) used were isolated from vegetables. The cultures were grown on potato dextrose agar (PDA) for 72 to 96 hr at 30 ± 1°C and spores were harvested and suspended in sterile water containing 0.1 per cent Tween 80.

The fractions of orange and lime oil and their derivaties were dissolved in ethanol and desired volume to contain 50-750 mg were mixed with 100 ml of PDA. Medium was poured into sterile petriplates and allowed to solidify. Maximum concentration of ethanol in the medium was below 0.75 per cent which is not inhibitory to the test organisms. One tenth of an ml of spore suspension containing 10,000 spores was spread on the surface of the media with a bent glass rod.

The results presented in Table 1 indicate that orange terpene at 250 mg per cent was inhibitory to all the test organisms. Orange non-terpene at 750 mg per cent retarded the growth of organisms. The size of developing fungal colonies upto 10 days was very small. It is seen from Table 2 that *Fusarium* sp. is very sensitive to lime non-terpene (LNT) even at 100 mg per cent level, while a concentration of 200 mg per cent is required to inhibit the growth of other two fungi. Test organisms exhibited poor growth at 750 mg per cent lime terpene concentration. The results obtained with the other oxygenated compounds are presented in Table 3. It is seen that terpene formate at 200 mg per cent was inhibitory to *Aspergillus* sp. and *Rhizopus* sp. while 300 mg per cent was required to inhibit *Fusarium* sp. Terpenyl acetate at 100 and 300 mg per cent level was inhibitory to *Rhizopus* sp. and *Fusarium* sp. respectively, but did not inhibit *Aspergillus* sp. Poor growth without sporulation was observed even after 10 days at 400 mg per cent level. Limonine oxide at 300 mg per cent level inhibited growth of all the test organisms, while at

TABLE 1. EFFECT OF ORANGE TERPENES AND ORANGE NON-TERPENES ON GROWTH OF MOULDS

Mould	Type of orange oil	Concn in mg%						
		50	100	150	250	250	500	750
Aspergillus sp	Terpene	++	++	++	+	—	—	—
	Terepeneless	++	++	++	++	++	++	+
Rhizopus sp.	Terpene	++	++	++	+	—	—	—
	Terepeneless	++	++	++	++	++	++	+
Fusarium sp.	Terpene	++	++	++	+	—	—	—
	Terepeneless	++	++	++	++	++	++	+

++ = Good growth + = Poor growth — = No growth

TABLE 2. EFFECT OF LIME TERPENES AND LIME NON-TERPENES ON THE GROWTH OF MOULDS

Mould	Type of lime oil	Concn in mg%						
		50	100	150	200	250	500	750
Aspergillus sp.	Terpene	++	++	++	++	++	++	+
	Terpeneless	++	+	+	—	—	—	—
Rhizopus sp.	Terpene	++	++	++	++	++	++	+
	Terpeneless	++	+	+	—	—	—	—
Fusarium sp.	Terpene	++	++	++	++	++	++	+
	Terpeneless	+	—	—	—	—	—	—

++ = Good growth + = Poor growth — = No growth

TABLE 3. EFFECT OF TERPENE FORMATE, TERPENE ACETATE AND LIMONINE OXIDE ON THE GROWTH OF MOULDS

Organisms	Substance	Concn in mg%				
		50	100	200	300	400
Aspergillus sp.	Terpene formate	++	++	—	—	—
	Terpene acetate	++	++ns	++ns	+ns	+ns
	Limonine oxide	+	++	++ns	—	—
Rhizopus sp.	Terpene formate	++	++	—	—	—
	Terpene acetate	++ns	—	—	—	—
	Limonine oxide	++ns	++ns	+ns	—	—
Fusarium sp.	Terpene formate	++	++ns	++ns	—	—
	Terpene acetate	++	++	++ns	—	—
	Limonine oxide	++	++	++ns	—	—

++ =Good growth + =Poor growth — =No growth
ns = No sporulation upto 5 days incubation.

lower concentrations growth without sporulation was noticed. The results indicate that the terpene fraction of orange oil, and the non-terpene fraction of lime oil are found to be antifungal. Terpenyl formate was inhibitory to all the test organisms and at sub-inhibitory levels, prevented spore formation in *Fusarium* sp. Terpenyl acetate and limonine oxide at sub-inhibitory levels prevented sporulation in all the test organisms. These compunds appear to affect the formal physiology of the sporulation in fungi.

The studies indicate the possible use of these antifungal compounds in the cosmetic, pharmaceutical, and in general antiseptic preparations, whereas lime non-terpene and limonine oxide may be used in food-processing.

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COLORIMETRIC DETERMINATION OF CARBARYL AND ITS RESIDUE IN VEGETABLES

A method has been developed for the determination of carbaryl (1-naphthyl-N-methyl carbamate) in formulations and vegetables. Carbaryl on hydrolysis with methanolic KOH gives 1-naphthol which when coupled with diazotised 4:4-diaminodiphenyl sulphone has an absorption maxima at 540 nm. Beer's law is obeyed in the range of 0.25-5.0 μg/ml. The method is sensitive and can detect as low as 0.5 ppm of carbaryl in cabbage, okra and beans.

