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Research Notes

Microbiological and Physico-chemical Changes in *Idli* Batter During Fermentation

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***Idli* batter is obtained by autofermentation of wet ground mixture of split, debusked blackgram (*Phaseolus mungo*) and rice (*Oryza sativa*). Natural microbial load of the ingredients of the environment serve as inoculum for the initiation of fermentation. Gas production and leavening of the batter are attributed to the heterofermentative *Leuconostoc mesenteroides*. Batter obtained from hotels and restaurants showed yeast participation in the leavening of *idli* batter, which was confirmed by the presence of CO_2 by GLC analysis. Yeasts involved have been identified as *Trichosporan pullulans*, *Torulopsis candida* and *T. holmii*.**

Idli is a fermented, steam cooked, breakfast food popular all over India. It is prepared from autofermented wet ground batter of decuticled blackgram dhal (*Phaseolus mungo*) and rice (*Oryza sativa*) mixed in the ratio of 2:1. The popularity of *idli* is due to its delicate spongy texture and its easy digestibility.

Lewis and Johar[1] reported *Oidium lactis*, *Torulopsis holmii*, *Lactobacillus delbruckii*, *L. lactis* and *Streptococcus lactis* as important microflora in *idli* fermentation. Deshikachar *et al.*[2] reported the predominance of yeasts by using penicillin and chlorotetracycline as bacterial inhibitors. Radhakrishna Murthy *et al.*[3] have reported autofermenting ability of soaked blackgram dhal to be better than that of rice. Ramakrishnan[4] has studied type of microflora in soy idli fermentation and has characterised *Leuconostoc mesenteroides* to be the dominant organism responsible for lowering the pH of the batter. Pandalai and Kurup[5] have isolated *Aerobacter cloacae* and two species of yeasts namely *Candida parapsilosis* and *C. pelliculosa* from *idli* batter. Batra and Milner[6] have isolated *Torulopsis candida* and *Trichosporon pullulans* in the fermenting *idli* batter. Yajurvedi[7] has reviewed microbiology of *idli* fermentations in which among other things the author has suggested to establish the extent of yeast involvement in *idli* fermentation.

Mukherjee *et al.*[8] and Steinkraus *et al.*[9] have reported *Leuconostoc mesenteroides* to be the principal organism producing gas during fermentation.

The present communication deals with the activities of microorganisms derived from different sources in *idli* fermentation.

Materials and Methods

Blackgram dhal (*Phaseolus mungo*) and rice (*Oryza sativa*) variety 'Bangara sanna' were procured from the market, cleaned and the batter was prepared in the laboratory by grinding the soaked ingredients intermittently for 5 min to the required consistency and carefully transferred to a sterile beaker covered with aluminium foil and kept at room temperature for fermentation.

Batter samples from catering establishments were collected in sterile beakers and brought in ice buckets to the laboratory.

Non-protein nitrogen: Samples of batter were weighed and diluted to a known volume and filtered through Whatman No. I filter paper. An aliquot of the filtrate was treated with an equal volume of 20 per cent trichloroacetic acid, filtered and used for nitrogen estimation by Microkjeldahl method.

Sugars: Free and total reducing sugars were estimated by di-nitro salicylic acid method.[10]

pH and O/R potential: Oxidation-reduction potential and pH were determined in a Systronics model No. 324 pH meter. Platinum electrode was used for O/R potential determination.

Acidity: Acidity was determined by titrating an aliquot of the sample with 0.04 N sodium hydroxide using bromothymol blue as indicator.

Measurement of batter volume: This was done in one litre sterilized measuring cylinders maintaining the initial volume at 100 ml.

Gas analysis: The evolved gas during *idli* batter fermentation was analysed for constituent gases using

Aimil Gas Chromatograph equipped with thermal conductivity detector and Parapack Q. Column.

Microbiological analysis: Aerobic mesophilic count, total count for coliforms were carried out as per Compendium of Methods for Microbiological Examination of Foods[11]. Total count of *Lactobacilli* were carried out using Rogosa agar (Oxoid composition[12]). Microaerophilic conditions were maintained by overlayering the inoculated plates.

Yeasts and moulds were isolated by antibiotic selection method using the media of the following composition(g): peptone 0.94; yeast extract-0.47; beef extract-0.24; sodium chloride-1; dextrose-1 and agar 2 per cent. pH of the medium was 6.1. Seitz filtered chlorotetracycline and chloroamphenicol were added at 100 ppm, level after sterilization.

Identification of yeast: Descrete colonies of yeasts were picked up and maintained on potato-dextrose-agar slants. Identification was carried out as per Lodder[13] scheme.

Results and Discussion

Physico-chemical characters and microbial load of ingredients used in *idli* fermentation, before and after washing in sterile water as well as those soaked in sterile water for 7 hr are given in Table-1. A drastic reduction of microbial load after washing and increase after soaking was noticed. Yeasts were absent in both the ingredients. Coliform buildup in blackgram dhal was much more compared to that of rice. Similarly the *Lacto bacilli* count increased many folds after soaking, though in soaked rice, the increase was not of the same order.

The pH in both the ingredients remain around 7.0. The release of free sugars as well as non-protein nitrogen during soaking is much higher in blackgram dhal than rice. Blackgram dhal soaked water has higher concentration of soluble nutrients to support the growth of lactic acid bacteria, confirming the earlier[3] observation.

Microbiological and physico-chemical data of batter samples derived from different sources are given in Table 2. Domestic samples of batter had been ground

TABLE 1. PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSIS OF INGREDIENTS USED IN *IDLI* FERMENTATION

Parameters	Blackgram dhal			Rice		
	Prior to washing	After washing	7 hr soaking	Prior to washing	After washing	7 hr soaking
pH	6.70	6.90	7.10	7.10	7.10	6.55
Free sugars (mg/ml)	0.38	0.04	1.15	1.04	0.06	0.35
Total sugars* (mg/ml)	1.54	1.10	2.77	1.87	1.87	1.87
Non-protein N (mg/ml)	0.012	0.010	0.108	0.020	0.018	0.005
Aerobic mesophiles (cfu$\times 10^2$/g)	273	115	4130	3380	225	10.480
Coliforms (cfu$\times 10^2$/g)	23	8	1200	52	1	137
Lactobacilli (cfu/g)	60	8	37200	Nil	Nil	570
Yeasts (cfu/g)	Nil	Nil	Nil	Nil	Nil	Nil
Moulds (cfu/g)	256	86	95	440	30	30

*As reducing sugars

TABLE 2. PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSIS OF *IDLI* BATTER FROM DIFFERENT SOURCES

Parameters	Domestic sample			Laboratory sample			Hotel sample		
	0 hr	6 hr	18 hr	0 hr	6 hr	18 hr	0 hr	6 hr	18 hr
pH	6.10	5.35	4.70	6.65	5.70	4.82	5.60	4.35	4.20
Acidity	0.90	1.48	3.18	0.72	1.20	2.01	1.24	2.12	3.40
O/R potential	−120	−245	−330	+60	−70	−400	0	1.50	−200
Batter vol. rise	100	150	175	100	135	170	100	155	195
Gas	H_2	H_2	H_2	H_2	H_2	H_2	CO_2	CO_2	CO_2
Lactobacilli (cfu/g)	426×10^3	896×10^8	9498×10^9	354×10^2	7395×10^4	9980×10^9	3756×10^4	6481×10^9	7149×10^{10}
Coliforms (cfu/g)	189×10^3	800×10^5	860×10^6	79×10^2	114×10^3	246×10^7	79×10^2	40×10^2	14×10^2
Yeasts (cfu/g)	Nil	Nil	Nil	Nil	Nil	Nil	518×10^2	914×10^2	1043×10^4
Moulds (cfu/g)	1×10^4	5×10^3	5×10^3	40	50	30	160	130	40

in clean granite stone mortar, the laboratory samples were ground in electric waring blenders maintaining the same ingredient proportions as in general practice, and the hotel samples had been prepared in a power driven granite mortar. The progress of fermentation was studied upto 18 hr. There was 6-7 log increase in total *Lactobacilli* in different types of sample studied. The maximum increase was in the laboratory samples. Highest increase in coliforms is noticed in the laboratory samples (five log), three log increase in domestic samples, and a decreasing trend in hotel samples. Coliforms in the batter may be helpful in releasing fermentable carbohydrates from starchy substrates thus enabling the *Lactobacilli* and yeasts to take over the main fermentation bringing about desirable changes like lowering of pH and production of gas. Yeasts were present in hotel samples from the start of fermentation, registering a two log increase towards completion. Yeasts were absent in the laboratory samples as well as in domestic samples. Generation of heat during grinding in electric blender, and scrupulously clean grinding surface of stone grinders used in the preparation of domestic samples, may be the reason for their absence. Source of yeast in hotel samples could be attributed to inadequately cleaned grinding surface. This has been verified by taking swab rinses of grinding surface from a number of hotels, (Fig. 1). It may be seen that yeasts are present slightly over five log level, as in the case of coliforms. Lowering of pH (4.20-4.82) is a common feature in all the samples studied and acidity increases. Oxidation-reduction potential changes in hotel samples has showed negative trend throughout, and the laboratory samples at the end. Oxidation-reduction potential

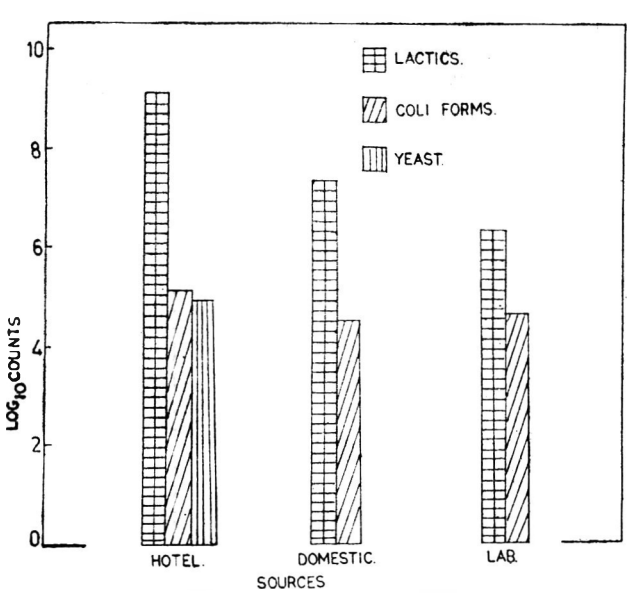

Fig. 1. Microbial load in the washings from grinders used for *idli* batter preparation collected from different sources

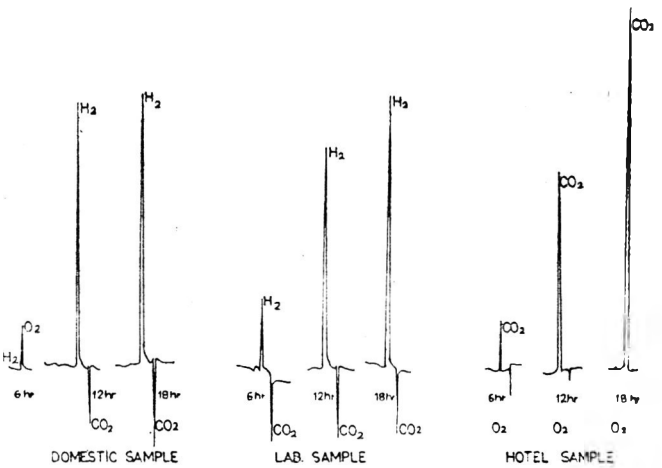

Fig 2. Gas analysis of fermenting *idli* batter using GLC.

values varied from 200 to 400 mv. at the end of fermentation in all the samples. Rise in batter volume was highest in hotel samples which contained a large number of yeasts. GLC analysis of the released gases gave a clear indication of distinct carbon dioxide peaks in samples where fermentation activity was due to yeasts and in other cases distinct hydrogen peaks were observed (Fig. 2.).

Table 3 gives data of dominant yeasts from hotel samples. The samples showed an average of three log increase in total *Lactobacilli*, one log decrease in coliforms and a steady state of yeast flora at three log level. Some samples showed pH values as low as 4.25 (av. 4.93) even before fermentation, which lowered to an average, 4.26 after fermentation. Rise in batter volume was in the range of 65-100 per cent and the average increase was 75 per cent. All the samples showed carbon dioxide peaks confirming the earlier observation.

Microflora involved in *idli* fermentation is a contravertial point. Mukherjee *et al*[7]., Steinkraus *et al*[9]. and Ramakrishnan[4] have claimed it to be entirely due to heterofermentative *Leuconostoc mesenteroides*, whereas Batra and Milner[5], Lewis and Johar[1] have shown yeast involvement in the fermentation. In the present study also involvement of yeasts has been established in *idli* fermentation in hotel samples only. Yeasts isolated from fermenting batter samples were *Trichosporon pullulans*, *Torulopsis holmii* and *T. candida* confirming earlier[1,5] observations. The important role of yeasts in gas production and leavening action was further corroborated by carrying out a set of experiments in which yeasts strains isolated in the above study and *Leuconostoc mesenteroides* were inoculated in sterile batter, and recording the rise in batter volume and gas analysis by GLC during the course of fermentation. The results indicate that yeasts are responsible for

TABLE 3. PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSIS OF *IDLI* BATTER FROM DIFFERENT CATERING ESTABLISHMENTS

Parameters	Initial			18 hr incubation		
		Range			Range	
pH	4.25	6.20	4.93	4.00	4.60	4.26
O/R potential	+80	+210	+129	0	−120	159
Batter vol. rise	100	100	100	165	200	176
Lactobacilli (cfu/g)	432×10^8	6744×10^8	3815×10^8	18×10^{11}	$58{,}460\times10^{11}$	1087×10^{11}
Coliforms (cfu$\times10^3$/g)	10	4240	677	4	1180	144
Yeasts (cfu$\times10^3$/g)	10	940	277	812	5260	2380
Moulds (cfu$\times10^2$/g)	2	30	7	1	500	56

CO_2 was the only gas detected by GLC

more than 50 per cent of the carbon dioxide and for the two fold increase in the volume of the batter.

References

1. Lewis, Y. S. and Johar, D. S., Microorganisms in fermenting grain mashes used for food preparations. *Bull. cent. Fd Technol. Res. Inst.*, Mysore, 1953, **2**, 288.
2. Desikachar, H. S. R., Radhakrishna Murthy R., Rama Rao, G., Kadkol, S. B., Sreenivasan, M. and Subrahmanyan, V., Studies on *Idli* fermentation, Part I. Some accompanying changes in the batter. *J. sci. ind. Res.* 1959, **19C**, 168.
3. Radhakrishna Murthy, R., Desikachar, H. S. R., Srinivasan, M. and Subrahmanyan, V., Studies on *Idli* fermentation, Part II. Relative participation of blackgram flour and rice semolina in the fermentation. *J. sci. ind. Res.* 1961, **20C**, 342.
4. Ramakrishnan, C. V., Studies on Indian fermented foods. *Baroda J. Nutr.*, 1979, **6**, 1.
5. Pandalai, N. G. and Kurup, P. V., Isolation of organisms from *idli* paste. *Indian J. Microbiol.* 1963, **3**, 155.
6. Batra, L. R. and Milner, P. D., Some Asian fermented foods and beverages and associated fungi. *Mycologia*, 1974, **66**, 942.
7. Yajurvedi, R. P., Microbiology of *idli* fermentation. *Indian Fd Pckr*, 1980, **34**, 33.
8. Mukherjee, S. K., Albury, M. N., Pederson, C. S., Van Veen, A. G. and Steinkraus, K. H., Role of *Leuconostoc mesenteroides* in leavening the batter of *idli*, a fermented food of India. *Appl. Microbiol.*, 1965, **13**, 227.
9. Steinkraus, K. H., Van Veena, A. G. and Thiebean, D. B., Studies on *idli*-an Indian fermented blackgram-rice food. *Food Technol.*, 1967, **21**, 916.
10. Miller, G. L., Use of dinitrosalicylic acid reagent for determination of reducing sugar *Annal. Chem.*, 1959, **31**, 426.
11. *Compendium of Methods for the Microbiological Examination of Food*, M. L. Speck, (Ed.) prepared by APHA Inter society/Agency Committee on Microbiological Methods for Foods, 1976, 417.
12. *The Oxoid Manual of Culture Media, Ingredients and Other Laboratory Services*, IV Ed. Oxoid Ltd., England, 1979, 310.
13. Lodder, J., *The Yeasts*, North-Holland publishing Co., Amsterdam 1970, 1385.

Studies on the Growth of *Fusarium* sp. on Citrus Waste for the Production of Single Cell Proteins

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Fusarium sp was grown on the clear filtrate of orange peels (4 per cent w/v) as substrate or after the addition of 0.15 per cent (w/v) of ammonium sulphate and 0.2 per cent (w/v) yeast extract as nitrogen and vitamin sources. The final protein production of the mycelium in control cultres was 1.2 to 1.4 g/l or 0.20 g of protein per g of sugar consumed, while in the cultures supplemented with nutrients the protein production was 3.7 to 4.6 g/l or 0.25 g of protein per g of sugar consumed. Protein production corresponded to almost 58 per cent of the final biomass. The fungus growth rate (μ) was (0.56 hr^{-1}) in cultures supplemented with the additives and (0.42 hr^{-1}) in control cultures respectively. (Ninety per cent and seventy per cent of the sugar in the nutrient supplemented medium and in the control cultures consumed respectively.) Temperature of 27°C and a pH of 4.6 were found to be optimum. The fermentation time was reduced to 5-6 hr when supplemented with the nutrients and a higher initial inoculum. The final biomass contained 26 per cent carbohydrates, 55.8 per cent proteins, 4.75 per cent RNA, 0.76 per cent DNA and 0.73 per cent phosphorus.