Carbaryl (1-naphthyl-N-methyl carbamate) is an important carbamate insecticide used for the control of crop pests. A number of spectrophotometric and gas chromatographic methods have been developed for the determination of carbaryl[1-7] from agricultural crops and formulations. Although gas chromatographic methods are ideal for determining and estimating pesticides, their use is often limited by nonavailability and cost. The non-innocuous nature and unavailability of the chromogenic reagents reported earlier[1-7] necessitated a search for an alternative sensitive reagent. The results of analysis of carbaryl employing 4:4-diaminodiphenyl sulphone for diazotisation are described in this paper.

Preparation of standard curve: Two ml of 1N methanolic KOH was added to each test tube containing carbaryl in methanol ranging between 5-100 μg, mixed, hydrolysed for 5 min in water at 60-70°C and cooled. 0.5 ml of a 0.5 per cent 4:4-diaminodiphenyl sulphone in methanol was diazotised by adding 0.5 ml of 1 per cent $NaNO_2$ and 1 ml 1N HCl. The diazotised reagent was added to hydrolysed carbaryl and mixed well for 2-3 min. The volume was made upto 20 ml by adding methanol and allowed 5 min for the development of full colour. The absorbance was read at 540 nm against a blank prepared similarly and the standard graph drawn.

Determination of residues from vegetables: Hundred grams each of blended cabbage (*Brassica oleracea*), okra (*Hibiscus esculentus*) and beans (*Phaseolus vulgaris*) were fortified with 50-200 μg carbaryl in chloroform and extracted twice with 100 ml of $CHCl_3$, the solvent layer was filtered through Whatman No. 1 filter paper and the residue was washed twice with 20 ml of $CHCl_3$. The combined $CHCl_8$ extract was evaporated completely in a vacuum evaporator and the residue was dissolved in 10 ml CH_3CN. The CH_3CN layer was washed thrice with 20 ml of hexane in a separatory funnel and the hexane layer was discarded. The CH_3CN layer was made up to a known volume and the colour was developed as mentioned earlier, before measuring the absorbance.

Formulation analysis: Quantities of 50 per cent W. P. carbaryl (supplied by Union Carbide Co., Bhopal, India) ranging from 5-20 mg were dissolved in 10 ml of methanol in a centrifuge tube and centrifuged at 5000 rpm for 5 min. The supernate was decanted into a 25 ml volumetric flask. The residue in the centrifuge tube was rinsed with another 10 ml portion of methanol and centrifuged as before. The methanolic extracts were combined and made up to volume with methanol. A known aliquot was taken from this and analysed as outlined in standard curve preparation

The linear relationship between absorbance at 540 nm and concentration is valid upto 5.0 μg/ml. It took 5 min for full colour development and the colour was stable for more than 24 hr.

To check the recovery of the fortified vegetable samples (cabbage, okra and beans) by this method,

TABLE 1. RECOVERY OF CARBARYL FROM SOME VEGETABLES

Vegetable	Added (μg)	Found[a] (μg)	Recovery (%)
Cabbage	50.0	48.75 $\pm$ 1.34	97.5
	100.0	96.00 $\pm$ 2.13	96.0
	150.0	143.40 $\pm$ 2.10	95.6
	200.0	188.00 $\pm$ 3.58	94.0
Okra	50.0	46.85 $\pm$ 1.30	93.7
	100.0	93.00 $\pm$ 2.28	93.0
	150.0	136.50 $\pm$ 3.82	91.0
	200.0	181.00 $\pm$ 1.67	90.5
Beans	50.0	49.25 $\pm$ 0.65	98.5
	100.0	97.10 $\pm$ 1.64	97.1
	150.0	144.00 $\pm$ 2.60	96.0
	200.0	185.00 $\pm$ 4.60	92.5

[a]Mean $\pm$ Standard deviation of five analysis.

samples were fortified with 50-200 μg of carbaryl. The percentage of recovery was 90.5-98.5 (Table 1). The analysis of 50 per cent W. P formulation of carbaryl revealed that the average carbaryl content was 49.07 per cent (average of 5 analysis) as against the declared value of 50 per cent.

The proposed colorimetric method can detect as low as 0.25 μg/ml as against 0.5-1 μg by other methods[1-4]. Although the method which specifies the use of 2,5-di-chloroaniline[5] is more sensitive (minimum detection limit of 0.1 μg) than the present method; it could be used only for formulation analysis and not for residue analysis. The interference by other phenolic substances in vegetable samples were overcome by substracting the blank values (0.01 optical density) from each carbaryl fortified samples. Further, the method does not involve elaborate clean up procedure as mentioned in other methods[1-7]. As the chromogenic reagent (4:4–diaminodiphenyl sulphone) used is innocuous and is sensitive, the present method could be used safely as an alternative sensitive method for the determination of carbaryl in vegetables (tolerance limit 5.0 ppm).

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BOOK REVIEWS

Developments in Food Microbiology-I: by R. Davies, (Ed.), Published by Applied Science Publishers, London-New Jersey; 1982; Pp. 219; Price: £ 24.

With change in socio-economic status, food industry and food microbiologists today are not only engaged in conservation and preservation of food but also desires to develop convenient, novel and gasteronomically pleasant food. Hand in hand, therefore goes the necessity to understand, monitor and control all those microbiological factors which control wholesomeness and safety of foods. Very appropriately therefore, four of the six chapters of this book are devoted to those aspects of food which contribute towards wholesomeness and safety of food.

T. A. McMeeken, in chapter on 'Microbial Spoilage of Meats', after giving the gist of the existing knowledge about contaminants of meats with special reference to psychrophiles, effect of storage and temperature on microbial population and the significance attached earlier to the proteolysis in aerobic spoilage, dives into critical details to introduce the new concept of mechanism of spoilage on the basis of the current research, corroborating certain findings of earlier workers, and concludes with a review and discusion on the earlier and the recent methods of evaluation of spoilage. The topic is dealt critically and gives insight into the recent trends in research on microbial spoilage of meats; however, the author has bypassed the mechanism of attachment of bacteria to surface of meat which should have been included.

The second chapter on Nurmi Concept which advocates *per ora* introduction of gastrointestinal flora from adult birds into newly hatched chicks or poults to control salmonella infections in poultry, with the aim to curb poultry borne salmonellosis in human beings, is dealt by H, Pivnic and E. Nurmi, the very workers on whose name the discovery is named. The review is exclusive, thought provoking and not only discusses the possible mechanism(s) by which gut flora (authochthonous microbiota) from adult birds protects chicks or poults from Salmonella (other than *S. pullorum* and *S. qallinarum*) infestions, but brings out in great detail the practical aspect of it giving sources of material to be used for treatment, preservation of the material, culturing of organisms to be used as treatment, inoculation of the chicks to be treated and also efficiency tests.

The third aspect considered as the bacteriology o fish handling and processing by G.Hobbs and W. Hadgkiss. It details the current trends in fish microbiology which emphasise the problems hygiene, food poisoning and microbiological specifications apart from the spoilage. Besides dealing with the handling and processing of preserved fish products, bacteriological problems associated with it and association of certain bacteria with particular spoilage changes are revealing.

The fourth aspect on the same theme considered is rapid and very rapid detection methods of microbial population in foods which anticipates the propensity to spoilage and potential to health hazard. The authors J. M. Wood and P. A. Gibbs give critical and comparative insight into various most recent rapid techniques like impedemetric, radiometric methods and very rapid tests like limulus assay, bioluminescene, chemiluminescence including separation of microorganisms from foods. These methods can replace the conventional methods like colony count. Brief description of various instruments and their operation with particular reference to impedimetric assays is informative.

The other two chapters consider altogether different aspects of food microbiology but none the less very important. One of the chapters on 'Lactose metabolism in dairy streptococci' by L. L. McKay deals with genetic and metabolic regulation of fermentation of lactose to lactic acid by dairy streptococci and how recent developments in molecular genetics are employed to develop improved lactose fermenting strains.

The other chapter by Brown, K. L. and Gelia A. Ayres on thermobacteriology of UHT processed foods summerises the literature relating to all aspects of ultra high temperature processing and the resistance of bacterial spores to high temperature under various topics.

The book is highly recommended as it is very useful to all those working in the field of food science irrespective of their major field of work, but especially more so to food microbiologists. Compilation and eddition of the latest trends in food microbiology, with the existing knowledge is commendable.

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Advances in Biochemical Engineering. Vol. 19. *Reactors and Reactions:* Edited by A. Fiechter, Spinger-Verlag, Berlin, Heidelberg, New York, 1981; Pp. 269, Price: $ 44.60.

This volume is an excellent compendium of critical reviews on five emerging frontier areas in Biochemical Engineering and is in tune with the tradition set by earlier volumes in the series.

The review on Design of Biochemical Reactors by M. Moo-Young and H. W. Blanch has very detailed and critical information on mass transfer criteria for simple and complex systems. Part I of the review deals with basic concepts and has information on mass transfer pathways, external particle mass transfer, intra particle mass transfer and physical properties of process materials. Equipment performance is covered in Part II where extensive information on gas-liquid contactors with or without mechanical agitation, agitation and power requirements; scale-up consideration on gas-liquid mass transfer and fluid flow basis is presented. The authors rightly point out that although extensive information has accumulated on the subject, more information is still needed to put process design on a rational basis before the special capabilities of different reactors and media configuration could be commercially exploited. The treatment is extensive and exhaustive as to stimulate scientific thinking in this area.

K. Schugerl in his review on oxygen transfer in highly viscous media takes into consideration problems involved in measuring OTR in high viscosity media. The theme is well developed with methodology, where techniques for measuring rheological behaviour, gas solubilities in media, OTR, volumetric mass transfer coefficient, mass transfer coefficient and power input are discussed. Also discussed in detail are the single bubble and swarm behaviour and different reactor systems like stirred tank reactors, single stage tower reactors and multi-stage tower reactors from the point of view of apparatus, instruments, mathematical models, hydrodynamic properties, OTR and KLa. Other novel reactors like loop reactors, plunging jet reactors and jet loop reactors are cited as also the various mixing devises. Comparisons with different reactor systems are made along with recommendations. The information presented here is very vital to a practicing biochemical engineer.

The review on machanisms and occurance of microbial oxidation of long chain alkanes by H. J. Rehm covers detailed information on the subject which could be of value in basic research as well as in controlling the production of primary and secondary metabolites. Introducing the theme, the review deals with primary oxidation steps in alkane molecule, metabolic pathways in the oxidation of long chain alkanes and their degradation pathways in different microbial systems. The review is made interesting by incorporation of suitable metabolic pathways and has exhaustive information on cellular fatty acid composition of bacteria degrading long-chain n-alkanes (C_{10}-C_{16}) as well as on extracellular fatty acids from different microorganisms. Information of this kind is very useful to a research worker in this field. The author also brings out the limitations of alkane degradation as a criteria for taxanomic purposes.