The need for more protein in recent years has led to the use of microorganisms grown on a variety of inexpensive substrates of low nutritional value, as a potential source of food and protein. In tropical countries the use of citric waste as a substrate might be very promising as it constitutes one of the highest industrial waste. A substrate tested for growth of fungi is the clear filtrate of orange peel extract. This substrate contains upto 65.75 per cent water and 34.25 per cent solid materials which constitute approximately 56 per cent of carbohydrates. 0.15 per cent of protein, 1.8 per cent of fats and 0.8 per cent phosphorus. The above analyses led us to hypothesize that *Fusarium* sp. grown on citrus waste may be able to give protein levels and an amino acid pattern which is comparable with those recorded for other fungi[1].

This paper presents the results of preliminary studies on the protein production and chemical composition of *Fusarium* sp. mycelium grown on cheap orange extract.

Materials and Methods

Organism: A laboratory strain of *Fusarium* sp. isolated from sweet orange peel was maintained on potato dextrose agar (Difco) slants and transferred monthly to fresh medium.

Substrate: The orange peel closest to the pulp was used. It was ground on a mixer (Osterizer), lyophilized, macerated and resuspended in destilled water at the required concentration. The solution was filtered through a Whatmen No. 1 filter paper three times, to remove insoluble residues of the orange peel. It was sterilized at 121°C for 15 min. The medium used consisted of 1 per cent (w/v) orange peel filtrate supplemented with 0.2 per cent ammonium sulphate (Merck), 0.1 per cent yeast extract (Difco) and monobasic potassium phosphate (Merck) at different concentrations (0.1-0.3 per cent). This medium was used for determination of optimum conditions of temperature and pH. The effect of substrate concentrations on total biomass production, yield and specific growth rate was also investigated. The concentrations of orange peel extracts used varied from 1 to 6 per cnet (w/v).

Inoculation and incubation: *Fusarium* sp. was grown in 300 ml flasks containing 50 ml of medium at a constant temperature in a reciprocating shaker bath (New Brunswick) at 120 strokes per min, with an amplitude of 3.7 cm. At the end of the incubation period pelet was obtained by centrifugation and suspended in 10 ml of fresh medium. One mililiter of the resultant mycelial suspension was used to start new flask culture by inoculating 300 ml flask containing 50 ml of liquid fresh medium. The flasks were inoculated in a reciprocating shaker bath for 24-48 hr. Under liquid shake culture conditions *Fusarium* sp. produce no mycelial mats, but a very fine suspension of small thalli.

Mycelium concentration: Mycelium concentration

was determined by gravimetric method and by measuring the optical density in a klett-summerson colorimeter provided with a N°54 filter (520-580 nm). The optical density was related to the cell concentration with a previously determined calibration curve. Yields were expressed as mg of cells produced per mg of sugars consumed. Since the curve generated by optical density measurements were similar to those of the gravimetric method thus the former method was used as an estimate of growth in some experiemnts.

Specific growth rate (μ)*:* The specific growth rates were calculated during the exponential growth phase from $\mu = (\ln X_2 - \ln X_1/t_2 - t_1)$; where X_1 and X_2 represent cell concentration at times t_1 and t_2 respectively. The units of μ are expressed as reciprocal hours.

Analytical procedure: Total sugars in the cells as well as in the medium were determined by the Anthrone method as described by Scott and Melvin[2], using glucose as a standard. Total protein was quantitatively determined according to the method of Herbert *et al*[3]. The nitrogen content in the medium was measured by the macro Kjeldahl method.[4] Total phosphorus in the cells as well as in the substrate was determined as described by Chen *et al*[5]. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in the total biomass were measured as described by Stewart[6]. Moisture was determined gravimetrically using the O'Haus automatic balance, and the crude fat content of the substrate was determined by the standard AOAC procedure[4] (Table 1).

Amino acid analysis: The samples were hydrolyzed in sealed ampules containing 0.5 ml of boiling HCl, 10 μ l of 0.1 M phenol and 10 μ l of 0.1 M dithiothreitol. Hydrolysis was done for 2 hr at 110°C in a continuous reflux of toluene. Amino acid analysis was performed in multichrom-Beckman liquid column chromatography 4225, (MBC Mannual).

Results

A. Effect of pH and temperature: A significant difference in the amounts of protein produced in 24 hr was observed when the pH was varied from 3 to 7, and temperature ranged from 25 to 40°C. The highest amount of protein as well as "μ" was obtained at pH values between 4 and 5, and at a temperature of 27°C (1.3 mg/ml). Since the pH of the medium itself was 4.6, this was considered later to be optimum for the growth of the microorganism. (Fig. 1, 2).

TABLE 1. CHEMICAL ANALYSIS OF PEEL EXTRACT

Constituent	Quantity (%)
Water	65.75
Total solids	34.25
Carbohydrates	56.83
Proteins	0.15
Fats	1.86
Phosphorus	0.75

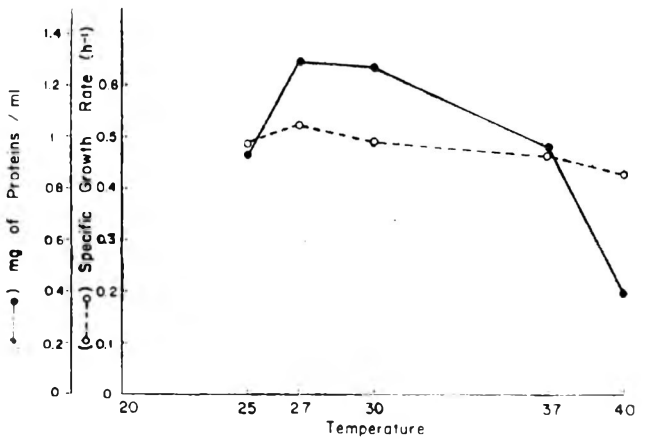

Fig. 1. Effect of temperature on protein production and specific growth rate

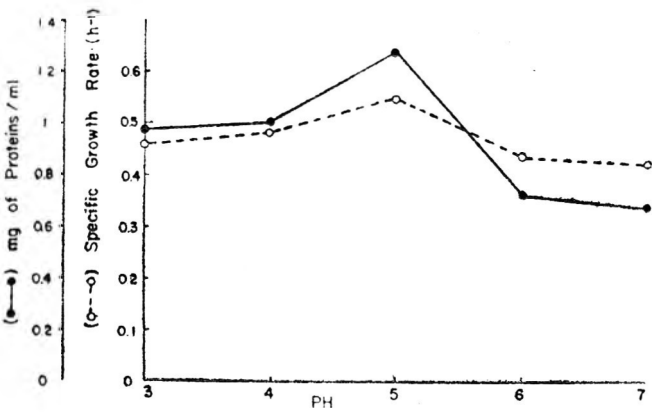

Fig. 2. Effect of pH of the culture medium on protein production and specific growth rate

B. Effect of substrate concentration: The effect of various substrate concentrations on the growth and protein production of *Fusarium* sp. was studied. Using substrate concentrations ranging from 1-6 per cent (w/v) we obtained optimum growth conditions at substrate concentration of 4 per cent (Table 2).

C. Effect of nitrogen, yeast extract and phosphorus: The growth of *Fusarium* sp. in liquid medium containing ammonium salts, yeast extract and phosphorus has been reported previously[7]. It was of interest to determine the required concentrations of these additives for maximum utilization of sugar, production of protein, and for obtaining maximum yield with the *Fusarium sp.*

TABLE 2. EFFECT OF ORANGE PEEL CONCENTRATION ON TOTAL BIOMASS PRODUCTION, YIELDS, AND SPECIFIC GROWTH RATE

Orange peel concn (%)	Final mycelium concn (mg/ml)	Glucose utilized (mg/ml)	Yield*	Specific rate (μ/hr)
1	0.97	2.0	0.45	0.31
2	1.35	2.9	0.46	0.31
3	1.96	3.8	0.51	0.34
4	2.38	4.6	0.51	0.42
5	2.04	4.3	0.46	0.38
6	2.09	4.1	0.48	0.39

*mg mycelium produced/mg of glucose utilized.

used in this study. In these experiments, the pH, temperature, concentration of substrate, and the concentration of two of the additives were kept constant while that of the third was varied.

After 24 hr of cultivation, the protein and sugar concentrations in the medium were determined. As shown in Fig 3, prtoein concentration and specific growth rate increased with the addition of ammonium sulphate at concentrations up to 0.15 per cent. Concentrations above 0.15 per cent did not result in an increase in protein production or growth rate. As seen in the Fig. 4, protein production and specific growth rate were higher when the concentration of the yeast extract was 0.2 per cent.

No significant differences in the concentration of protein and the specific growth rate were observed with the various concentrations of phosphate, indicating that no addition of supplementary phosphorus is necessary. The fungal mycelium grown in the culture medium without added phosphate contained 0.7 per cent phosphorus while there was 0.75 per cent of phosphorus available in the substrate (Fig. 5).

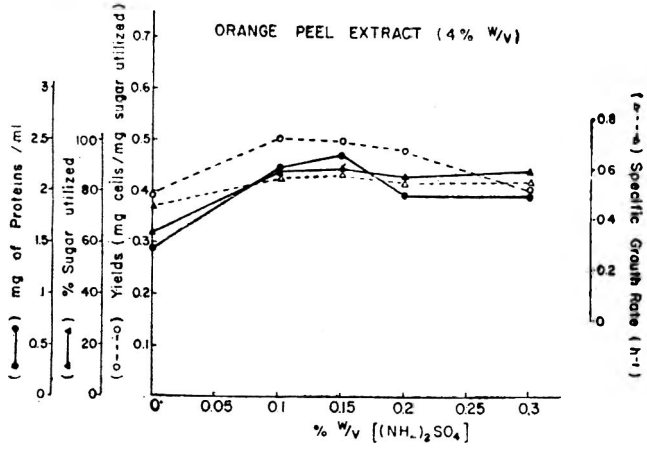

Fig. 3. Effect of ammonium sulphate at different concentrations on the protein production and yield of *Fusarium* sp.

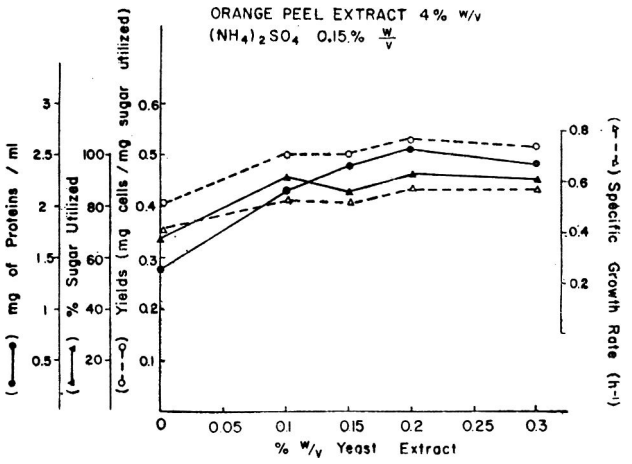

Fig. 4. Effect of yeast extract at different concentrations on the protein production and yield of *Fusarium* sp. mycelium.

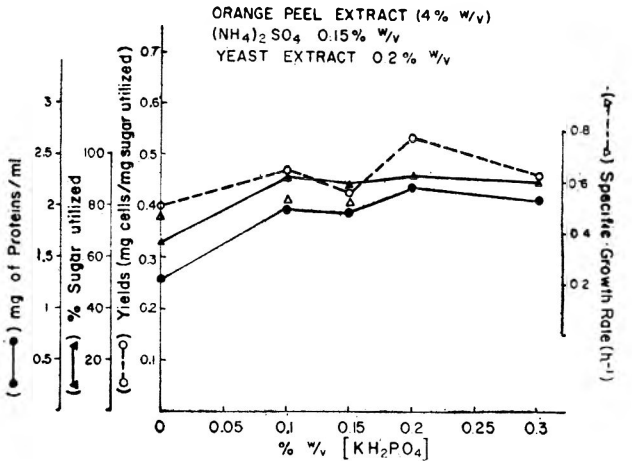

Fig. 5. Effect of monobasic potassium phosphate at different concentrations on the protein production and yield of *Fusarium* sp. mycelium

In view of the results obtained in the previous experiments, 0.15 per cent ammonium sulphate and 0.2 per cent yeast extract were selected as the optimum concentrations for the growth of *Fusarium* in citrus waste.

D. Effect of Inoculum: The previous experiments were carried out with inocula that gave initial concentration of approximately 0.45 mg mycelium/ml. In the next experiment, the inoculum was increased to 1.4 mg/ml by a factor proportional to the concentration of the nutrients. Under these conditions over 85 per cent of the sugar was utilized in 5 to 6 hr. The time for the

TABLE 3. EFFECT OF INITIAL INOCULUM SIZE ON TOTAL BIOMASS PRODUCTION AND THE LENGTH OF THE PROCESS

Period (hr)	Mycelium concn. (mg/ml)	
	Exp. 1	Exp. 1
0	0.5	1.4
2	0.7	4.2
4	0.9	6.8
6	1.2	9.2
8	1.5	9.9
10	2.1	9.4
12	2.4	—
14	2.7	—
16	3.2	—
18	—	—
20	—	—
22	—	—
24	3.6	9.4

fungus to reach the stationary phase of growth was significantly reduced from 16 to 7 hr (Table 3).

Substrate analysis: Table 1 gives the approximate composition of the citrus peel extracts which were utilized as the culture media, both, with and without additives for the growth of *Fusarium*.

Mycelium composition: The mycelium obtained was found to contain 58 per cent proteins, 26 per cent total carbohydrates, 4.75 per cent RNA, 0.76 per cent DNA and 0.73 per cent of phosphorus.

Amino acid contents: The amino acid composition of the *Fusarium* sp. used in this experiment is in close agreement with the amino acid composition of other *Fusarium* sp.[8] It is also very similar to the amino acid content of soy bean flour, and can be compared with the acceptable established values reported by Food and Agriculture Organisation (FAO), with the exception of low amounts of methionine and cysteine, as has been reported for other microbial proteins[7,9,10] (Table 4).

TABLE 4. AMINO ACID CONTENT OF *FUSARIUM* SP. MYCELIUM

Amino acid	Quantity (M moles/g)
Lysine	0,061
Histidine	0,018
Arginine	0,042
Aspartic acid	0,065
Threonine	0,036
Serine	0,047
Glutamic acid	0,072
Proline	0,028
Glycine	0,061
Alanine	0,085
Cystine	0,010
Valine	0,042
Methionine	0,008
Isoleucine	0,031
Leucine	0,051
Tyrosine	0,033
Phenylalanine	0,106

Discussion

The growth of fungi on agricultural wastes for its use as a potential source of proteins has been reported by several investigatiors. Among these agricultural wastes, the orange peel extract appeal to be very suitable for this purpose[8-11,13,14].

In our experiments we observed an increase by a factor of two in the concentration of protein when nutritents were added to the medium at their optimal concentrations.

The need for additives has also been confirmed by other investigators utilizing agro industrial wastes[8-10,12,15] Yeast extract at a concentration of 0.2 per cent and above, in 4 per cent orange peel wastes gave maximum glucose utilization and cell yield. Although good growth was obtained without added nitrogen, protein production was doubled with addition of ammonium sulphate. This means that the medium itself does not contain enough assimilable nitrogen for the fungus to be able to grow and consume all the available glucose. It was observed that 0.15 per cent ammonium sulphate was the optimal concentration for the salt for maximal sugar utilization and protein production. Our data suggest that the supplementation of the medium with phosphate is not necessary, and seems to indicate that phosphorus is not a limiting factor in the medium used in these experiments.

Though it has been reported that the presence of essential oils in the orange peels have an inhibitory effect on microorganisms, Hernandez *et al.*,[15] found that they do not interfere with the production of proteins, sugar consumption, and cell yields. We therefore did not attempt to extract the oil from the orange peel. We found that of the total crude lipids in the peels 1.86 per cent is oil. This proportion decreases in the process of lyophilization of the orange peels by evaporation of the volatile oils. In the process of preparation of the medium at 4 per cent (w/v), the oil content is reduced to 25 per cent, and after sterilizing the liquid medium the final concentrations of the essential oils will remain in the range of 0.2 per cent of the total lipids.

Fusarium sp. is known to grow well at 26-30°C[7,8]. We obtained the best results at 27°C. The optimum protein production was achieved at a pH range of 4 to 5. It has been shown in other microorganisms that

seeding with a high inoculum reduces the length of the fermentation process[16]. Knight *et al*[17] have reported that the inoculum volume influences growth rate and showed that higher inoculum volumes gave faster growth and higher C.O.D. reduction. In our experiments we found that as the inoculum size increased the time required for complete removal of the sugar dropped from over 17 to 6 hr. Demonstrating a better growth with a larger inocula, while longer periods of time were required with the smaller quantities of seed. Since economic factor is an important consideration in the growth of fungi decreased processing costs would tend to increase the commercial feasibility of this operation.

In general, fungi grown on different kinds of substrate in batch cultures have been reported to contain between 30 and 60 per cent protein.[10,18] *Fusarium moniliforme*[9], was reported to contain 38 per cent protein, *Fusarium oxysporum*[7], 35 per cent protein; and for other species of *Fusarium* values of 30 to 60 per cent were reported. In our studies the fungus contained 55.8 per cent protein. The total carbohydrate content was 26 per cent, and the nucleic acid content 5.51 per cent which is in agreement with that reported earlier (2.5-6 per cent)[10]. Among the various types of microorganisms yeast and filamentous fungi have been the most commonly used for microbial protein production. Filamentous fungi possess certain qualities like texture, higher amounts of essential amino acids, protein content, fast rate of growth, lower nucleic acid content which make them preferable to yeast specific cases.[5,19,20]

Though it has been reported that some *Fusarium* sp. are mycotoxin producers[1,21] experiments done feeding rats with *Fusarium* sp as a source of protein have shown no apparent toxic effect[9].

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References

1. Moreau, C., *Moulds, Toxins and Food*, John Wiley & Sons. New York, Toronto, 1979, 217.
2. Scott, T. A. and Melvin, E. H., Determination of dextran with anthrone. *Anal. Chem.*, 1953, **25**, 1656.
3. Herbert, D.. Phipps, P. J. and Strang, R. E., *Methods in Microbiology*, Academic Press, London, New York, Vol. 5b, 1971, 209.
4. *Official Methods of Analysis*, Association of Official Agricultural Chemists, 11th ed., Washington D. C., 1970.
5. Chen, P. S., Toribara, T. A. and Warner, H., Microdetermination of phosphorus. *Anal. Chem.*, 1956, **28**, 1756.
6. Stewart, P. R., *Methods in Cell Biology*, Academic Press, New York, Vol. XII, 111, 1975.
7. Christias, C., Couvarak, C., Georgopoulos, S. G., Macris, B. and Vomvoyanni, V., Protein content and amino acid composition of certain fungi evaluated for microbial production. *Appl. Microbiol.*, 1975, **29**, 250.
8. *Production of Single Cell Protein from Agricultural and Food Processing Wastes*, Phillip Lyle Memorial Research Laboratory. P. O. Box 68, England, 1975.
9. Drouliscos, N. J., Macris, B. J. and Kokke, R., Growth of *Fusarium moniliforme* on carob aqueous extract and nutritional evaluation of its biomass. *Appl. Environ. Microbiol* 1976, **31**, 691.
10. Labaneiah, M. E. O., Abou-Donia, S. A., Mohamad, M. S. and El-Zalaki, E. M., Utilization of citrus wastes for the production of fungal protein. *J. Fd Technol.*, 1979, **14**, 95.
11. Church, B. D., Nash, H. A. and Brosz, W., Use of fungi imperfecti in treating food processing wastes. *Development Ind. Microbiol.*, 1972, **13**, 30.
12. Janardhanan, K. K., Kaul, T. N. and Husain, A., Use of vegetable wastes for the production of fungal protein from *Morchella species. J. Fd., Sci. Technol.*, 1970, **7**, 197.
13. Macris, B. J. and Kokke, R., Effect of growth rate on the amino acid composition of *Fusarium moniliforme* cultivated continuously for fungal protein production. *Biotech. Bioenging.*, 1979, **21**, 707.
14. Manachine, P. L., Screening of edible higher fungi for production of biomass and extracellular metabolites in submerged culture. *Technologie Alimentari*, 1979, **11**, 17.
15. Hernandez, E., Legorburo, M. B., Lequerica, J. L., Martin, F. and Lafuente, B., Aprovechamiento de subproductos citricos. I. Enriquecimiento de proteinas del pienso de corteza de naranja mediante el desarrollo de leva duras. *Revista de Agroquimicay Technologia de Ali-mentos*, 1975, **15**, 415.
16. Wasserman, A. E., Hopkins, W. J. and Porges, N., Whey utilization-growth conditions for *Saccharomyces fragilis*, *Sewage Ind. Wastes*, 1958, **30**, 913.
17. Knight, S., Smith, W. and Mickle, J. B., Cheese whey disposal using *Saccharomyces fragilis*, *Dairy Prod., J.*, 1972, **1**, 17.
18. Dabbah, R., Protein from microorganism. *Fd Technol.*, 1970, **24**, 659.
19. Reed, G., use of microbial culturs. Yeast products. *Fd Technol.*, 1981, **35**, 89.
20. Spicer, A., Protein production by micro-fungi. *Trop. Sci.*, 1971, **13**, 239.
21. Beuchat, L. P., *Food and Beverage Mycology*, AVI Publishing Connecticut U.S.A., 1979, 415.

Physico-chemical Changes in Whole Wheat Flour *(Atta)* and Resultant *Atta* During Storage

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Changes observed during five months storage of *atta* (whole wheat flour) and resultant *atta* showed that free fatty acids (FFA) and alcoholic acidity increased rapidly in resultant *atta* (0.19 and 0.30 percent respectively) than in *atta* (0.05 and 0.15 per cent respectively). While *atta* was in good condition, resultant *atta* developed slight bitter taste and musty odour within 2 months. The poor storage stability of resultant *atta* can be attributed to higher moisture content (12.1 percent) as well as to the activities of lipase, lipoxidase and protease. The adverse changes during storage of resultant *atta* were minimised by reducing its moisture level from 12.1 per cent to that of *atta* (7-8 percent).

The main raw material used in the preparation of chapati, one of the major staple food is the whole wheat flour (*atta*) or resultant *atta* also known as mill *atta*, a by-product from roller flour mill. Though more suited for chapati making[1]. *Atta* is more expensive than the resultant *atta*, which has better nutritional characteristics, as it contains the higher amountsof some of the essential amino acids and some vitamins[2] of B group but has comparatively poor shelf-life. The changes in the chemical and dough characteristics of *atta* during storage have been reported by several workers[3–5], while information on those for resultant *atta* is lacking. Hence, comparative storage behaviour of *atta* and resultant *atta* and the factors responsible for the rapid deterioration of the latter are presented in this paper.

Materials and Methods

Materials: Samples of resultant *atta* and wheat grist processed to obtain the same, were procured from a commercial roller flour mill. The wheat grist was ground in a disc mill (*chakki*) to obtain *atta*. Resultant *atta* had a moisture content of 12 per cent and the atta 7.7 per cent. Moisture content of a part of each of these flour was adjusted to that of the other either by drying or keeping in a humid chamber. Prepared samples were stored in air tight tin containers at ambient temperature (27-29°C) and relative humidity (60-70 per cent). Materials were periodically inspected for infestation and samples drawn at frequent intervals for analysis and evaluation.

Chemical characteristics: Moisture, total ash and ether extractives in the samples were determined according to AACC method[6]. Micro-Kjeldahl method was used for estimating the crude protein and AOAC method[7] was used for determining the FFA. Alcoholic acidity was estimated by the method described in ISI[8]. Proteolytic activity and lipase activity, were determined by the method of Lusena and Mc Farlane[9] and Kantharaj Urs *et al*[10]. respectively. Lipoxidase activity was determined by the AACC method[6] using differential respirometer.

Dough characteristics: Farinograph characteristics of *atta* samples were determined at a chapati dough consistency of 450 BU using lever position of 1:3, according to the method standardized by Haridas Rao *et al*[11]. The extensograph characteristics of such a dough were determined, using 75 g dough[11] instead of 150g recommended in the AACC[5] procedure. The farinograms as well as the extensograms were evaluated according to standard procedures.

Organoleptic quality: The stored flour samples were evaluated at monthly intervals by a panel of six judges for odour, taste and for infestation.

Results and Discussion

Chemical characteristics: Some of the important chemical characteristics of *atta* and resultant *atta* are given in Table 1.

Moisture content of resultant *atta* was higher by about 4 per cent than that reported earlier for *atta* due to the conditioning of wheat from 10 per cent moisture to about 15 per cent before milling. However, in the case of *atta*, a loss of about 2 per cent was observed during grinding in *chakki* due to frictional heat.

Free fatty acid content was about 3 times higher and alcoholic acidity about 2 times higher in resultant *atta*, as compared to those of *atta*. The alcoholic acidity of

TABLE 1. SOME CHEMICAL CHARACTERISTICS OF WHOLE WHEAT FLOUR* (*ATTA*) AND RESULTANT *ATTA*

Flour samples	*Atta*	Resultant *atta*
Moisture (%)	7.70	12.00
Total ash (%)	1.58	1.57
Protein (N×5.7) (%)	10.60	11.10
Ether extractives (%)	1.81	1.84
Free fatty acids (%)	0.031	0.087
Alcoholic acidity (%)	0.059	0.098
Lipase activity** (ml)	5.20	9.68
Proteolytic activity+	4.21	8.92
Lipoxidase activity‡ (μl)	210	505

All values are on 14% moisture

*The same wheat was used for processing of whole wheat flour (*atta*) and resultant *atta*.

**Of 0.1 N NaOH

+Expressed as milliequivalents of tyrosine per kg of flour

‡Of oxygen per gram sample per 20 min.

the resultant *atta* (0.098 per cent) was conforming to ISI specification.

The activities of lipase, lipoxidase and protease were 2-2½ times higher in R. *atta* than in *atta*. The higher enzyme activities in resultant *atta* may probably be due to the presence of higher amounts of enzyme rich parts like germ and aleurone layers of wheat.

There was no significant change in the moisture content of both *atta* and resultant *atta* during storage.

Generally, FFA content of both the samples increased with time, the rate of increase being higher in resultant *atta*. (0.265 per cent) than in *atta* (0.11 per cent) after 5 months storage (Fig. 1). The greater increase in resultant *atta* might probably be due to the higher activity of lipase and liposidase (Table 1). which are reported to accelerate the release of free fatty acids[11]. Samples having higher moisture content showed more FFA.

This was further confirmed when the *atta* sample was stored at higher initial moisture level of 12.1 per cent.

In the case of resultant *atta* having 12.1 per cent moisture, the alcoholic acidity increased from 0.098 to 0.4 per cent after 5 months storage, while the increase in *atta* was from 0.048 to 0.205 per cent. It may also be noted that the alcoholic acidity exceeded the maximum limit prescribed in Prevention of Food Adultration Act, India (0.18 per cent) within 5 weeks of storage in the case of resultant *atta* while for *atta* 16 weeks were required to cross the limit. This indicated again the better storage stability of *atta*.

The increase in alcoholic acidity during storage was considerably reduced when the resultant *atta* was

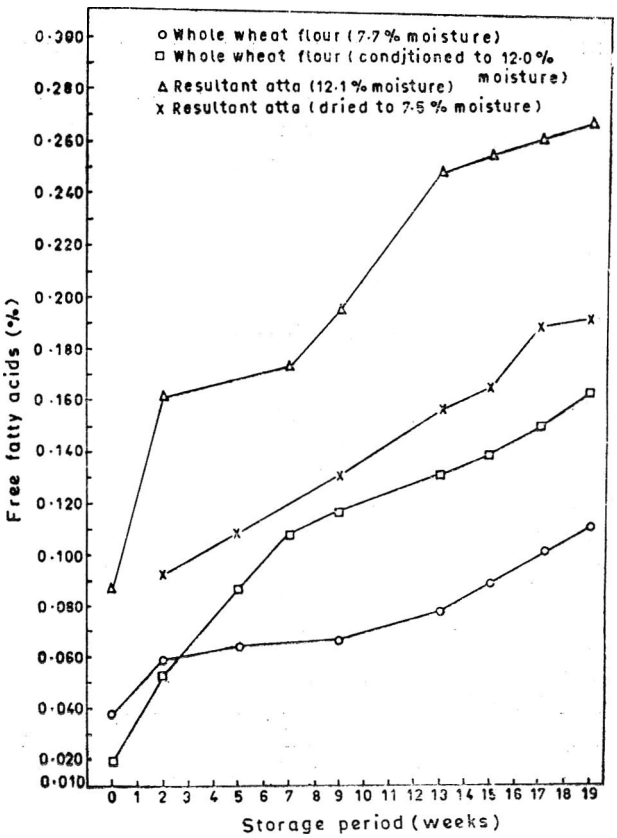

Fig. 1. Changes in free fatty acids during storage of whole wheat flour (*atta*) and resultant *atta*

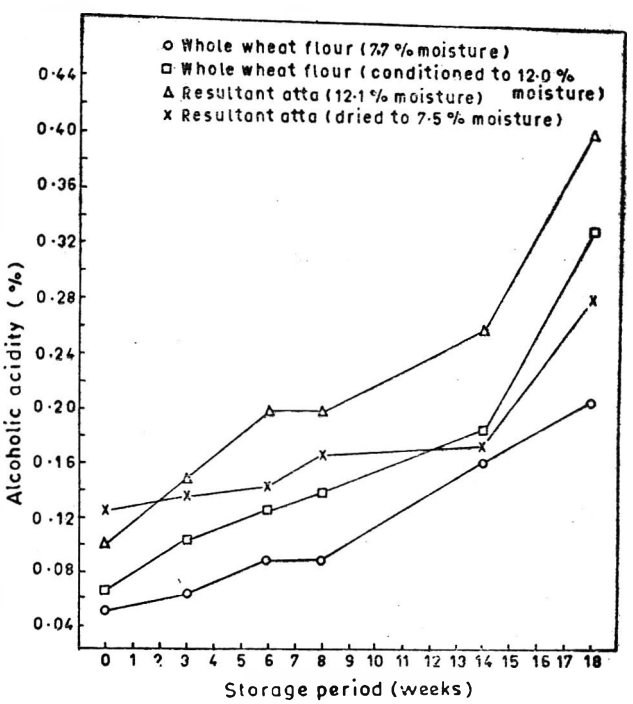

Fig. 2. Changes in alcoholic acidity during storage of whole wheat flour (*atta*) and resultant *atta*

TABLE 2. CHANGES IN THE FARINOGRAPH CHARACTERISTICS OF WHOLE WHEAT FLOUR (*ATTA*) AND RESULTANT *ATTA* DURING STORAGE

Type of flour	Chapathi water absorption (%)*		Dough development time (min)		Dough stability (min)	
	Initial	4 months	Initial	4 months	Initial	4 months
Atta	63.3	60.4	10.0	10.5	5.0	5.0
Resultant *atta*	64.4	60.6	6.0	10.0	3.5	7.0

*Values expressed on 14 per cent moisture basis

conditioned to 7.5 per cent moisture before storage. However, the increase was always higher in resultant *atta* as compared to *atta* (Fig. 2).

Dough characteristics: Changes in farinograph characteristics of *atta* and resultant *atta* stored at their original moisture levels are given in Table 2. The water required to make the chapati dough of optimum consistency (450 BU at lever position of 1:3) decreased by about 3.0 per cent at the end of storage period in both the samples. The dough development time and dough stability were however, not affected due to storage in the case of *atta*. while a considerable increase in the above characteristics was observed in resultant *atta*. Similar increase in the stored resultant *atta* samples has also been reported by Arya *et al*[5]. The resistance to extension increased and the extensibility decreased in resultant *atta* and *atta* samples during storage (Table 3). However, the extent of increase was higher in resultant *atta*.

Even after altering the moisture content there was no effective change in the farinograph or in the extensograph characteristics.

Organoleptic quality: The data on organoleptic evaluation of *atta* and resultant *atta* having different moisture levels are given in Table 4. The *atta* kept well for two months but musty odour was detected after 3 months. It was free from insect infestation even after 5 months. On the other hand, resultant *atta* developed musty odour within a month, bitter taste after two months which intensified further on storage. Insect infestation was also observed after 3 months which increased with storage. However, the resultant *atta* in which the moisture level was adjusted to 7.5 per cent kept well for 2 months without development of any musty odour or bitter taste. Onset of insect infestation was also considerably delayed from 3 to 5 months. However, its quality with respect to odour and taste was inferior to that of *atta* stored under similar conditions.

TABLE 3. CHANGES IN THE EXTENSOGRAPH CHARACTERISTICS OF WHOLE WHEAT FLOUR (*ATTA*) AND RESULTANT *ATTA* DURING STORAGE

Type of flour	Resistance to extension (R) (BU)		Extensibility (E) (mm)		Ratio of R/E		Area (cm^2)	
	Initial	4 months	Initial	4 months	Initial	4 months	Initial	4 months
Atta	680	760	40	37	17.0	20.5	33.5	37.0
Resultant *atta*	700	960	47	41	14.9	23.4	40.2	45.5

TABLE 4. ORGANOLEPTIC QUALITY OF WHOLE WHEAT FLOUR (*ATTA*) AND RESULTANT *ATTA* STORED AT AMBIENT CONDITIONS

Type of flour	Initial moisture content (%)	Flavour[a] after indicated period (months) of storage						Infestation[b] after indicated period (months) of storage					
		0	1	2	3	4	5	0	1	2	3	4	5
Atta	7.7	PTW	PTW	PTW	M	M	M	—	—	—	—	—	—
Atta	12.0	PTW	PTW	PTW	M	M & SB	M & SB	—	—	—	—	+	+
Resultant *atta*	12.1	MTW	M	M & SB	M & B	B	B	—	—	—	+	++	+++
Resultant *atta*	7.5	MTW	MTW	MTW	MTW	M	M	—	—	—	—	—	+

a. PTW —Pronounced and typical of wheat
MTW—Mild and typical of wheat
M —Musty
SB —Slightly bitter
B —Bitter

b. + Slight infestation with tendency to form lumps
++ Infestation with formation of lumps
+++ Infestation with the presence of live insects.

Similar observation was made with *atta* samples having high moisture content.

It could be concluded that the *atta* has a poor shelf life when compared to *atta* due to the higher content of moisture and lipolytic enzymes. The shelf life of resultant *atta* could be considerably improved by reducing the moisture. But even at equal moisture levels. *atta* had better shelf life on storage possibly due to its lower enzyme activity.