S. Fukuil and A. Tanaka in their review on metabolism of alkanes by yeasts bring out the conspicuous appearance of peroxisomes in alkane degrading yeast, their role in the formation of acetyl CoA and co-operative action of peroxisomes, mitochondria and microsomes in lipid biogenesis. The theme is developed along the following lines, uptake of alkanes, initial oxidation, oxidation of higher alcohols to fatty acids, appearance of peroxisomes and formation of acetyl CoA, synthesis of cellular fatty acids and tricarboxylic acid cycle intermediates and discusses biochemical basis of conversion of citric acid to polyols, erythritol to mannitol. The review also stresses specific differences in metabolic regulation between fermentative saccharomyces and oxidative candida yeasts. Physiological studies on alkane utilising yeasts is expected to give new information on the general physiology of living organisms.

The review on Protoplasts in genetic modification of plant, is covered competently by O. L. Cramborg and P. J. Bottino. This very promising thrust area in biotechnology is going to revolutionise our agricultural practices, a social and industrial forestry and control of plant diseases. Introducing the theme the authors have discussed plant protoplast isolation with respect to enzymes and isolation medium, protoplast culture and development and uses of protoplasts. The authors then discuss the production of plant cells mutants, metabolic and auxotrophic and chlorophyll deficient mutants, which have been of great value in elucidating problems in genetics and biochmistry. The technique of somatic hybridisation to produce wide crosses for plant breeding to transfer resistance to diseases and pests, tolerence to stress conditions, improve product quality and growth characteristics is systematically developed and aspects like protoplast fusion, hybrid development, hybrid selection and plant development are thoroughly discussed. Finally the review has information on the use of protoplasts for gene transfer through DNA uptake and uptake of organelles and single cells, along with its wide spread implications.

This valuable review aptly titled 'reactors and reaction' would be extremely useful for biochemical engineers, and researchers interested in the field of microbial metabolisms, industrial fermentation and genetic engineering.

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Advances in Biochemical Engineering. Vol. 21: by Fiechter A. (Ed), Springer-Verlag, New York, 1982; Pp: 232; Price: $ 42.90.

This volume has four articles which are of current relavence. The first one is on the microbial biomass from renewables with emphasis on alternate substrates and processes. Unlike earlier reviews on SCP production, this review presents alternate processes available keeping in mind the cost economics involved in SCP production. Processes that generate multiple end products in addition to the biomass perhaps have a future and from this point of view, the article is timely for all those interested in SCP production. The article is exhaustive and contains 700 references and it appears that the authors, Rolz & Humphrey have left nothing untouched in this area. The second article by Harder and Roels is a review on application of simple structural models in Bioengineering. It points out certain difficulties that are encountered in applying mathematics to complex living systems and highlights the use of continum models which are applicable to complex systems where Monod's classical model fails. The third review by Gutschick deals with the Energetics of microbial nitrogen fixation in various biological systems. The energy balances are calculated keeping in mind synthesis, asimilation and maintenance of the organisms under *in vivo* and *in vitro* conditions. Many of the areas in nitrogen fixation that need further studies have also been pointed out. The fourth article by Viesturs & others on Foam control deals with an important aspect of microbial fermentations which sometimes is unavoidable but problematic. Although antifoam agents are available, mechanical breaking has many advantages. Keeping this in mind the various types of mechanical foam breakers have been discussed. In general, this volume has much information both for Microbiologists and for Bioengineers.

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Advances in Biochemical Engineering. Vol. 24: by A. Fiechter (Ed) Springer-Verlag, Berlin, Heidelberg, New York. p. 178.

This volume besides comprising of reviews on four important areas in Biochemical Engineering has author's index with titles of topics covered in all the 24 volumes in the series.

The review by A. Schumpe, G. Quicker, and W. D. Deckwer covers extensive information on the subject of gas solubilities particularly oxygen and carbon dioxide so vital to fermentation processes. Stressing the importance of gas solubilities to establish mass balances, to calculate yield coefficients, to determine volumetric mass transfer coefficient and to scale up of bioreactors, the review deals with theories of gas solubilities, parameters affecting gas solubilities inclusive of media constituents like single and mixed electrolytes adsorption effects. The solubility parametes for oxygen have been summarised. Also discussed is the use of predictive methods both direct and indirect for estimating solubilities during actual bioreaction and their limitation.

Immobilised biocatalysts are replacing conventional fermentors rapidly and Klaus Buchholz's review on reaction engineering parameters for immobilised biocatalysts is apt and timely. Introducing the subject with possible reactor configurations available for the purpose, the author discusses the parameters affecting the performance of heterogenous immobilised biocatalysts. The discussion on mechanical and physical parameters of carrier-bound biocatalysts concerns with particle diameter, swelling behaviour, pressure drop, practicle compression behaviour and abrassion. Kinetics and the effectiveness of the sytem as well as operational stability are discussed in great detail. The author also discusses new concepts like specific designs, coimmobilisation of enzymes.