References

1. Tara, K. A., Haridas Rao, P. and Bains G. S., Composition and rheological and baking quality of a by-product of wheat milling simulating whole wheat meal. *J. Sci. Fd Agric.*, 1969, **20**, 370.
2. Kumar K. R. and Anandaswamy, B., Packaging studies of *atta* and *Soji* in unit packages. *Indian Miller*, 1978, **9**, 7.
3. Arya, S.S., Mohan, M.S. and Nath, H., Studies on packaging and storage of *atta* (wheat flour) under tropical conditions. *J. Fd Sci. Technol.*, 1971, **8**, 134.
4. Premavalli, K. S., Leela, R. K., Arya, S. S., Parihar, D. B. and Nath, H., Studies on packaging and storage of wheat flour (*atta*) under tropical conditions. *J. Fd Sci. Technol.*, 1972, **9**, 27.
5. *Approved Methods*, American Association of Cereal Chemists, 7th edn., St. Paul, Minnesta, USA, 1969.
6. *Official Methods of Analysis*. Association of Official Analytical Chemists, Washington, D. C., 20044, 12th edn., 1975.
7. *Specification for Wheat Atta*, IS: 1155-1968, Indian Standards Institution, New Delhi.
8. Lusena, C. V., and McFarlane, W. D., Studies on the processing of wheat germ. *Can. J. Biochem.*, 1945, **23**, 201.
9. Kantharaj Urs, M., Bains, G. S. and Bhatia, D.S., Triaceitin as substrate for peanut lipase. *Sci. Ault.*, 1962, **28**, 581.
10. Haridas Rao, P.. *Studies on Chapati and Similar Traditional Foods* Ph.D., Thesis, University of Mysore, Mysore, 1982.
11. Pomeranz, Y., *Wheat Chemistry and Technology*, American Association of Cereal Chemists, St. Paul, Minnesota, 1971.

Study of Proteolytic Inhibitor in Indian Wheats and Response to Proteolytic Supplements

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Bread wheats, 'WG 357' and 'S 308' and their milled fractions, showed significant resistance to proteolysis by papain, ficin and Rhozyme A-4 tested at a concentration of 60 to 300 Casein units (CU/100g). Inhibition of ficin activity was less, when compared with papain and Rhozyme A-4. Farinography, gluten recovery and enzymes when used at a dose of 30 to 120 CU/100g. Decreasing the levels to 10 to 40 CU but increasung the reaction time to one and a half hr indicated papain to be more damaging to gluten. 'WG 357', withstood proteolytic action better than 'S 308'. Ficin doughs were found normal to handling during baking. Papain was deleterious to loaf qualify whereas ficin and Rhozyme A-4 improved crumb grain and texture. Evidence is presented to show that soluble N values as an index of proteolytic inhibition is inadequate to refflect the mechanics of action in relation to dough and gluten properties of flours.

The presence of protease inhibitors in different grades of English wheat flour, capable of inhibiting papain action without any effect on the native proteolytic enzymes of wheat was reported by Hites *et al.*[1] Johnson and Miller[2] confirmed the presence of a papain inhibitory factor in wheat flour, using farinograph technique. The inhibitor fraction extracted from wheat was shown to be non-dialyzable by Hites *et al*[1]. and by Shymala and Lyman[3]. The distribution of proteolytic inhibitor in various mill fractions has not received sufficient attention. Low proteolytic acitivity in Indian wheats has been reported earlier by Singh *et al*[4]. In this paper, results of the distribution of protease inhibitor in milled products of two extensively cultivated varieties of Indian wheats, 'WG 357' and 'S 308', and effect of plant and fungal proteolytic enzyme supplementation as determined rheologically and by baking tests, are presented.

Materials and Methods

Straight-grade flours (Table 1) of 'WG 357' and 'S' 308' were prepared in the pneumatic Buhler laboratory mill (MLU-202) after conditioning to 15.5 per cent

TABLE 1. YIELD AND COMPOSITION OF STRAIGHT-GRADE FLOURS OF WG 357 AND S 308 VARIETIES OF WHEAT

Variety	Straight grade (%)	Protein (N×5.7) (%)	Ash (%)	Diastatic activity (mg maltose/10g)	Damaged starch (%)
WG 357	69.3	9.5	0.46	316	11.9
S 308	71.2	9.1	0.43	160	7.4

moisture. The milled fraction were collected separately. Break, reduction and dusted bran flour fractions were combined to obtain straight grade flour. Bran, shorts and flour were collected separately, weighed and kept in individual air-tight containers.

Enzyme supplements included fungal protease, Rhozyme A-4 (M/s Rohm Haas, Philadelphia, USA), papain and ficin. They were used according to their activity determined by the AACC procedure[5]. The casein used as substrate was Hammarstein grade (BDH) and the activity expressed in casein units (CU).

The inhibitor was extracted from flour and bran (finely ground) by maceration with two and two and a half volumes of distilled water respectively, in a glass pestle and mortar for 5 min. The slurry was centrifuged at 10,000 rpm for 10 min and the supernatant decanted.

Proteolytic inhibitor activity was determined by the modified Ayre-Anderson[6] method as described by Hitres *et al.*[1] Soluble N was determined by the method of Lowry *et al.*[7] as modified by Skupin and Warchalewaski[8].

Gluten recovery, as affected by the proteolytic supplements, was studied using plain doughs. In the first series, the proteolytic levels, 30-120 CU/100g, were used as in the farinograph studies. Thirty minutes rest was given to the dough as prescribed by the AACC[5]. In the second series, the dough was rested for 90 min before washing gluten. This simulated bulk fermentation time used in baking. The levels of protease supplements varied from 10 to 40 CU/100g. Gluten strength was evaluated according to the Hanford[9] scale. The farinograph was operated in accordance with constant flour weight method of AACC[5] using a 50 g mixing bowl. Calculated quantity of enzyme in solution was added to doughs and mixed for 15 min to decipher changes occurring in consistency. Farinograph water absorption of the control was used in these tests. Dough handling properties were evaluated subjectively at different stages in baking. Straight dough method of baking with remixing[10] was followed. Baking formula included(g): flour, 100; yeast, 2.5, sugar, 2.5; shortening 1.0; salt, 0 and 1.5; and proteolytic enzymes, 0 to 40 CU. The dough was mixed optimally, fermented for 90 min remixed for 15 sec, followed by a recovery for 25

TABLE 2. INHIBITORY EFFECT OF WHEAT VARIETIES AND THEIR MILLED FRACTIONS ON THE ACTIVITY OF PROTEOLYTIC SUPPLEMENTS

Proteolytic supplement	Dosage (CU/100g)	Increase in soluble N[1]							
		WG 357				S 308			
		Wheat	Bran	Shorts	Flour	Wheat	Bran	Shorts	Flour
Papain	60	0.0	5.8	0.0	0.0	0.0	0.0	0.0	0.0
	120	0.0	10.8	1.8	1.9	0.0	0.0	0.0	0.5
	180	0.2	21.1	3.2	3.3	0.0	0.0	0.0	3.7
	240	0.4	36.0	4.6	4.3	0.5	3.6	2.2	7.0
	300	0.7	40.8	6.5	5.2	1.5	8.4	4.1	12.5
Ficin	60	0.9	3.6	4.6	5.7	0.3	3.6	1.3	0.0
	120	1.7	13.9	10.3	10.7	0.9	7.2	3.2	0.5
	180	2.5	25.7	16.2	15.4	1.2	13.2	4.6	4.3
	240	3.8	37.2	19.7	18.2	1.7	17.5	7.4	6.5
	300	4.9	43.2	25.0	22.9	2.0	23.9	10.1	13.4
Rhozyme A-4	60	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	120	0.0	0.0	0.5	0.0	0.0	2.4	0.0	0.0
	180	0.4	0.0	0.5	2.4	0.3	4.8	0.0	1.9
	240	0.6	0.0	1.3	3.3	0.5	11.8	2.2	4.8
	300	0.7	5.8	2.2	4.3	0.8	18.7	3.6	7.0

1 'F' ratio—Varieties, 12.24; mill fractions, 18.03; proteolytic supplements, 19.54; dosages, 11.19; ($P < 0.01$)

min. After proofing for 55 min at 30°C, the loaves were baked for 25 min at 232°C, cooled and volume taken by the rape seed displacement method[5]. Next day they were scored for crust and crumb characteristics.

Soluble N and loaf volume data were examined statistically by the analysis of variance using 'F' ratio as criterion of significance.

Results and Discussion

Soluble N: Statistically significant ($p<0.01$) differences existed in the protease inhibitory effect of the varieties, their milled fractions and the proteolytic supplements (Table 2). The extract of 'WG 357' bran showed no inhibitory effect on Rhozyme A-4 upto 240 CU concentration. Greater inhibition of papain and ficin by the bran extract of 'S 308' than 'WG 357' was observed. Extract of 'S 308' shorts inhibited papain more than ficin. The flour extract of 'WG 357' showed minimal inhibitory effect as compared to 'S 308'.

Gluten: Ficin affected the gluten strength of both the varieties than papain which affected more the weaker 'S 308' gluten in the range of 30-120 CU/100g (Table 3). Rhozyme A-4 had little effect either on the quality or the percentage recovery of gluten. However, with increased level of the enzyme, these parameters were affected. There seemed to be hardly any correspondence between the results of soluble N as an index of proteolytic inhibitor activity with the physical changes in gluten evaluted by the Hanfords' method.[9] The liquefying action of ficin on gluten was attributed to its faster action. A gradual decrease in the recovery of gluten from doughs having increasing levels of the proteolytic supplements (10-40 CU/100g) and extended rest time simulating bulk fermentation of dough was observed (Table 4). The loss of gluten from 'S 308' having

TABLE 3. EFFECT OF PROTEOLYTIC SUPPLEMENTS ON THE RECOVERY AND STRENGTH OF GLUTEN

Proteolytic supplement	Dosage (CU/100g)	WG 357			S 308		
		Dry gluten (%)	Decrease (%)	Gluten[9] strength score	Dry gluten (%)	Decrease (%)	Gluten[9] strength score
Control	0	9.0	—	6	9.2	—	6
Papain	30	9.0	0.0	6	9.1	1.1	5
	60	9.0	0.0	6	9.0	2.2	4
	120	8.8	2.2	6	9.0	2.2	2
Ficin	30	8.9	1.1	6	9.0	2.2	5
	60	8.4	6.7	4	8.5	7.8	2
	120	8.1	10.0	2	—	—	0
Rhozyme A-4	30	9.0	0.0	6	9.2	0.0	6
	60	8.7	3.3	6	9.1	1.1	6
	120	8.9	1.1	6	9.2	0.0	6

See Reference 9.

TABLE 4. EFFECT OF PROTEOLYTC SUPPLEMENTS ON THE RECOVERY AND STRENGTH OF GLUTEN FROM DOUGH, RESTED FOR NINETY MINUTES

Proteolytic supplement	Dosage (CU/100g)	WG 357			S 308		
		Dry gluten (%)	Decrease (%)	Gluten[9] strength score	Dry gluten (%)	Decrease (%)	Gluten[9] strength score
Control	0	8.9	—	6	10.0	—	5
Papain	10	8.8	1.1	6	9.2	8.0	5
	20	8.8	1.1	6	9.1	9.0	5
	30	8.7	2.3	5	9.0	10.0	5
	40	8.2	7.8	5	8.6	14.0	4
Ficin	10	8.9	0.0	6	9.8	2.0	5
	20	8.7	2.3	6	9.6	4.0	5
	30	8.6	3.4	6	9.4	6.0	5
	40	8.4	5.6	6	9.4	6.0	4
Rhozyme A-4	10	8.9	0.0	6	10.0	0.0	5
	20	8.9	0.0	6	9.8	2.0	5
	30	8.7	2.3	6	9.7	3.0	5
	40	8.6	3.4	6	9.6	4.0	5

See Reference 9.

papain (40 CU/100g) was upto 14 per cent. The gluten was largely disrupted by papain than either by ficin or by Rhozyme A-4. 'WG 357' gluten was rated stronger according to Hanford[9] scale than 'S 308'. Upto 40 CU/100g of the proteolytic supplements, the strength of its gluten was hardly affected. It was commonly observed that the supplements mellowed the glutens even below the inhibitory levels.

Farinograms: 'WG 357' flour showed better rheological quality by the farinograph technique than 'S 308' characterised by poor absorption, dough development time,-mixing tolerance index and marked tendency to softening (Table 5). At different levels of the proteolytic supplements, peak consistencies hardly changed. The dough development times decreased gradually as the level of papain was increased from 30 to 120 CU/100g unlike the negligible effect of ficin and Rhozyme A-4 which hardly affected the properties of 'WG 357' dough. The results indicated a substantial latitude in the rheological behaviour of dough in case of ficin than papain and Rhozyme A-4. This was despite the fact that ficin showed considerable softening of gluten in isolated systems.

Dough handling: Dough of 'S 308' having 10 CU/100g of papain was sticky which became more sticky with increased concentration of the supplements (Table 6)

TABLE 5. EFFECT OF PROTEOLYTIC SUPPLEMENTS ON THE FARINOGRAPH CURVE CHARACTERISTICS OF WG 357 AND S 308 FLOURS

Proteolytic supplements	Dosage (CU/100g)	Dough development time (min)		Stability, (min)		Mixing tolerance index (B.U.)		Softening (B.U.)	
		WG 357	S 308	WG 357	S 308	WG 357	S 308	WG 357	S 308
Control	0	3.8	2.2	6.0	1.7	95	160	80	150
Papain	30	3.5	2.1	4.8	1.4	90	155	85	170
	60	3.0	2.0	4.0	1.5	105	170	95	180
	120	1.5	1.9	2.7	0.8	105	155	160	220
Ficin	30	4.0	2.1	5.7	1.7	95	155	70	165
	60	3.7	2.1	6.7	1.5	85	160	70	155
	120	3.5	2.1	6.6	1.7	95	155	75	170
Rhozyme A-4	30	3.2	2.0	3.8	1.5	75	160	125	170
	60	3.5	2.0	2.9	1.6	125	160	150	185
	120	3.5	2.1	4.8	1.0	165	160	135	190

Farinograph water absorption—WG 357, 58.8%, S 308, 55.2%

TABLE 6. EFFECT OF PROTEOLYTIC SUPPLEMENTS ON THE DOUGH HANDLING PROPERTIES[1] OF WG 357 AND S 308 FLOURS AT DIFFERENT STAGES IN BANKING

Proteolytic supplement	Stage	Proteolytic supplements treated at indicated dosages (CU/100g)									
		WG 357					S 308				
		0	10	20	30	40	0	10	20	30	40
Papain	Mixing	N	N	N	N	N	N	N	N	N	N
	Remixing	N	N	N	SS	VS	N	SS	S	VS	VVS
	Sheeting/moulding	N	N	N	E	R	N	F	F	VF	R
Ficin	Mixing	N	N	N	N	N	N	N	N	N	N
	Remixing	N	N	N	N	N	N	N	N	N	N
	Sheeting/moulding	N	N	N	P	P	N	N	N	N	E
Rhozyme A-4	Mixing	N	N	N	N	N	N	N	N	N	N
	Remixing	N	N	N	N	N	N	N	N	N	N
	Sheeting/moulding	N	N	N	P	P	N	N	N	N	N

1. N—normal P—pliable E—extensible SS—slightly sticky S—sticky VS—very sticky VVS—very very sticky F—Flowy VF—Very flowy R—rotten

TABLE 7. EFFECT OF PROTEOLYTIC SUPPLEMENTS WITH AND WITHOUT SALT ON THE BAKING CHARACTERISTICS OF WG 357 AND S 308 FLOURS

Proteolytic supplement	Dosage (CU/100g)	WG 357						S 308		
		Salt O.O.%			Salt 1.5%			Salt 1.5%		
		Loaf vol. (ml)	Crumb		Loaf vol. (ml)	Crumb		Loaf vol. (ml)	Crumb	
			Grain	Texture		Grain	Texture		Grain	Texture
Control	0	515	F	S	520	F	S	475	F	S
Papain	10	500	F	S	495	F	S	475	F	S
	20	465	F	S	455	F	S	425	F	H
	30	425	C	H	420	C	H	405	C	H
	40	—	—	—	400	C	H	400	C	H
Ficin	10	495	F	S	520	F	S	510	F	S
	20	500	F	S	520	F	S	495	F	S
	30	505	F	S	520	F	S	495	F	S
	40	495	F	S	500	F	S	500	F	S
Rhozyme A-4	10	505	F	S	515	F	S	515	F	S
	20	500	F	S	515	F	S	515	F	S
	30	500	F	S	515	F	S	515	F	S
	40	505	F	S	495	F	S	520	F	S

F—fine C—coarse S—soft H—hard

'F' ratio—varieties, 2.95 (non-significant); —proteolytic supplements, 19-79 (significant at P=0.01); —dosages, 1.79 (non-significant)

Under similar conditions, 'WG 357' dough was less sticky. Ficin and Rhozyme A-4 doughs were judged normal at different stages of handling in baking. 'WG 357' doughs, without salt, were more sticky than with salt, specially when papain (40 CU/100g) was used. The improving effect of salt on the 'WG 357' doughs having Rhozyme A-4 was also observed. Collating the results of gluten softening and dough handling properties, it seemed that plant proteases had greater effect on dough than the fungal protease, which contrasted with the proteolytic inhibitor concept based on soluble N values.