Maria-Ragina, Karl Heinz Kroner and Helmut Hustedt in their review on purification of enzymes by liquid-liquid extraction bring out the current status on the application of extractive purification of enzymes on a large scale. Aspects like properties of acqueous phase, polymers constituting the phase system, improvements in selective extraction through affinity partition and liquid ion exchangers are discussed in detail. The treatment on single step partition has examples of enzyme purification and scale-up consideration while that on multistage extraction deals with Grasser contactors and centrifugal separators. The review also discusses separation of acqueous phases systems, removal of polymers and economic considerations. Basic and practical information presented here is of great use

to enzymologists and biochemical engineers interested in producing enzymes for setting up enzyme reactors.

The review on biomass separations from liquids by sedimentation and centrifugation by Udo Wiesmann and Hubert Binder discusses this important step in product recovery in fermentation systems. The topic is discussed in detail under three heads, sedimentation, centrifugation and flocculation. Discussion on sedimentation tanks has information on different systems including the lamella type separators and sedimentation in open and closed systems. The treatment on centrifugation centers round disc, decanter and tube centrifuges and clarification in open and closed systems. The review also covers information on application of these systems to biotechnology processes and has a short discussion on floculation a process in vogue for separation purposes.

This is an excellent volume of use to biochemical engineers, chemical engineers, and research workers in the field of Biotechnology.

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Elements of Food Spoilage and Preservation: by Awan and Okaka, Published by Institute of Management and Technology, P. M. B. 01079, Enugu, Nigeria; 1983; Pp: 160, Price: $ 12.00+postage.

This is the first publication in the field of Food Preservation written by professional food scientists/ technologists in Nigeria. This book written specially for the benefit of Nigerian students of food science and technology on spoilage and preservation of food.

History of food processing, importance of food processing and preservation, classification of foods, spoilage of foods and spoilage agents, principles of food preservation, preparatory operations in food preservation, use of high and low temperature, removal or binding of moisture, use of chemical additives and use of fermentations and irradications have been dealt in the introduction.

Definition of food processing, interdisciplinary approach in food science and technology and the knowledge required for a food technologist have been incorporated in the first chapter.

The second chapter deals with the ancient and modern methods of food processing. In the third chapter, the importance of food processing and preservation have been briefly explained. The fourth chapter includes the classification of foods based on composition, functions and their origin.

The spoilage of foods by autolysis, microbial activities, insects, rodents and other factors have been highlighted in the fifth chapter. The spoilage agents including the enzymes, microorganisms, and the factors affecting the growth of microorganisms have been incorporated in the sixth chapter. The principles of food preservation and the preparatory operations in food preservation have been covered in the Seventh and eighth chapters respectively.

In chapter nine, use of high temperature in pasteurization, tyndallization and canning operations have been explained. Freezing preservation of food with equipment and procedure have been included in the tenth chapter. The principle of dehydration, types of dehydrators, evaporation and concentration, intermediate moisture food technology have been explained in the eleventh chapter. In chapter twelve, the use of chemical additives and the factors influencing their effectiveness in the preservation of food products have been highlighted.

Thirteenth chapter explains the use of fermentations in the production and preservation of food and types of fermentation. The latest methods of preservation by irradiations, such as ultraviolet and ionizing radiations indicating the effect of irradiation on foods and the future prospects of irradiation have been briefly summarised in the last chapter.

The presentation is simple and can be followed by those who does not have any background of food preservation and technology. The book is useful to the students of Food Science and Technology, Home Science and Agriculture. It is also useful to those in the allied disciplines such as Nutrition and Dietetics, Hotel and catering Management and Home Economics.

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Human Milk Banking: Science et Technique Du Froid, Series Proceedings of the Meeting of Commission C, at Kradic Kralove, Czechoslovakia 1981-82, pp: 192, Price: 100 FF.

This book contains the papers presented at the international meeting on Human Milk Banking at Kradic Kralove in Czechoslovakia in 1981. Initially, the conclusions and recommendation of the meeting are presented. The papers discuss the problems of milk banking: frequent regular collections, methods of milk collection from donors, contamination of milk and safety measures a problem even under favourable hygienic conditions. Heat treatment destroys or changes

many of the important and unique components of human milk, mainly the cell constituents and their produces. The specific composition of human milk is highlighted in a number of papers. Also investigations on the bacterial and viral content of the collected breast milk are reported. The problem of excretion of pathogens (bacteria or virus) and toxins e.g. pesticides or metals except lead in one paper, have not been discussed. The possible excretion of drugs has only been touched en-passant but had not been emphasised. Instead of reproducing the papers as they were presented at the meeting, it sould have been helpful for the reader if they had been arranged under subjects e.g. papers dealing with the composition of breast milk, the bacteriology, technology and the maintenance of milk banks.

The papers are concerned with milk banking in developed countries where refrigerators are common household assets and milk collected by the donors can be stored and frozen in the domestic refrigerator. For developing countries this mode of collection would not be feasible. A "Walking donor", practically the equivalent of a wet nurse, could be only a short term solution. Fortunately in India breast feeding is the common practice. In big hospitals a milk bank would be required for special, selected cases but the cost of such a setup seem to be prohibitive.

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HPLC in Food Analysis: by R. Macrae (Ed), Academic Press, London, 1982; Pp: 340+xii; Price: £ 28.00, $ 50.00.

High pressure liquid chromatography is an excellent area for the interplay of analytical chemists who are more interested in the fundamental aspects and the application oriented chromatographers. This monograph edited by R. Macre is a shining example of such an effort in the area of food science. The first few chapters deal with the theory and instrumentation of HPLC and the later chapters deal with the analysis of individual components of food using HPLC.

Chapter I by R. Macrae deals with the essential elements of the theory cutting down significantly the mathematical treatment usually not assimilated by food chemists. One of the major bottle-necks viz. band broadening has been diagramatically explained. Knox plot useful to find out the usefulness of the column has been dealt with, in required detail. Tabulation of methods of quantification and their physical significance will be useful for actual users.

Chapter II by R. Newton describes the various parts of HPLC instrumentation in precise details. The classification of detectors into solute specific detectors, bulk property detectors, desolvation detectors, LC-MS detectors, etc. is quite appropriate. Instrumentation for preparative HPLC, column switching and recycling having potential industrial ramifications in future could have been dealt in little more detail. Additional bibliographic references and clear diagramatic representations have made a complicated subject simple.

The Chapter on separation modes by C. F. Simpson discusses the different types of HPLC modes like, adsorption, partition, bonded phase, ion exchange, ion-pair, gel permeation modes. Tabulation of characteristics of some polar bonded phases and of commercially available reveresed phase packings will be very helpful to beginners. The role of solvents in HPLC separations and inclusion of a UV cut off data chart would have been of immense value to actual users.

The chapter on data handling and automation by C. R. Loscombe is topical and educative. Food Scientists in industry would have derived some benefit by an automated on line analytical illustration. An introduction to food applications provided by R. Macrae, in the second portion of the book can be rightly called a buffer chapter between the two parts.

The applications of HPLC to the analysis of various food components are excellently reviewed by respective area specialists: Carbohydrates (D. J. Folkes and P. W. Taylor), Lipids (E. W. Hammond), Vitamins (P. J. Van Niekerk), Food Additives (K. Saag), Mycotoxins (D. C. Hunt), and Amino acids and peptides (A. P. Williams).

The futurology of HPLC vis-a-vis food analysis. forms the thesis of the final chapter by R. Macrae and H. E. Nursten. Microbore packed columns, packed capillary columns, open tubular columns, HPLC/MS are some of the areas which will witness intense activity in the near future. The tables on silica based and organic based packings will be of immediate relevance to the users.

The format of the book, type setting and illustrations highlight the excellence in publishing standards expected from an Academic Press Publication. The arrangements of topics flows into a logical pattern.

However, some important topics like HPLC analysis of naturally occuring toxins, pesticide residues in foods etc. are absent. Though the Editor himself has pointed out in page 146 that there should be more emphasis on the analysis of food samples rather than the chromatographic separation of food components, this book also lays emphasis on the latter at the expense of the former. An attempt to give a few illustrations of complete analysis of a particular food would have been more intersting.

The price of the publication is rather high and can best serve as a reference monograph in the library.

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

Meat Microbiology: by M. H. Brosn (Ed), Applied Science Publishers, England, 1982, pp: 529, Price: £ 46.00.

A rapidly perishable and costly commodity like meat draws the interests of researchers and technologists alike but the onus of responsibility for prescribing and guiding the quality control and hygiene in the production of meat rests squarely on the microbiologist. This group of researchers must collect, sift and interpret an enormous amount of data piling up through the efforts of several laboratories engaged in studying every conceivable aspect of meat microbiology and use what is most pertinent to the production or processing of a particular meat commodity. To them specially is this comprehensive and informative book on meat microbiology going to be of special interest.

The contents of this work beginning with an illuminating preface and introduction by MH Brown of Unilever Research Laboratory, who is also the editor, are presented in eleven chapters clearly identifiable as commodity chapters and quality assurance chapters.

The commodities may vary depending on the technological innovations but well established products and methods and their microbiological implications have been very lucidly described. Carcass meat, bacon and ham, the chilling, freezing and thawing, the meat industry by products and of course the packaged meat have different microbiological status the significance of which has been brought out.

The Chapter on microbial interaction with meat summarising the present state of knowledge on the degradative effects of bacterial action will be welcomed by researchers in meat science.

The organisms of public health significance and the type of commodities most affected including the epidemiological manifestations are well described although more emphasis could have been laid on the prevention and control of contamination measures. There is a most useful and exhaustive bibliography at the end of this chapter.

The sampling and sample examination techniques no doubt are going to be of immediate and enormous interest to the quality control personnel but a discussion however brief on the present thinking on microbial standards for meat and meat products and especially raw meat would have been received most enthusiastically by researchers in countries where meat is not an organized industry as yet and the control of raw meat quality is an immense and often an obtuse task fraught with difficulties. This really is a grey area which the eminent contributors in this very readable book could have attempted to put in the correct perspective.

Elegantly bound and attractively covered, this book deserves a pride of place amongst reference volumes with any one aspiring to deal with meat as commodity and study it as a subject.

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Developments in Food Proteins-1: by B.J.F. Hudson (Ed), Applied Science Publishers, England, 1982, Pp. 335, Price: £ 35.00

The shift in the thrust on proteins in food of the present decade has not deterred the publication of books on various aspects of food proteins. The book under review contains information ranging from conventional to speculative proteins, and analytical techniques for protein analysis.