Baking: As the level of papain in the dough was increased from 0-40 CU/100g, the loaf volumes decreased considerably (Table 7). The loaf volume of 'S 308' was decreased even at 10 CU level of papain, being more pronounced with 40 CU in the dough. The loaf volumes were hardly affected by the corresponding levels of ficin and Rhozyme A-4 supplements. Overall, differences in loaf volume of the varieties of wheat were non-significant. However, among the proteloytic supplements, the loaf volume differences were statistically significant ($P < 0.01$). The damaging effect of papain on loaf volume has been reported by Hites *et al.*[1] The crumb grain of 'WG 357' control and of 10-20 of papain loaves was fine but became coarse at 30 CU level. The grain of 'S 308' control loaves was fine but became coarse even at 10 CU of papain, and more so when 20-40 CU of the enzyme was used. With ficin and Rhozyme A-4 in the range of 10-40 CU, the crumb grain of loaves of both the varieties was fine and texture softer. 'WG 357' loaves baked without salt had characteristics similar to the loaves without salt, but tasted incipid. Improvement in crumb characteristics using judicious levels of proteolytic supplements was reported by Singh *et al*[4]. The present study has shown that the inhibitory effect of milled wheat fractions was largely confined to the soluble N producing action. The gluten softening action was less obvious over the optimal range of supplements used with accompanying improvement of loaf characteristics.

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References

1. Hites, B. D., Sandstedt, R. M. and Schaumburg, L., Studies of proteolytic activity in wheat flour doughs and suspensions. II. A Papain inhibitor in flour. *Cereal Chem.*, 1951, **28**, 1.

2. Johnson, J. A. and Miller, B. S., The relationship between dough consistency and proteolytic activity. *Cereal Chem.*, 1953, **30**, 471.
3. Shyamala, G. and Lyman, R. L., The isolation and purification of a trypsin inhibitor from whole wheat flour. *Can. J. Biochem.*, 1964, **42**, 1825.
4. Singh, R. P., Singh G. P. and Bains, G. S., Baking response of Punjab wheats to proteolytic supplements. *J. Fd Sci. Technol.*, 1978, **15**, 259.
5. *AACC Approved Methods*, American Association of Cereal Chemists, Inc. St. Paul, Minn, USA, 1972.
6. Ayre, C. A. and Anderson J. A., Varietal differences in barleys and malts. VI. Autolytic proteolytic digestion of malt and its correlation with wort nitrogen and barley nitrogen fractions. *Can. J. Res.*, 1939, **17C**, 239.
7. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R. J., Protein measurement with the Folin-phenol reagent. *J. biol. Chem.*, 1951, **193**, 265.
8. Skupin, J. and Warchaleswki, J., Isolation and properties of protease A from wheat grain. *J. Sci. Fd Agric.*, 1971, **22**, 11.
9. Hanford, J., The proteolytic enzymes of wheat and flour and their effect on bread quality in the United Kingdom. *Cereal Chem.*, 1967, **44**, 499.
10. Irvine, G. N. and McMullan, M. E., The 'remix' baking test. *Cereal Chem.*, 1960, **37**, 603.

Effect of Roasting on Amylose Content and Textural Quality of Cassava (*Manihot esculenta* Crantz)

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Tubers from seven cultivars of cassava were processed by pan-roasting of the flour and their amylose content and organoleptic quality evaluated. The roasted samples were less Sticky when steam-cooked, than unroasted samples. Both total and insoluble amylose content were higher in the roasted samples. Intervarietal relationship between insoluble amylose content and stickiness score was not consistent. In addition to amylose, unbound fat may also contribute to the cooking quality of cassava.

Cassava flour becomes sticky during cooking[1]. This in turn, lowers the acceptability of the product. Studies in this laboratory have shown that cooking quality of cassava could be improved by heat-moisture-treatment[2]. Cereal flour prepared from wheat, and rice are often roasted prior to steam-cooking to reduce the development of stickiness. In the present study the effect of roasting on amylose content and textural quality of the roasted cassava flour in terms of stickiness have been studied using a few selected cultivars.

Materials and Methods

The cultivars used for the study were two local varieties 'Malayan-4' (M-4) and 'Local White', and also the high-yielding varieties 'H-97,' 'H-2304', 'H-165', 'H-226' and 'H-1687' released by the Central Tuber Crops Research Institute (CTCRI), Trivandrum. 'Malayan-4' and 'Local White' were procured from the local farms while the high-yielding varieties were collected from CTCRI Experimental Farm.

Potato amylose used as the standard was obtained from the Vallabhai Patel Chest Institute, New Delhi. A C-2 spectrophotometer Model VSU-2P was used for reading the absorbance.

Heat treatment by roasting: Fifty gram lots of flour were moistened to raise the moisture content to 28±2 per cent, hand-mixed and kept in stoppered glass bottles for 1 to 2 hr for moisture equilibration. Samples were then roasted till the product gave out a fried aroma. Experiments were done in triplicate with each variety. The parameters studied are given in Table 1.

Amylose estimation: Total and hot-water soluble amylose contents were estimated in both untreated and roasted samples. The difference between the two values was taken as the insoluble amylose content of each sample. Total amylose was estimated by the method described by Sowbhagya and Bhattacharya[3].

The soluble amylose was determined by the method of Hall and Johnson[4] with the following modification arrived at after standardisation of particle size, extraction time and size of the aliquot.

Fifty milligrams of flour sample 60-100 mesh (British Mesh No.) was extracted with 100 ml distilled water in a boiling water bath for 10 min. Aliquots (5 ml)

TABLE 1. EXPERIMENTAL PARAMETERS OF HEAT TREATMENT OF CASSAVA FLOUR

Cassava variety	Initial Moisture content (%)	Moisture after equilibration (%)	Roasting temp. (°C)	Roasting period (min)	Moisture of roasted sample (%)
M-4	10.2	27.0	102	15	2.6
Local white	8.2	26.0	102	15	1.4
H-97	7.6	25.8	102	15	1.2
H-2304	7.6	26.0	102	14	1.4
H-165	9.6	26.6	101	14	1.8
H-226	9.2	26.0	104	15	2.1
H-1687	9.4	26.8	102	14	1.2

were taken for development of colour. The absorbance was read at 600 nm. Analyses were done in triplicate and the mean value was taken in each case.

Cooking quality studies: Cooking quality of the steam-cooked products prepared from roasted samples were evaluated by a selected panel of 8 judges. The products prepared from plain (not roasted) cassava flour, and rice flour were used as reference samples for lowest and highest acceptability respectively.

Results and Discussion

Results of the analyses of untreated and roasted samples for their amylose contents and their organoleptic score for stickiness are presented in Table 2.

Changes in amylose content: An increase in the values of both total and insoluble amylose content was noticed in all the varieties after roasting. The total amylose content in the roasted samples ranged from 20.6 to 22.5 per cent among the varieties studied. These values were more or less indentical with the values obtained for the same varieties after defatting with chloroform-methanol (2:1) as already reported by Raja *et al*[5]. In the latter case the values ranged from 20.3 to 21.9 per cent. This possibly suggests that like defatting, heat treatment by roasting may also enable the cleavage of lipid-amylose complex, thereby enhancing the free amylose content in the roasted samples.

The per cent increase of total amylose content in the roasted samples over that of untreated ones differed significantly among the varieties. Eventhough varieties like 'M-4' and 'H-165'; 'H-97; 'H-226', and 'H-1687' had almost similar levels of amylose contents in their untreated samples, the per cent increase of amylose after roasting was widely different. This could be due to the difference in the nature of lipid-amylose complex which may be characteristic of each variety. It has already been reported by these authors that the extractable total lipids from the varieties used for the study, ranged from 0.9 to 1.2 per cent when the samples were extracted using solvent system consisting of chloroform-methanol, (2:1)[5].

Amylose content and textural quality: In rice flour, studies carried out with different varieties of rice by Bhattacharya *et al*[6]. have indicated that insoluble amylose content could be considered as an index of cooking quality. In this study on cassava flour also, increase in amylose contents after roasting was found to be generally followed by reduction in stickiness. However, it was slightly different in that the relationship among different varieties was not so close or direct as in the case of rice. The reduction of stickiness during roasting may also be due to the presence of unbound

TABLE 2. AMYLOSE CONTENT* AND COOKING QUALITY OF CASSAVA FLOUR

Cassava Variety	Raw flour				Roasted flour				
	Total amylose (%)	Insoluble amylose %	Insoluble amylose As % of total amylose	Stickiness (score)@	Total amylose %	Total amylose % increase	Insoluble amylose %	Insoluble amylose As % of total amylose	Stickiness (score)
M-4	18.8	5.6	29.7	2.6	20.6	9.5	7.2	34.9	4.7
Local white	19.4	5.6	28.8	3.4	21.6	10.2	8.8	40.7	5.2
H-97	19.1	3.7	19.3	2.7	21.5	12.6	7.9	36.7	4.0
H-2304	20.3	5.3	26.1	3.5	21.4	5.4	7.4	34.6	4.4
H-165	18.9	5.1	26.9	1.5	22.5	19.0	9.0	40.0	2.5
H-226	19.7	5.2	26.4	1.5	21.4	8.6	7.8	36.4	4.0
H-1687	19.6	7.2	36.7	3.2	20.9	6.6	9.6	45.9	4.1

*Expressed on moisture free basis

@Expressed on a score scale: 0 to 10=lowest to highest acceptability

fat (polar lipids) caused by the cleavage of lipid-amylose complex as it is already known that amylose forms inclusion compounds with polar lipids[7]. It has already been reported in our earlier study[5] that defatting of cassava increases the insoluble amylose content but results in increased stickiness possibly due to quicker gelatinisation and also total removal of fat.

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References

1. Emilia Abraham, T., Raja, K. C. M., Sreedharan, V. P. and Sreemula Nathan., H., Some quality aspects of a few varieties of cassava. *J. Fd Sci. Technol.*, 1979, **16**, 237.
2. Emilia Abraham, T., Raja, K. C. M., Manoharan, E. P. and Mathew, A.G., Effect of heat-moisture-treatment on textural characteristics of cassava flour. *Cereal Chem.*, 1983, **60**, 7.
3. Sowbhagya, C. M. and Bhattacharya, K. R., A simplified colorimetric method for determination of amylose content in rice. *Starch*, 1971, **23**, 53.
4. Hall, V. L. and Johnson, J.R., A revised starch-iodine blue test for raw milled rice. *Cereal Chem.*, 1966, **43**, 97.
5. Raja, K. C. M., Emilia Abraham, T. and Mathew, A. G., Effect of defatting on amylose contents, viscosity characteristics and organoleptic quality of cassava (*Manihot esculenta* Crantz). *J. Fd Technol.*, 1982, **17**, 61.
6. Bhattacharya, K. R., Sowbhagya, C. M. and Indudharaswamy Y. M., Importance of insoluble amylose as a determinant of rice quality. *J. Sci. Fd Agric.*, 1978, **29**, 359.
7. Hudson, B. J. F. and Ogunusua, A. O., Lipids of cassava tubers (*Manihot esculenta* Crantz). *J. Sci. Fd Agric.*, 1974, **25**, 1503.

Composition and Storage Characteristics of *Khoa* Made from Lactose Hydrolysed Buffalo Milk

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Lactozyme a β-D-galactosidase preparation (NOVO Industries, Denmark) when added at the rate of 1 ml/1 of buffalo milk at 40°C hydrolysed about 50, 71 and 90 per cent of lactose in 30, 60 and 120 min correspondingly. Significant differences ($P<0.05$) were noted in lactose and hydroxymethyl furfural (HMF) contents of *Khoa* prepared from lactose hydrolysed (LHM) and lactose unhydrolysed (LUM) milk. *Khoa* from LHM contained 2.56 per cent lactose as compared to 23.4 per cent of the one prepared from LUM. The HMF content (61.28 μM/100 g) was also significantly higher ($P<0.05$) than the *khoa* made from LUM (14.28 μM/100 g). *Khoa* prepared from LUM exhibited higher proteolysis and lipolysis, and was also susceptible to mould attack within 3-4 days. *Khoa* prepared from LHM showed higher peroxide value, resistance to mould attack till 30 days. It was sweet in taste, soft, brown in colour and had uniform body and texture.

The problem of lactose intolerance due to the deficiency of β-D-galactosidase in the brush border of intestinal mucosa[1,2] has necessitated development of milk products based on lactose degraded milk[3]. The incidence of lactose intolerance vary with different races and geographical condition[4]. Among Indians and people of the far east, lactose intolerance ranges from 50 to 85 per cent[5]. So far attempts have not been made to utilize lactose hydrolysed milk for the manufacture of dairy products in India. *Khoa* is one of the important products made from whole milk which forms a base for many Indian sweets. About 7 per cent of total milk produced in the country is converted into *Khoa*[6]. Studies were undertaken on the enzymatic hydrolysis of lactose in buffalo milk and conversion of such milk to *khoa*. The results are presented here.

Materials and Methods

Buffalo milk was obtained from the student's Training Dairy of this college and also from the Hadgud village Co-operative Society of Amul Dairy, Anand.

Lactose hydrolysis: Lactozyme of NOVO Industries,

Denmark (1500 lactase units/ml) was used at the rate of 1 ml per 1 of milk. Reaction was carried out at 40°C for 2 hr and the extent of hydrolysis recorded at 30 min intervals according to the procedure of Nickerson *et al*[7]. Samples of 50 ml were boiled to arrest the reaction. Milk used for manufacture of *khoa* was incubated at 40°C for one hour after addition of lactozyme.

Khoa was prepared from both unaltered (LUM) and lactose hydrolysed (LHM) raw milk, by the open pan desiccation method, in batches of 3 l, as described by Srinivasan and Anantakrishnan[8].

Total solids, protein, ash, calcium and total reducing value of milk and *khoa* samples were determined as described in ISI[9]. Fat in milk[10] and *Khoa*[11] was estimated by ISI methods. The acidity of milk and reconstituted *khoa* (20 g *khoa*/100 ml water) is expressed as lactic acid[10]. Lactose, glucose and galactose in milk and *khoa* were estimated by the method of Nickerson *et al*[7]. Free fat in *khoa* was estimated by the method of Hall and Hedrick[12], free fatty acids (FFA) by the method of Thomas *et al*[13], proteolysis by the method of Lowry *et al*[14]. peroxide value by A.O.A.C.[15] procedure and the hydroxy methyl furfural (HMF), by the method of Keeney and Bassette[16]. Comparison between the two *khoa* preparations was carried out by the application of 't' test[17].

Results and Discussion

The rate of hydrolysis of lactose in buffalo milk by lactozyme is shown in Fig. 1. It is observed that about 71 and 90 percent of lactose is hydrolysed with 60 and 120 min of incubation respectively. Percentage hydrolysis of lactose in six samples of raw milk used for preparation of *khoa* ranged from 71.2 to 74.2 with an average of 72.8±1.09.

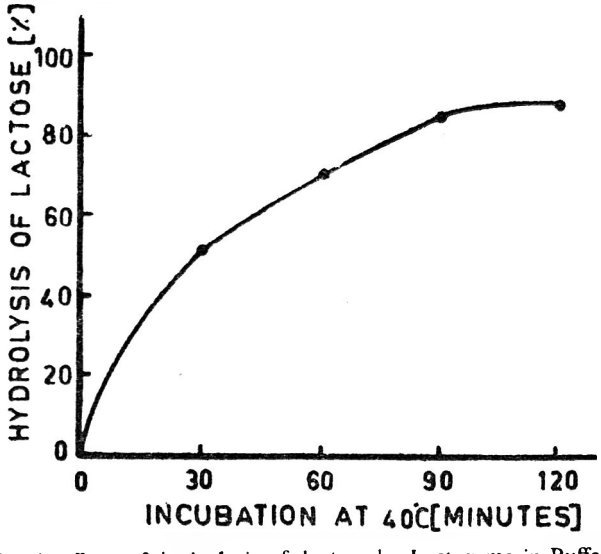

Fig. 1. Rate of hydrolysis of lactose by Lactozyme in Buffalo milk.

The results on the chemical composition of milk, *khoa* made from LUM and LHM are presented in Table 1. Very low moisture content of *khoa* samples may be attributed to the prolonged desiccation period required to achieve proper body and texture in LHM-*khoa*. Lactose content of LHM-*khoa* (2.56 per cent) is significantly ($P<0.05$) lower than that of LUM-*khoa* (23.40

TABLE 1. CHEMICAL COMPOSITION OF MILK AND KHOA MADE FROM LACTOSE HYDROLYSED (LHM) AND LACTOSE UNHYDROLYSED (LUM) MILK

Constituent	Milk	LUM-*Khoa*	LHM-*Khoa*
Total solids (g/100g)	15.63	81.29	82.97
Fat (g/100 g)	6.76	36.37	39.67
Protein (g/100 g)	3.51	17.73	18.05
Lactose (g/100 g)	4.62	23.40	2.56*
Glucose+galactose (g/100 g)	—	—	18.90
Ash (g/100g)	0.69	3.67	3.77
Calcium (mg/100g)	155.00	854.30	844.30
Phosphorus (mg/100 g)	98.00	620.80	607.50
Acidity (% lactic acid)	0.135	0.63	0.65
Total reducing capacity (% glucose)	3.600	17.71	19.23
Hydroxy methyl furtfural (μm/100 g)	—	14.28	61.28*
Free fat (% of total fat)	—	65.91*	55.10
Free fatty acids (% oleic acid)	—	0.22	0.20

Average of six samples. *Significant at 5 per cent level

TABLE 2. CHANGES IN ACIDITY, TYROSINE RELEASE (PROTEOLYSIS), FREE FATTY ACIDS RELEASE (LIPOLYSIS) AND PEROXIDE VALUE OF LACTOSE UNHYDROLYSED (LUM) AND LACTOSE HYDROLYSED (LHM) *KHOA* DURING STORAGE AT 30°C

Storage period (hr)	Acidity (% lactic acid)		Tyrosine (mg/g)		Free fatty acids (% oleic acid)		Peroxide value (ml of 0.002 N ($Na_2S_2O_3$/g)	
	LUM-Khoa	LHM-Khoa	LUM-Khoa	LHM-Khoa	LUM Khoa	LHM-Khoa	LUM-Khoa	LHM-Khoa
0	0.62	0.64	1.98	1.80	0.22	0.20	0.24	0.51
24	0.69	0.75	2.16	1.89	0.24	0.21	0.57	0.79
48	0.74	0.79	2.83	2.06	0.33	0.30	0.67	1.08
72	0.78	0.81	3.59	2.37	0.47	0.39	0.72	1.42
96	0.81	0.83	4.20	2.49	0.55	0.44	1.05	1.92
120	0.84	0.85	4.57	2.68	0.63	0.49	1.21	1.98
144	0.89	0.90	4.76	2.90	0.70	0.54	1.36	2.19

Each value is the average of six replications.

per cent). It is also observed that there is further hydrolysis of lactose probably due to continuance of activity even during evaporation till the enzyme is inactivated at elevated temperature. The net hydrolysis of lactose during these two stages therefore, totalled to about 89 per cent. The chemical composition of LUM-*khoa* is comparable with reported values[8]. It is also seen in Table 1, that when milk is converted into *khoa*, there is increase in acidity. This could be attributed to lactose-protein interactions[18], phosphate bond cleavage[19] thermal decomposition of lactose[20] and the displacement of ionic calcium phosphate equilibrium[21] which also lead to the heat coagulation of milk during preparation of *khoa* and such changes appear to be more pronounced in LHM-milk during *khoa* making.