Though inadvertent modification of proteins during food processing is known for ages, the modern approach is to modify them with desirable characteristics. There have also been efforts to modify vegetable proteins so that they simulate animal proteins. Modifications of the functional properties of food proteins like casein, muscle protein, wheat protein and isolated proteins, and preparation of hydrolytes as achieved by enzymes and chemicals are adequately discussed in chapter 1.

The practical aspects in the use of the technique of differential scanning calorimeter (DSC) for better understanding of food proteins is detailed in chapter 2. This technique is capable of providing detailed information on macromolecular interactions and the phenomenon of denaturation of protein. However this technique has found limited application. Ultra filtration is a new technique to concentrate proteins and has a wide range of applications, and can be used at different scales of operation. This appears also to be useful in concentration of proteins. The principle of the technique has been discussed in detail in chapter 3.

Considering the volume of information on milk proteins, the data provided in chapter 4 is very limited and confined to its use in formulated foods. Information on the modification of milk proteins to obtain novel and newer functional proteins is interesting.

Estimation of non-meat proteins in formulated foods based on meat is a complex problem particularly after cooking which leads to denaturation of proteins. In chapter 5 varied methods, extending from microscopy to physicochemical methods, are critically assessed for their reliability to estimate non-meat proteins in meat preparation.

The complexity of assessing the response of users of novel protein products is briefly discussed in chapter 6. But the treatment of the subject is not effective since a number of aspects ranging from the need for nutritional quality to health criteria are included. The use of texturised vegetable protein based on soya in catering industry is detailed as a case study in chapter 7. This is presented as a project report with emphasis on the value of textured vegetable proteins.

Chapter 8 and 9 are on the speculative unconventional proteins from leafy material and algae respectively. The discussion on leaf protein (LP) is confined to the preparation of colourless LP for use in human food. The emphasis on colorless LP is difficult to understand in the context of the failure of LP to find large scale application anywhere. This aspect has not been discussed at all. In contrast, algae as a future protein source is considered for feed purposes with food uses coming later. The details of algal production and its evaluation are quite general and limited in coverage but literature references are extensive.

The book is intended to focus the attention of researchers on recent trends in different areas of food proteins. Each chapter is a review on a specific aspect dealing with a technique, a product or a protein source though the coverage is comprehensive always. The book will certainly be of interest to R & D workers in food proteins. They will find the literature cited at the end of each chapter valuable.

L. V. VENKATARAMAN
M. S. NARASINGA RAO
C.F.T.R.I., MYSORE

Developments in Food Proteins-2: by B. J. F. Hudson, (Ed), Applied Science Publishers, England, 1983; Pp: 339; Price: £ 36.00.

This is the Second Volume following the first which contained reviews on milk proteins and their modifications, analytical techniques and the other speculative proteins from leaves and algae. In this volume, wheat, soya, rapeseed, lupin, legumes, fish and yeast proteins are reviewed in seven chapters with a final on estimation of protein quality. The emphasis in each has been on the technological aspects and food uses.

Chapter 1 details wheat proteins and thier technological significance being the world's largest cereal. Gluten proteins of wheat includes gliadinis, glutenins and residue proteins and are unique technologically in bread making and other baked products. The nature of the gulten proteins and how these properties are responsible for its technological application has been effectively brought out.

Chapter 2 is on the well-known soy proteins. In this brief review the nature of proteins and other biologically active components like protease inhibitors, haemagglutinins, goitrogens, estrogens etc., and their effect on nutritional value are discussed.

Rapeseed, an important oil seed protein has been discussed in Chapter 3. The development of low glucosinolate variety has enhanced its use. The preparation and properties of protein concentrates and also of isolates and their economic feasibility have been discussed. These however, result in low product yields and suffer from desirable functional properties. The data given are very informative.

Chapter 4 covers lupin which is a legume; it has high protein content and low levels of toxic and bitter principles. The details on the extractibility, characterization, functional properties and nutritional value based on essential amino acid pattern and *in vitro* digestibility are given in detail. There are possibilities to use lupin proteins for poultry.

Air classification of legume proteins and its relative merit over more complex and expensive solvent extraction is discussed in Chapter 5. Pin milling and air classification can isolate nearly cent per cent starch and other fractions containing crude protein fraction with other constituents which has wide and varied application. The functional properties of the flour fractions have also been detailed. Starch fractions have only limited food uses.

Chapter 6 describes the new approaches in the use of fish proteins. There is more information on fish resources and composition of various parts than on fish proteins. The new approaches include protein spinning, hydrolysates and use of chemical and enzymic modifications to obtain favourable functional properties. The information, however, is not exhaustive.

Chapter 7 deals with a Single Cell Protein, Yeast. The important processes for production, composition and nutritional value and limited details on safety are given. The arguments for considering it as potential protein in food formulations is week both in terms of safety and cost not to mention of its acceptability.

Chapter 8 deals with the need for a rapid and easy method for evaluating protein quality. Animal bioassay

procedures are time consuming and expensive. In this context, a combination of amino acid composition and *in vitro* digestibility data may be used to compute a combined index. However, how reliable this can be, needs confirmation by workerss in the field.

The book contains useful reviews on varied protein sources and each Chapter has extensive bibliography on specific proteins and will be quite useful to researchers in the field.

L. V. VENKATARAMAN
C.F.T.R.I., MYSORE

YOUNG SCIENTIST AWARD FOR 1983

Association of Food Scientists and Technologists (India) invites nominations for the Young Scientist Award for distinguished scientific research and technological contributions to the field of Food Science and Technology.

The award consists of a cash prize of Rs. 1,000/- and a certificate.

Nomination for the Award is open to aspirants fulfilling the following conditions:

1. The candidate should be an Indian National below the age of 35 years on the date of application, working in the area of food science and technology.
2. The candidate should furnish evidence of either,
   (*a*) Original scientific research of high quality, primarily by way of published research papers and (especially if the papers are under joint authorship) the candidates own contribution to the work.

   OR

   (*b*) Technological contributions of a high order, for example in product development, process design etc., substantiated with documentary evidence.

The application along with details of contributions and bio-data (4 copies) may be sent by Registered Post, (envelope should be superscribed as 'Nomination for Young Scientist Award') so as to reach Dr. S. C. BASAPPA, Hony Secretary, Association of Food Scientists and Technologists (India), CFTRI campus, Mysore-570 013, before 31 January 1984.

SUMAN FOOD CONSULTANTS TRAVEL AWARD FOR 1983

The Association of Food Scientists and Technologists (India) has instituted a Travel Award in the name of "Suman Food Consultants" to Post-Graduate Degree/Diploma students in Food Science/Technology. The Award will be of Rs. 500/- which will enable the awardee to attend the Annual General Body Meeting and the Technical Seminar/Symposium of the AFST(I) in that year.

The selection of the Award will be based on an essay competition. The subject for the essay is "ROLE OF FOOD SCIENCE AND TECHNOLOGY IN RURAL DEVELOPMENT". Four copies of the essay are to be submitted to the AFST(I) office, Mysore before 31st January 1984. The essay may contain 15–20 pages of typed matter and should be comprehensive. A certificate from the Head of the Department under whom the student is working should be enclosed along with the essay.

PROF. V. SUBRHAMANYAN INDUSTRIAL ACHIEVEMENT AWARD FOR THE YEAR 1983.

The Association of Food Scientists and Technologists(I) has instituted this Award. Nominations for this award for the year 1983 are invited. The guidelines for the award are as follows:

1. Indian Nationals engaged in the field of Food Science and Technology will be considered for the award.
2. The Nominee should have contributed to the field of Food Science and Technology, for the development of Agro-based food and allied industries or to basic food science and technology with immediate prospect and/or future potential for industrial application.
3. The nomination should be proposed by a member of the Association. The bio-data of the candidate together with his consent should be given in detail including the work done by him and for which he is to be considered for the award.
4. The Awardee will be selected (from the names thus sponsored) by an Expert panel constituted for the above purpose by the Executive Committee.

Nominations along with bio-data and contributions should be sent by Registered Post, so as to reach Dr. S. C. BASAPPA, Honarary Executive Secretary, Association of Food Scientists and Technologists (India), Central Food Technological Research Institute Campus, Mysore-570 013, before 31st January 1984. The envelope should be superscribed as 'Nomination for Prof. V. Subrahmanyan Industrial Achivement Award'.

BEST STUDENT AWARD

The Association of Food Scientists and Technologists (India) has instituted a BEST STUDENT AWARD to be given every year to two students with distinguished academic record and undergoing Final Year Course in Food Science and Technology. The aim of the award is to recognise the best talent in the field and to ensure wider recognition of food science and technology as professional discipline.

There are two awards each comprising a cash award of Rs. 500/- and a certificate.

The candidates to be considered for the awards should fulfil the following conditions:

1. They must be Indian nationals
2. They must be students of one of the following:
   (*a*) M.Sc. (Food Science)/(Food Technology).
   (*b*) B.Tech., B.Sc. Tech., B.Sc. Chem. Tech., in Food Technology.
   (*c*) B.Tech., in food sciences
3. They should not have completed 25 years of age on 31st December of the year preceding the announcement when their names are sponsored.

Heads of Post-graduate Departments in Food Science and Technology may sponsor the name of one student from each Institution supported by the candidate's bio-data, details starting from High School onwards, including date of birth and his post graduate performance to date (4 copies).

Nominations for the year 1983 may be sent by Registered Post. (the envelope should be superscribed as 'Nomination for Best Student Award') so as to reach Dr. S. C. BASAPPA, Hony. Exec., Secretary, AFST(I), Central Food Technological Research Institute Campus, Mysore-570 013, before 31 January 1984.

INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (*in triplicate*) should be typewritten in double space on one side of bond paper. The manuscripts should be complete and in final form, since only minor corrections are allowed at the proof stage. The paper submitted should not have been published or data communicated for publication anywhere else. Review papers are also accepted.

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. Superscripts and subscripts should be legibly and carefully placed. Foot notes especially for text should be avoided as far as possible.

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

5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. They should be typed on separate paper. Nil results should be indicated and distinguished clearly from absence of data, which is indicated by '—' sign. Tables should not have more than nine columns.

6. **Illustrations:** Graphs and other line drawings should be drawn in *Indian ink* on tracing paper or white drawing paper preferably art paper. The lettering should be in double the size of printed letters. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size 16cms (ox axis) × 20cms (oy axis); photographs must be on glossy paper and must have good contrast; *three copies* should be sent.

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

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

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

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

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

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

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

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

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

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

9. Consult the latest copy of the *Journal* for guidance.

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