Increase in the total reducing value in LHM-*khoa* is due to the relase of monosaccharides (glucose and galactose). Higher concentration of HMF (Table 1) in case of LHM-*khoa* may be due to the release of monosaccharides which interact with proteins during processing[22]. The resulting complexes on constant heating during *khoa* making decompose faster and produce substantial amount of HMF and other intermediates[23] causing extensive browning in the product as compared to the LUM-*khoa*. The free fat in *khoa* is due to the rupture of fat globule membrane as affected by agitation of heated milk[24]. Free fat to the extent of 82 per cent in *khoa* prepared from buffalo milk has been reported[25]. The differential status of free fat could be attributed to the status of lactose in two types of *khoa* preparations[26]. However no significant difference is noted in the initial free fatty acid (FFA) content of the two types of *khoa*.

The changes in the composition of *khoa* during storage are presented in Table 2. Acidity, proteolysis, lipolysis and peroxide value increased with time leading to the changes in the organoleptic characteristics of the product. Proteolysis (release of tyrosine) and lipolysis (release of FFA) was higher in LUM-*khoa* because of higher microbial activity as evidenced by early appearance of yeasts and moulds on LUM-*khoa* (3rd or 4th day of storage) than the LHM-*khoa*. The higher peroxide value in case of LHM-*khoa* may be due to higher HMF content which is produced at a faster rate. The rate of development of lactic acid in the two types of *khoa* was almost similar during storage. The observations with respect to acidity, FFA and peroxide value of LUM-*khoa* are in close agreement with the observations of Rudreshappa and De[27].

Mould growth was observed on the surface of LUM-*khoa* on the 3rd or 4th day while LHM *khoa* remained sound for one month. This may be due to the presence of browning intermediates particularly the higher concentrarion of HMF in LHM-*khoa*. Other factor may be change in the water activity of the product due to the generation of extra molecules of glucose and galactose upon hydrolysis of lactose in milk.

The *khoa* prepared from LHM was appreciably sweet in taste, considerably brown in colour and had soft, smooth and fairly unifrom body and texture.

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References

1. Pyke, M., *Success in Nutrition*, John Murray Ltd., London, 1975, 35.
2. Eastman, E. F. and Walker, W. A., Effect of cow's milk on the gastrointestinal tract; A persistent dilemma for the pediatrician. *Pediatrics*, 1977, **60**, 477.
3. Holsinger, V. H. and Roberts, N. E., New Products from lactose hydrolysed milk. *Dairy Ice Cream Field*, 1976, **159**, 30.
4. Pyke, M., Milk technology in the light of new views on nutrition. *J. Soc. Dairy Technol.*, 1971, **24**, 82.
5. Desai, H. G., Chitre, A. V. and Jeejeebhoy, K. N., Lactose loading—A simple test for detecting intestinal lactose. Evaluation of different methods. *Gastroenterologia*, 1967, **108**, 177.
6. Rajorhia, G. S. and Srinivasan, M. R., Technology of *Khoa*-A review. *Indian J. Dairy Sci.*, 1979, **32**, 209.
7. Nickerson, T. A., Vujicic, I. F. and Lin, A. Y., Colorimetric estimation of lactose and its hydrolytic products. *J. Dairy Sci.*, 1976, **59**, 386.
8. Srinivasan, M. R. and Anantakrishnan, C. P., *Milk Products of India*. ICAR, New Delhi, 1964, 3.
9. *Methods for Rapid Examination of Milk*, *IS*:1479 (*Part—I*), Indian Standards Institution, New Delhi, 1960.
10. *Methods of Test for Dairy Industry*, *IS*:1479 (*Part—II*), Indian Standards Institution, New Delhi, 1961.
11. *Specifications for Hard Cheese, Processed Cheese and Processed Cheese Spread*. Indian Standards Institution, New Delhi, 1964.
12. Hall, C. W. and Hedrick, T. I., *Drying of Milk and Milk Products*, AVI Publ Co., Westport, Connecticut, 1971, 229.
13. Thomas, W. R., Harper, W. J. and Gould, I. A., Free fatty acid content of fresh milk as related to portions of milk drawn. *J. Dairy Sci.*, 1954, **37**, 830.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. L., Protein measurement with the Folin-phenol reagent *J. biol. Chem.*, 1951, **193**, 265.
15. *Official Methods of Analysis*, Association of Official Agricultural Chemists, Washington, 1970.
16. Keeney, M. and Bassette, R., Detection of intermediate compounds in the early stages of browning reaction in milk products. *J. Dairy Sci.*, 1959, **42**, 945.
17. Snedecor, G. W. and Cochran, W. G., *Statistical Methods*, Oxford and IBH Publishing Co., New Delhi, 1968, 59.
18. Grimblby, F. H., Heat induced acidity in milk., *J. Dairy Res.*, 1954, **21**, 207.
19. Pyne, G. T. and McHenry, K. A., The heat coagulation of milk. *J. Dairy Res.*, 1955, **22**, 60.

20. Pyne, G. T. Some new factors in the heat coagulation of milk. *XIII Int. Dairy Proc.*, 1953, **3**, 1032.
21. Smeets, W. T. G. M. and Seekles, L., Determination of the content of calcium ions in milk ultrafiltrate. *Nature, Lond.*, 1952, **169**, 802.
22. Lewis, V. M. and Lea, C. H., A note on the relative rates of reaction of several reducing sugars and sugar derivatives with casein. *Biochem. Biophys. Acta*, 1950, **4**, 532.
23. Reynolds, T. M., Non enzymatic browning. *Adv. Fd Res.*, 1965, **14**, 219.
24. Choi, R. P., Tatler, C. W. and Malley, C. M. Lactose crystallization in dry products of milk. II. The effect of moisture and alcohol. *J. Dairy Sci.*, 1951, **31**, 850.
25. Boghra, V.R. and Rajorhia, G.S., Utilization of pre-concentrated milk for khoa making. *Aust. J. Dairy Res.*, 1982, **1**, 6.
26. Reinke, E., Brunner, J. R. and Trout, G. M., Effect of variation in processing on free-fat, self dispersion and flavour of whole milk powder. *Milk Prod. J.*, 1960, **51**, 6, 24.
27. Rudreshappa, K. G. and De, S., Studies on preservation of khoa. *J. Fd Sci. Technol.*, 1971, **8**, 50.

Preparation of a Yoghurt-like Product from Soybeans*

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Yoghurt-like fermented product was prepared from soymilk using *Streptococcus thermophilus* and *Lactobacillus acidophilus* after fortification with 1 per cent sucrose. A mixture of the two cultures produced more acid (0.7 per cent) after 24 hr than any one, of the cultures individually. The pH was found to be 4.3. Sensory evaluation revealed absence of beany flavour and the products had desirable body and texture. Incorporation of 10–20 per cent skim milk enhanced acid production and flavour, without impairing the body of the product while a higher level weakened the body.

Traditionally lactic acid bacteria are used to prepare fermented milk products. Soymilk fermentation utilizing lactic cultures have been carried out by many workers[1-5]. Hang and Jackson[6] prepared a cheese-like product from soybeans using *S. thermophilus*. Yamanaka *et al*[7] prepared a sour milk beverage from a mixture of soy protein, cows milk, sucrose and amino acids using *S. thermophilus* and *L. bulgaricus*. Wang *et al*[8]. prepared (refershing sweet sour taste) a fermented soy milk drink using *L. acidophilus*. Mital and Steinkraus[9] prepared soya fermented product with acceptable flavonr and yoghurt-like texture but with low acidity.

The major fermentable carbohydrates in soybeans are sucrose (5.0 per cent), raffinose (1.1 per cent) and stachyose (3.8 per cent)[10], but in soy milk the concentration of these carbohydrates is less than 1 per cent and nearly half of which is not utilized by lactic acid bacteria[11]. Certain lactobacilli possessing alpha galactosidase are able to utilize these carbohydrates. Thus approximately 0.5 per cent of carbohydrates are available in soy milk for fermentation as compared to about 5 per cent in cow or in buffalo milk which may explain for low acid production. Therefore, supplementation of soy milk with a suitable carbohydrate like sucrose ensures[5,12] sufficient acid production.

Supplementation of sucrose and skim milk to soy milk on acid production and acceptability of the fermented product were investigated.

Materials and Methods

Cultures: Lactobacillus acidophilus (NRRL-B-629) obtained from Northern Regional Research Laboratory, Peoria, Illinois (U.S.A.) and *Streptococcus thermophilus* obtained from National Dairy Research Institute, Karnal (India) were used in this study. The cultures were grown in sterile soy milk at 37°C and were maintained at 5°C between monthly transfers.

Preparation of soy milk: Dry, whole soybeans (Bragg variety) obtained from Crop Research Centre of the University were cleaned, washed and soaked in tap water over night in three times the weight (w/v). After decanting the soak water, the beans were washed again with fresh tap water. The wet soybeans were then

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TABLE 1. PROXIMATE COMPOSITION OF SOY MILK[1]

Constituent	Per cent
Moisture	91.00
Protein	3.10
Fat	2.00
Ash	0.43
Carbohydrates[2] (by diff.)	3.47

[1]Average of duplicate experiments [2]Contains 1% added sucrose w/v

ground with nine times the weight of hot water (89-90°C) in a colloid mill. Boiling water inactivates lipoxigenase during grinding[13]. The ground material was then filtered using double layered cheese cloth. Soy milk so obtained was supplemented with 1 per cent sucrose (w/v). Two hundred millilitres of soy milk dispensed in 500 ml conical flask was sterilized at 121°C for 15 min. Table 1 shows the proximate composition of soy milk.

Fresh skim milk obtained from Live Stock Research Centre of the University was added to soy milk at 10, 20 and 30 per cent level and sterilized at 121°C for 15 min.

Protein was determined by Kjeldahl method[14]. Fat moisture and ash were determined according to AOAC procedures[13].

Fortified Soy milk was manufactured at 37°C inoculated with 16-18 hr culture at 1 per cent level. The mixed culture consisted of 0.5 per cent of each and samples were withdrawn at specified intervals and analysed for growth, acid production and pH.

Lactic agar[15] plates, in duplicate were used to determine viable counts, after incubating at 37°C for 24-48 hr. Ten gram samples were titrated against 0.1 N NaOH using phenolphthalein as the indicator and acidity calculated.

Sensory evaluation: Fermented Yoghurt-like products were evaluated for their body, texture and flavour. A taste panel consisting of 6 members rated the samples on Hedonic scale[16] from 1 to 9 where 1 and 9 represented dislike extremely and like extremely, respectively.

Results and Discussion

As seen from Table 2, *S. thermophilus* was more (3.9×10^8 ml) in soy milk than in soy milk-skim milk combinations (9.8×10^7-1.80×10^8/ml). Besides this, the time taken to attain higher population was less in soy milk than in the other samples. In contrast, *L. acidophilus* attained greater numbers in soy milk-skim milk combinations (7.8×10^8/ml) than in soy milk (3.7×10^8/ml) (Table 3). The mixed cultures of *S. thermophilus*

TABLE 2. *S. THERMOPHILUS* (PER ML) IN SOY MILK AND SOY MILK-SKIM MILK COMBINATIONS[1]

Time (hr)	Soy milk	Soy milk + 10% skim milk	Soy milk + 20% skim milk	Soy milk + 30% skim milk
0	1.0×10^3	2.0×10^2	5.6×10^2	5.5×10^2
4	7.0×10^7	7.4×10^7	3.6×10^2	7.5×10^7
8	3.9×10^8	8.9×10^7	8.1×10^7	1.8×10^8
12	2.6×10^8	1.0×10^8	9.8×10^7	1.4×10^8
16	1.8×10^8	1.4×10^8	6.0×10^7	1.3×10^8

[1]Average of duplicate experiments

TABLE 3. *L. ACIDOPHILUS* (PER ML) IN SOY MILK AND SOY MILK-SKIM MILK COMBINATIONS[1]

Time (hr)	Soy milk	Soy milk + 10% skim milk	Soy milk + 20% skim milk	Soy milk + 30% skim milk
0	1.5×10^3	7.2×10^2	1.1×10^3	1.0×10^3
4	4.5×10^7	3.6×10^7	1.0×10^7	1.8×10^3
8	2.2×10^8	6.1×10^8	2.9×10^8	5.3×10^7
12	3.7×10^8	6.2×10^8	4.3×10^8	7.4×10^8
16	2.6×10^8	2.9×10^8	2.9×10^8	7.8×10^8

[1]Average of duplicate experiments

and *L. acidophilus* exhibited growth pattern similar to that of *S. thermophilus*. It indicates that *S. thermophilus* grew faster than *L. acidophilus*. The mixed culture attained higher numbers (2.9×10^8/ml) in soy milk than in soy milk-skim milk combinations (1.8×10^8-2.1×10^8/ml) (Table 4).

S. thermophilus and *L. acidophilus* ferment sucrose[17]. There are reports[6,7,18,19] to indicate that *S. thermophilus* produces more acid and *L. acidophilus* produce sufficient acid in soy milk.

TABLE 4. GROWTH OF MIXED CULTURE OF *S. THERMOPHILUS* AND *L. ACIDOPHILUS* (PER ML) IN SOY MILK AND SOY MILK-SKIM MILK COMBINATIONS

Time (hr)	Soy milk	Soy milk + 10% skim milk	Soy milk + 20% skim milk	Soy milk + 30% skim milk
0	1.3×10^3	3.0×10^2	1.0×10^3	1.1×10^3
4	5.5×10^7	6.2×10^7	9.2×10^7	9.3×10^6
8	2.8×10^8	1.1×10^8	1.1×10^8	1.8×10^8
12	2.9×10^8	1.5×10^8	2.0×10^8	1.7×10^8
16	2.8×10^8	2.1×10^8	1.0×10^8	8.8×10^7

[1]Average of duplicate experiment

TABLE 5. TITRATABLE ACIDITY (TA) AND PH OF SOYMILK AND SOYMILK-SKIM MILK COMBINATIONS FERMENTED WITH LACTIC ACID BACTERIA[1]

Organism	Soy milk		Soy milk+10% skim milk		Soy milk+20% skim milk		Soy milk+30% skim milk	
	%TA	pH	%TA	pH	%TA	pH	%TA	pH
Initial	0.15	6.3	0.13	6.4	0.12	6.4	0.13	6.40
S. thermophilus	0.58	4.5	0.63	4.4	0.69	4.3	0.70	4.20
L. acidophilus	0.63	4.5	0.68	4.5	0.76	4.3	0.81	4.20
S. thermophilus+ *L. acidophilus*	0.70	4.3	0.78	4.2	0.83	4.1	0.88	4.10

[1]Average of duplicate experiments

Per cent titratable acidity and pH were determined after 24 hr.

TABLE 6. TASTE PANEL EVALUATION OF SOY MILK FERMENTED WITH DIFFERENT CULTURES

Culture	Flavour score[1]
S. thermophilus	6.16
L. acidophilus	6.60
S. thermophilus+*L. acidophilus*	7.50

All samples had desirable body and texture

[1]The samples were rated on Hedonic scale numerical values ranging from 1 to 9 where 1 represented dislike extremely and 9 represented like extremely.

TABLE 7. TASTE PANEL EVALUATION OF SOY MILK AND SOY MILK+ SKIM MILK COMBINATIONS FEREMENTED WITH MIXED CULTURE OF *S. THERMOPHILUS* AND *L. ACIDOPHILUS*

Treatment	Flavour score[1]
Soymilk	6.63
Soymilk+10% skim milk	6.72
Soymilk+20% skim milk	7.18
Soymilk+30% skim milk	7.81

[1]The samples were rated on Hedonic scale numerical values ranging from 1 to 9 where 1 represented cislike extremely and 9 represented like extremely.

All samples showed desirable body except the 30% skim milk used sample which showed a week body.

Acid production and changes in pH brought about by *S. thermophilus*, *L acidophilus* and their combinations in soy milk are presented in Table 5. Mixed culture of *S. thermophilus* and *L. acidophilus* produced greater amount of acid in soy milk than by either of the culture. Addition of skim milk to soy milk further enhanced acid production. Yamanaka and Furukawa[20] have also reported that addition of skim milk to soy milk upto 30 per cent enhanced acid production by lactic cultures. The changes in pH were in conformity with acid production by the culture.

The results of sensory evaluation of fermented products are presented in Table 6. Soy milk fermented with mixed culture of *S. thermophilus* and *L. acidophilus* (acidity 0.70 per cent, pH 4.3) was rated superior to the soy milk fermented either with *S. thermophilus* (acidity 0.58 per cent, pH 4.5) or *L. acidophilus* (acidity 0.63 per cent, pH 4.5). Greater acid production has been recorded as the main criterion for higher rating of soy milk. All the three samples were found to be devoid of beany flavour and exhibited clean, slightly acidic taste and desirable body and texture.

Though addition of skim milk to soy milk enhanced acceptability of the product, it was found that incorporation of skim milk more than 20.0 per cent weakened the body of the product (Table 7).

Thus an acceptable yoghurt-like product can be prepared by fermenting 1 per cent sucrose fortified soy milk with *S. thermophilus* and *L. acidophilus* or their combination. Incorporation of skim milk at 20 per cent in soy milk enhanced acceptability of the product.

References

1. Kellogg, H., Method of making acidophilus milk, *U. S. Patent*, 1934-1982941.
2. Angeles, A. G. and Marth, E. H., Growth and activity of lactic acid bacteria in soy milk, I. Growth and acid Production. *J. Milk Fd Technol.*, 1971, **34**, 30.
3. Gehrke, C. and Weiser, H. H., A Comparative study of the biochemical activity of *Streptococcus lactis*, *Streptococcus citrovorus*, and *Streptococcus paracitrovorus* when grown in cow's milk and soybean milk. *J. Dairy Sci.*, 1948, **32**, 213.
4. Mital, B. K., Shallenberger, R. S. and Steinkraus, K. N., Alpha-galactosidase activity of *Lactobacilli. Appl. Microbiol*, 1973, **26**, 787.
5. Mital B. K., Steinkraus, K. H. and Naylor N. B., Growth of lactic acid bacteria in soy milks. *J. Fd Sci.*, 1974, **39**, 1018.
6. Hang, Y. D. and Jackson, M., Preparation of soybean cheese using lactic starter organisms. II. Effects of addition of rennet extract and skim milk. *Fd Technol.*, 1967, **21**, 97.
7. Yamanaka, Y., Okamura, O. and Hasegawa, Y., Method of preparing sour milk beverage. U. S. Patent, 1970–3535177.

8. Wang, H. L., Kraidej, L. and Hesseltine, C. W., Lactic fermentation of soybean milk. *J. Milk Fd Technol.*, 1974, **37**, 71.
9. Mital, B. K. and Steinkraus, K. H., Flavour acceptability of unfermented and lactic fermented soy milk. *J. Milk Fd Technol.*, 1976, **39**, 342.
10. Kawamura, S., Review of PL-480 Work on soybean carbohydrates, *Proceedings of International Conference on Soybean Protein Foods*, Peoria, Illinois, ARS 71-35, U.S. Dept. Agr., May 1967, 249.
11. Mital, B. K. and Steinkraus, K. H., Utilization of oligosaccharides by lactic acid bacteria during fermentation of soy milk. *J. Fd Sci.*, 1975, **40**, 114.
12. Mital, B. K., Singh, R. and Singh, S., Effect of carbohydrates and phosphates on acid production by lactic acid bacteria in soy milk. *J. Fd Sci. Technol. India.*, 1977, **14**, 182.
13. Wilkens, W. F., Mattick, L. R. and Hand, D. B., Effects of Processing methods on oxidative flavours of soybean milk. *Fd Technol.*, 1967, **21**, 86.
14. *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, D. C., 11th Edn., 1970.
15. Elliker, P. R., Anderson, A. W. and Hannesson, G., An agar culture medium for lactic acid *Streptococci* and *Lactobacilli*. *J. Dairy Sci.*, 1956, **39**, 1611.
16. Larmond, E., *Laboratory Methods for Sensory Evaluation of Food*, Publ. No. 1637, Research Branch, Canada Dept. of Agric., Ottawa, Canada, 1977.
17. Buchanan, R. E. and Gibbons, N. E., *Bergey's Manual of Determinative Bacteriology*, The Williams and Wilkins Co., Baltimore, 8th Edn., 1974.
18. Kim, C. S. and Shin, S. H., Studies on preparation of a cheese-like product from soybean milk. *Korean J. Sci. Technol.*, 1971, **3**, 57.
19. Matsuoka, H., Sasago, K. and Sekiguchi, M., Manufacturing a cheese- like product from soybean milk. *J. Fd Sci., Technol., Tokyo*, 1968, **15**, 103.
20. Yamanaka, Y. and Furukawa, N., Studies on utilization of soybean protein for food manufacturing II. Influence of soy milk added to skim milk on the acidity and hardness of curd produced by lactic bacteria for dairy use. *J. Fd Sci. Technol., Tokyo*, 1970, **17**, 456.

Effect of *Khoa* Preparation on Cell Population of *Staphylococcus aureus*

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The effect of heat treatment on the cell populations of *Staphylococcus aureus* during preparation of *Khoa* at 98°C for 15-20 min was studied. A cell population of 1×10^6 (colony forming units) CFU/ml in milk of *S. aureus* reached a level of 10^8-10^9 CFU/ml in 6 hr of incubation at 37°C. *Khoa* prepared out of such a milk showed staphylococcal counts of 2×10^2 to 1×10^4 CFU/g in 4 samples wherein, the staphylococcal colonies were smaller in diameter (1 mm) on Baird-Parker medium even after 72 hr of incubation at 37°C indicating the probable recovery from heat injury. Colonies of K-283 and K-213, the two wild strains of *S. aureus* from market samples *O. Khoa* showed a curled margin as against smooth margin of normal colonies. As the level of initial inoculum of test strain was lowered from 1×10^6 to 1×10^3 CFU/ml of milk, there was gradual decrease in the staphylococcal counts of fresh *Khoa* samples, further with the lower level of initial inoculum, staphylococcal colonies were absent at 10^{-1} dilution. *Staphylococci* which were heat injured during *Khoa* preparation did recover and grow in *Khoa* stored at room temperature (25-35°C) for a week as well as, when the samples were enriched with brain heart infusion broth for 24 hr at 37°C.

Khoa is a heat concentrated (98°C for 15-20 min) Indian milk product having a moisture level of 26-28 per cent and used as a base in the preparation of several Indian milk sweets. A high incidence of *Staphylococci* in market *Khoa*[1-3] indicates the unhygienic conditions prevailing during the manufacture and handling of *Khoa*. Often, low grade raw milk containing a high level of such organism constitute the raw materials for *Khoa* manufacture.

Being thermostable, staphylococcal enterotoxins preformed in the raw materials are easily carried over to the final products without much loss in their activity. Though *Staphylococci* have been known to be killed at near pasteurisation temperatures[4-9], the ability of sta-

phylococcal cells to survive the heat treatment encountered in Khoa preparation has been examined.,

Materials and Methods

Staphylococcal cultures: The staphylococcal test, cultures used were 4 wild strains of *S. aureus* (K-283, K-192, K-213 and K-208) isolated from market *Khoa* samples producing enterotoxin(s) namely, A(SEA), E(SEE), BE(SEB and E) and CE(SEC and E), respectively and two standard strains of *S. aureus* (A_{100} and B_{S-6}) producing SEA and SEB, obtained from Dr. M. S. Bergdoll, Food Research Institute, Madison, Wisconsin.

Preparation of Khoa: One litre aliquot of milk sample was brought to boil in an open pan over a low fire with continuous stirring and scraping till the viscous produce reached a pasty consistency and began to dry up. The pan was removed off the fire and after scrapping for a few minutes, cooled to room temperature and stored in previously sterilised glass stoppered bottles avoiding any contamination.

Model experiment: A suspension of 1×10^6 colony forming units (CFU) of test strain per ml of milk in 1 ml of saline was inoculated into 1 l aliquots of raw cow's milk and incubated for 6 hr at 37°C. *Khoa* was prepared from such a sample and stored for one week at room temperature (RT) (25-35°C) and refrigeration temperature (RFGT) (4-5°C).

In another trial, a wild strain of *S. aureus* K-283 was inoculated at three levels viz., 1×10^5, 1×10^4 and 1×10^3 CFU/ml of milk into 1 l of raw cow's milk and then *Khoa* was prepared.

Enrichment: With a view to activate any heat injured staphylococcal cells that might be present, 1 g sample of fresh and stored *Khoa* were enriched with 99 ml of 3.7 per cent Difco brain heart infusion (BHI) broth and incubated at 37°C for 24 hr.

Enumeration of Staphylococci: Samples of raw and incubated milk, fresh, stored and enriched *Khoa* were analysed for staphylococcal counts. Appropriate dilutions of the samples were surface plated on egg yolk-tellurite-glycine-pyruvate agar (ETGPA) of Baird-Parker[10] and the plates were incubated at 37°C for 24 to 72 hr. Black colonies of *S. aureus* with a clearing zone of surrounding egg yolk formed on the incubated agar plates were counted and expressed as staphylococcal colony forming units per ml/g.

Results and Discussion

Staphylococcal populations in milk and Khoa: The results presented in Table 1 show that the test strains grew well in raw milk during the incubation period of 6 hr at 37°C, reaching 6×10^7 to 6×10^9 CFU/ml from an intitial 1×10^6 CFU/ml. When these milk samples were converted into *Khoa* it was observed that in 4 fresh samples of *Khoa* (strains 'K-283, 'K-213', A_{100} and B_{S-6}), staphylococccal counts ranging from 2×10^2 to 1×10^4 CFU/g were recorded. The colonies of *Staphylococci* were smaller (1 mm diameter) on ETGPA medium even after 72 hr of incubation at 37°C, indicating that the organism survived processing but could not grow as well as they did in raw milk. Staphylococcal colonies on ETGPA plates from *Khoa* samples having wild strains K-283 and K-213, possessed curled margin as against the smooth margin of the colonies formed by normal cells.

It was surprising to observe staphylococcal counts

TABLE 1. STAPHYLOCOCCAL POPULATIONS IN MILK AND *KHOA*

Type of sample	Incubation/storage		Staphylococcal counts (CFU/ml/g)					
	Period	Temp. (°C)	K-283	K-192	K-213	K-208	A_{100}	B_{S-6}
Milk*	6 hr	37	6×10^9	4×10^9	3×10^9	4×10^9	6×10^7	6×10^8
Khoa	Fresh	—	2×10^2	$<10^1$	2×10^2	$<10^1$	1×10^4	2×10^2
Khoa	7 days	25-35	1×10^4	5×10^5	4×10^5	$<10^1$	$<10^1$	$<10^1$
Khoa	7 days	4-5	1×10^4	$<10^2$	$<10^1$	$<10^1$	$<10^1$	$<10^1$
Khoa in enriched media	Fresh	—	3×10^5	2×10^6	1×10^5	$<10^2$	6×10^6	1×10^4
RT stored *Khoa* in enriched media	24 hr	37	1×10^6	1.6×10^5	8×10^5	2×10^4	1×10^6	1×10^5
RFGT stored *Khoa* in enriched media	24 hr	37	2×10^7	1.2×10^5	$<10^2$	$<10^1$	$<10^1$	$<10^1$

*Test cultures inoculated at a level of 1×10^6 CFU/ml
Raw cow milk samples showed the absence of *Staphylococci* in 10^{-1} dilution.

in a few fresh *Khoa* samples, which were supposed to have been killed during heating at/or near pasteurisation temperatures[4-9]. The chances of staphylococcal cells surviving the heat treatment encountered during *Khoa* preparation are very less. The counts observed in fresh samples of *Khoa* may be attributed to the phenomenon of heat injury of staphylococcal cells and their subsequent recovery in suitable environments like medium consisting of enriched source of nutrients.

It may be observed from Table 1 that during the storage of *Khoa* at RT for 1 week as well as, when samples were enriched by mixing the samples in BHI broth and subsequently incubated, slight increase in the counts occurred in a few samples, while in 2 other samples *Staphylococci* appeared where they were initially absent. Here the injured cells would have recovered either during storage or on enrichment in BHI broth. The rate of recovery depends upon the extent of injury caused to the cells and the ability of these injured cells to make use of the nutrients available so as to revive their lost metabolic functions. Certain essential nutritents are required for the complete recovery of cells from heat injury. According to Iandolo and Ordal[8], the requirements for reactivation of heat stressed cells are an energy source (glucose), a mixture of amino acids and a phosphate. The ETGPA plating medium[10] used in this study is quite suitable for the recovery of injured cells, as it contains not only the above requirements, but also a rapidly metabolizable energy source like pyruvate which has been shown to enhance the recovery of heat stressed cells[11]. The staphylococcal counts observed in stored/enriched *Khoa* samples may represent just the recovered cells from heat injury or growth of these recovered cells. The differences observed in the case of revived test strains may be attributed to the variations in the extent of heat injury.

It was reported by Hansen and Riemann[12] that thermal resistance of bacteria is influenced by composition of culture medium in which the organisms are grown before heating, density of suspension, the menstruum in which the organisms are heated and the composition of recovery medium. Walker and Harmon[13] in their heat treatment studies on *S. aureus* observed that although the order of death was logarithmic until about 99.9 per cent of the cells were inactivated, there was a decrease in the rate of inactivation, suggesting that the surviving cells were more heat resistant. Similar observations have also been made in relation to spore forming bacteria[14].

In the present study, the initial populations of *Staphylococci* in milk before conversion into *Khoa* were very high in the range of 10^8-10^{10} CFU/ml. It is possible that out of such a large number of cells, a majority of them would have undergone injury. It may be seen from Table 2 that with a decrease in the initial population of *Staphylococci* in milk obtained by inoculating milk initially with lesser numbers, there was the gradual reduction in the staphylococcal counts of fresh *Khoa* samples. Staphylococcal counts of 1.1×10^2 and 4×10^1 CFU/g were recorded in fresh *Khoa* samples prepared out of milk inoculated at a level of 1×10^5 and 1×10^4 CFU/ml of milk, while no staphylococcal colonies were formed in 10^{-1} dilution of *Khoa* sample prepared from milk inoculated with 1×10^3 CFU/ml.

The method of *Khoa* making in open pan by a continuous stirring of milk results in alternate heating and cooling. Although during preparation the temperature may go as high as 98°C, the actual thermal effect is much less. Actual measurement made during *Khoa*

TABLE 2. STAPHYLOCOCCAL POPULATIONS IN MILK AND KHOA USING THREE LEVELS OF INOCULUM OF *S. AUREUS* K-283 IN MILK

Type of sample	Incubation/Storage		Staphylococcal counts (CFU/ml/g) at the initial inoculum level of		
	Period	Temp (°C)	(1×10^5)	(1×10^4)	(1×10^3)
Milk	6 hr	37	6×10^8	4×10^7	6×10^6
Khoa	Fresh	—	1.1×10^2	4×10^1	$\times 10^1$
Khoa	7 days	25-35	4×10^4	4×10^3	4×10^2
Khoa	7 days	4-5	1×10^2	$< 10^1$	$< 10^1$
Khoa in enriched media	Fresh	—	1×10^3	1×10^2	$< 10^1$
Stored *Khoa* in enriched media	24 hr	37	1×10^6	2×10^4	6×10^3
REGT stored *Khoa* in enriched media	24 hr	37	2×10^5	1×10^4	$< 10^1$

making showed that the temperatures at various stages ranged from 62 to 98°C. In the final stages as the product becomes viscous tending towards a pasty consistency, the temperature gradually decreases. On this basis, it is quite clear that the actual thermal effect on *Staphylococci* is much less than that of the heat treatment of 98°C for 15-20 min. It may be analogous to what happens during milk spray, even-though hot air at temperatures in the range of 150-170°C is used for the removal of water from the concentrate the thermoduric organisms survive because of the short period to which the cells are exposed to the heat.

The role of coagulated proteins of *Khoa* acting as insulators of heat and providing protection for the staphylococcal cells against the heat treatment cannot be ruled out here. In cheese preparation, during cooking it was observed by Takahashi and Johns[15] that the staphylococcal cells get embedded in between the curd particles.

On the basis of the above points, it would be appropriate to attribute the staphylococcal counts observed in samples of fresh, stored and enriched *Khoa* either to the recovery of heat injured cells or survival of few staphylococcal cells to the heat treatment encountered during *Khoa* preparation.

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References

1. Kudchodkar, B. G. and Singh, I. P., Incidence of enterotoxigenic type *Staphylococci* in indigenous milk products. *Indian J. Dairy Sci.*, 1964, **17**, 144.
2. Ghodekar, D. R., Dudani, A. T. and Ranganathan, B., Microbiological quality of Indian milk products. *J. Milk Fd Technol.*, 1974, **37**, 119.
3. Varadaraj, M. C. and Nambudripad, V. K. N., Staphylococcal incidence in market *Khoa* and their entertoxins production. *J. Fd Sci. Technol.*, 1982, **19**, 53.
4. Jackson, H. and Woodbine, M., The effect of sublethal heat treatment on the growth of *S. aureus*. *J. appl. Bacteriol.*, 1963, **26**, 152.
5. Zottola, E. A. and Jezeski, J. J., Effect of heat on *Staphylococcus aureus* in milk and cheese during commercial manufacture. *J. Dairy Sci.*, 1963, **46**, 600.
6. Bhatt, U. A. and Bennett, R. W., Thermal death time studies of *Staphylococci* in milk. *J. Dairy Sci.*, 1964, **47**, 666.
7. Stiles, M. E. and Witter, L. D., Thermal inactivation, heat injury and recovery of *Staphylococcus aureus*. *J. Dairy Sci.*, 1965, **48**, 677.
8. Iandolo, J. J. and Ordal, Z. J., Repair of thermal injury of *Staphylococcus aureus*. *J. Bacteriol.*, 1966, **91**, 134.
9. Zottola, E. A., Jezeski, J. J. and Al-Dulaimi, A. N., Effect of short time sub-pasteurisztion treatments on the destruction of *Staphylococcus aureus* in milk for cheese manufacture. *J. Dairy Sci.*, 1969, **52**, 1707.
10. Baird-Parker, A. C., An improved diagnostic and selective medium for isolating coagulase positive *Staphylococci J. appl. Bacteriol.*, 1962, **25**, 12.
11. Baird-Parker, A. C. and Davenport, E., The effect of recovery medium on the isolation of *Staphylococcus aureus* after heat treatment and after the storage of frozen of dried cells. *J. appl. Bacteriol.*, 1965, **28**, 390.
12. Hansen, N. H. and Riemann, H., Factors affecting the heat resistance of non-sporing organisms. *J. appl. Bacteriol.*, 1963, **26**, 314.
13. Walker, G. C. and Harmon, L. G., Thermal resistance of *Staphylococcus aureus* in milk, whey and phosphate buffer. *Appl. Microbiol.*, 1966, **14**, 584.
14. Galesloot, E., *Milk Hygiene*, World Health Organisation, Monograph Series No. 48, WHO Geneva, 1962, 269.
15. Takahashi, I. and Johns, C. K., *Staphylococcus aureus* in Cheddar cheese. *J. Dairy Sci.*, 1959, **42**, 1032.

Tamar Eddin — A New Product of Date

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Tamar Eddin, a dried date product resembling *Kamar Eddin* made from apricot juice and highly popular during the *Ramadan* festival has been made from the 'Rezaiz' variety of date which is abundantly grown in Saudi Arabia. The effect of citric acid, ascorbic acid, can syrup and sulphur dioxide on the quality of the product has been studied. Addition of about one per cent of citric acid and a moderate amount of sulphur dioxide to the homogenate gave a dried product having good texture, taste and colour. Addition of can syrup resulted in an elastic product. Data have been analysed statistically.

Production of dates is the highest among the agricultural crops in Saudi Arabia[1]. They are very popular in the fresh as well as dried form. They are usually sun-dried in the open with the result that the product suffers in quality when compared with imported fresh, canned, frozen and dried fruits. A large proportion of them is either wasted or used as animal feed. Consumption of imported fruits and vegetable in Saudi Arabia have steadily increased[2] The 'Rezaiz' variety is nearly sixty per cent of the total 'Al-Hassa' date production[3].

An attempt has, therefore, been made to develop from these dates, a dried product in the form of sheets, similar to *Kamar Eddin* which is made from apricot juice and is highly popular for consumption during the fasting month of *Ramadan* in the Islamic World.

Materials and Methods

'Reazaiz' variety of dates, pressed and packed in polythene bags was procured from the local market and kept at 5°C in a refrigerator for subsequent use. Can syrup of 73 per cent total solids was purchased locally. (Tamar) dates were depitted manually and the edible portion homogenized with water in the ratio of 5:7 for 2 min at high speed in an Osterizer blender. The homogenate was filtered through cheese cloth and divided into 12 aliquots to study the effect of different additives on the dehydrated product.

The product was spread (5.98 kg homogenate/sqm.) evenly on stainless oiled trays (65 g olive oil/sqm.) and dehydrated in an air-forced oven at 65°C until sheets of Tamar Eddin were formed. The final moisture content was approximately 18 per cent. Details regarding the treatments are given in the flow sheet Table 1 and Fig. 1).

Methods of analyses: Moisture was determined by drying 8-15 g of the homogenised sample in an aluminium dish in a vacuum oven at 65°C. Total sugar, fibre and ash contents were determined according to A.O.A.C. methods[4]. Colour was determined by spectrophotometry. For this, a 10 per cent solution of *Tamar Eddin* was prepared in warm water and allowed to settle. The clear solution was centrifuged and filtered to obtain an optically clear yellow solution and its transmission was measured at 430 and 700 nm. Optical density was calculated according to the equation given by Ray Junk *et al*[5]. Acidity of the product was determined as citric acid, by potentiometric titration to a pH of 8.3 using 0.1N sodium hydroxide solution. Sensory evaluation of the product was carried out by assessment of the

TABLE 1. TREATMENT SCHEDULE FOR THE FILTRATE

Treatment	Citric acid (g/kg homogenate)	Ascorbic acid (g/kg homogenate)	SO_2 (ppm)	Corn syrup (g/kg homogenate)
1.	—	—	—	—
2.	10	—	—	—
3.	—	5	—	—
4.	—	—	3350	—
5.	5	—	4020	—
6.	2.5	—	2345	—
7.	0.87	—	335	—
8.	0.87	—	167	—
9.	0.87	—	84	—
10.	0.82	—	1220	220
11.	0.82	—	240	110
12.	1.8	—	360	110

The concentration of additives are in g/kg of homogenate.

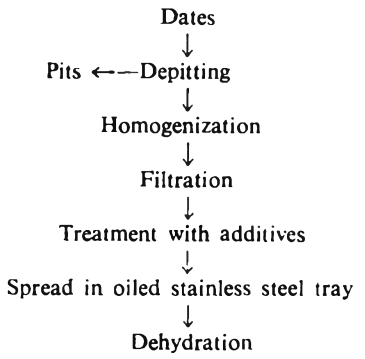

Fig. 1. Scheme for processing dried sheet of *Tamar* (*Tamar Eddin*)

taste panel of 7 faculty members of King Faisal University, Hofuf. The members were just familiarised with the product. A square (8 cm×8 cm) piece of the dried product was served to each member for evaluation on the basis of 40 points for taste, 40 points for colour and 20 points for texture (total 100 points)[6].

The data were analysed for least significant difference at 5 per cent probability by the method of Snedecor and Cochran[8].

Residual SO_2 content was determiued by the Monier-Williams procedure as described by Pearson[7].

Results and Discussion

Moisture content of the product varied from 16.52 to 19.42 per cent total solids from 80.62 to 83.52 per cent (Table 2). Total sugars varied from 74.3 to 78.2 per cent which is higher than in *Kamar Eddin* (59.2 to 64.92 per cent). Moisture content also was slightly more. *Tamar Eddin* without additives (control) had an aicdity of 0.42 per cent as citric acid and was sweet in taste. Sample with the highest acidity, namely 2.63 per cent was judged by the panel members to have a taste similar to that of *Kamar Eddin*. Thus degree of acidity is close to the minimum found in *Kamar Eddin* whose acidity ranged from 2.19 to 6.63 per cent, the product with the higher acidity having a strong acid taste. The ash content of the control sample of *Tamar Eddin* was 1.94 per cent whereas that of *Kamar Eddin* varied widely from 1.57 to 4.60 per cent. The ash content of *Tamar Eddin* to which inorganic salts had been added, ranged from 3.26 to 3.60 per cent. The higher ash content in the *Kamar Edddin* might be due to a high sulphate content and imperfect washing of the fruit used.

Effect of added SO_2 on the optical density of the product: The optical density of the water extract of the untreated product was quite high (0.507) in comparison with that of the treated products (Table 3). Addition of ascorbic acid reduced the optical density to 0.430. Addition of citric acid improved the colour to a slightly lesser degree (OD. 0.460)

TABLE 2. EFFECT OF ADDITIVES TO THE DATE HOMOGENATE ON THE COMPOSITION OF *TAMAR EDDIN*

Treatment‡	Moisture (%)	Total solids (%)	Total sugar (%)	Titratable acidity (as % citric acid)	Ash (%)	Fiber (%)
			Tamar Eddin			
1	18.7	81.3	76.1	0.42	1.94	1.09
2	17.2	82.8	74.7	2.63	1.68	1.01
3	16.5	83.5	76.1	0.87	1.84	1.00
4	18.5	81.5	75.8	0.64	3.36	1.01
5	19.4	80.6	74.3	1.18	3.60	0.90
6	18.7	81.3	75.4	0.69	3.26	1.01
7	17.5	82.5	76.2	0.67	2.00	0.95
8	17.0	83.0	76.3	0.77	1.90	1.05
9	17.2	82.8	76.4	0.63	1.88	0.96
10	16.5	83.5	74.4	0.42	1.93	0.96
11	17.1	82.9	77.8	0.43	1.89	0.94
12	17.2	82.8	78.2	0.52	1.84	1.02
LSD	0.82	0.85	1.03	0.52	0.61	0.047
CV	5.18	1.10	1.52	7.10	29.60	0.050
			Kamar Eddin			
1	15.50	84.5	59.2	6.63	4.60	3.90
2	17.50	82.8	64.9	2.19	1.57	0.92
LSD*	0.90	0.90	4.57	1.29	0.76	2.26
C.V.	5.76	1.22	6.89	11.96	37.40	6.3

*Total LSD and C.V. for both *Tamar Eddin* Products and *Kamar Eddin* samples.

‡See Table 1 for treatment details.

Higher levels of additions of SO_2 (3354-4020 ppm) resulted in an excellent colour to the product, but the taste was not acceptable. Additions at lower levels of SO_2 (220-335 ppm) gave acceptable colour (O.D. 0.373-0.332) and the improvement was better than in the case of ascorbic acid (O.D. 0.430) (Table 3). The O.D. method of colour determination of the product corresponded closely with taste panel evaluation. SO_2 has long been used to retain colour in dried fruits[9] and it is the most effective agent, retarding non-enzymatic browning during the drying process[10].

The loss of SO_2 in samples with higher doses of SO_2 ranged from 1095 to 3022 ppm in the pH range of 4.12 to 5.50. According to McWeeny[11] loss in SO_2 in food with greater than pH 4 is due to chemical changes rather than to physical changes, while in the case of less than

TABLE 3. LEVELS OF ADDED SO_2 IN HOMOGENATE AND THE CORRESPONDING OPTICAL DENSITY OF THE PRODUCT EXTRACT

Treatments*	Added SO_2 (ppm)	Calculated O.D.‡
1	—	0.507
2	—	0.460
3	—	0.430
4	3350	0.259
5	4020	0.201
6	2345	0.219
7	335	0.332
8	167	0.382
9	84	0.401
10	220	0.373
11	244	0.395
12	362	0.392

‡Optical density was calculated according to the following equation:

$$O.D.=\frac{\text{Log \% T at 700 nm—Log \% T at 430 nm}}{\text{2 cm (cell legnth)}}$$

*See Table 1 for treatment details

pH 4, it may still be predominantly due to chemical changes. The mechanism of SO_2 loss is however being examined in greater detail.

Effect of different treatments on organoleptic quality: Average scores and calculated percentage of acceptability for colour, taste and texture of the product got from different treatments are given in Table 4.

Colour: The product made using ascorbic acid as additive scored 32 points and was acceptable to 87 per cent of the members. Addition of larger doses of SO_2 gave a lighter coloured product (36-39 points) and was found acceptable by 87-100 per cent of the panel members. A smaller addition of SO_2 in addition to citric acid, gave a darker, coloured product although the taste was better as judged by the panel (43-71 per cent).

Taste: Addition of citric acid imparted an acid taste that did not resemble that of date but was practically close to that of dried apricot juice (*Kamr Eddin*). Addition of SO_2 imparted an undesirable SO_2 taste to the product. Addition of a small amount of citric acid however, enhanced the date flavour of the product (71-83 per cent acceptance).

Texture: Addition of citric acid or ascorbic acid did not affect texture of the product to any noticeable extent. Addition of SO_2 had a small favourable effect on this texture. With less addition of SO_2 as well as of citric acid resulted in a sticky texture (Acceptability 43-57

TABLE 4. AVERAGE SCORING AND CALCULATED PERCENTAGE OF ACCEPTABILITY OF COLOUR, TASTE AND TEXTURE OF *TAMAR EDDIN* TREATMENTS FOUND BY PANEL MEMBERS

Treatment	Colour		Taste		Texture		Total score	Remarks
	Score	Acceptability (%)	Score	Acceptability (%)	Score	Acceptability (%)		
1	26	57	30	71	17	71	73	Date taste
2	32	71	30	71	17	83	79	Slightly acid
3	32	87	32	83	16	71	80	
4	39	87	28	45	18	71	85	Taste of SO_2
5	38	100	27	30	18	87	83	and a light
6	36	87	29	43	17	87	82	colour
7	30	71	33	83	8	57	71	
8	28	43	34	83	9	30	71	
9	25	43	34	71	8	57	67	
10	29	43	30	71	9	57	68	Elastic texture
11	31	57	29	71	10	43	70	
12	33	71	29	71	10	57	72	
LSD	3.79	16.86	1.99	19.46	3.72	14.99	5.41	
C.V.	23.63	27.64	7.28	36.25	31.75	26.04	8.03	

per cent). Incorporation of can syrup along with SO_2 and citric acid led to a non-acceptable gummy texture resembling that of chewing gum.

In general, *Tamar Eddin* can be a good substitute for the apricot based *Kamar Eddin*. Addition of one per cent citric acid to the date pulp and of a moderate amount of SO_2 (167-335 ppm) to improve the colour helps in preparing a product having acceptable colour, texture and taste. Addition of can syrup makes the product elastic.

This product can be considered as one of the dry fruit products. It can be used either in dry form or rehydrated by soaking or simmering in water. *Tamar Eddin* has been proved, by the panel, to be more attractive than the very sweety taste of the original Tamar.

Generally, the preparation of the product is simple. The stages involved are those of common practice in food industry, which give this *Tamar Eddin* an advantage over raw date packaging.

References

1. *Statistical Yearbook*, Central Department of Statistics, Ministry of Finance and National Economy, Riyadh, Saudi Arabia, Tenth Issue, 1974, 393.
2. *Trade Yearbook*, Food and Agriculture Organization of the United Nations, Rome, Vol. 33,1979.
3. Matter, Bassam, Process Engineering, Date Processing Plant in Al-Hassa, Saudi Arabia, (Personal communication).
4. *Official Methods of Analysis*, Association of Official Analytical Chemists, 12th ed. Washington, D.C. 1975.
5. Ray Junk, W. and Pancoast, H. M., *Handbook of Sugar for Processors, Chemists and Technologist*, AVI Publishing Company, West Port, Connecticut, 1973, 266.
6. Amerine, M. A., Pangborn, R. M. and Roessler, E., *Principles of Sensory Evaluation of Food*, Academic Press, New York, 1965, 275.
7. Pearson, D., *The Chemical Analysis of Food*, Churchill Livingstone, Edingburgh, London and New York, 7th ed. 1976, 29.
8. Snedecor, G. W. and Cochran, W. G., *Statistical Methods*, Iowa State University Press, Ames, Iowa, USA, 6th ed. 1974
9. Long, J. D., Mrak, E. M. and Fisher, C. D., *Investigations in the Sulfuring of Fruits for Drying*. California Agriculture Experimental Station Bulletin, 636-56, 1940, 11.
10. Reynolds, T. M., *Chemistry of Non-enzymatic Browning, Adv. Food Res.*, 1965, **14**, 241.
11. McWeeny, D. J., *Sulphur Dioxide and Maillard Reaction in Food*, Pergamon Press, Oxford, Vol. 5, 1981, 395.

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RESEARCH NOTES

A NOTE ON THE EFFECT OF ETHREL AND HOT WATER DIP TREATMENT ON THE RIPENING AND RESPIRATORY ACTIVITIES OF MANGO VARIETY DASHEHARI

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Studies on ethrel and hot water treatment on the ripening of mango cultivar 'Dashehari' revealed that either hot water treatment or ethrel upto 500 ppm concentration could be employed for inducing ripening without impairing taste and flavour. Hot water treatment was however, the better of the two treatments as the microbial spoilage was also reduced considerably. Ethrel at more than 500 ppm level caused development of off flavour, altered taste and reduced shelf life of fruits.

Development of attractive colour makes the fruits more appealing to the eye, enhances general acceptability and market value. Traditional practice of ripening the fruits between layers of straw and paper (PAL method) is not effective in inducing proper ripening. Calcium carbide treatment is found to alter the aroma and flavour and reduce the shelf life of fruits. Bhatnagar and Subramanyam[1] have successfully applied ethrel and hot water treatments for ripening and proper development of carotene content in some south Indian varieties of mangoes. Effect of ethrel and hot water treatment on the ripening of 'Dashehari' varieties of mangoes cultivated in Uttar Pradesh was carried out during 1976 and 1977 in this laboratory and the results are recorded here.

Uniformly mature green fruits selected by float and sink methods, were procured from Malihabad, of Lucknow District. The following seven treatments were given, five replicates, of ten fruits each were utilised for each treatment.

1. Control (dipped in cold water for 5 min)
2. Dipped in hot water (53±1°C) for 5 min
3. Dipped in ethrel (250 ppm) for 5 min
4. Dipped in ethrel (500 ppm) for 5 min
5. Dipped in ethrel (750 ppm) for 5 min
6. Dipped in ethrel (1000 ppm) for 5 min
7. Dipped in hot water containing ethrel (500ppm) for 5 min

Treated fruits were stored at ambient temperature (29-35°C) and relative humidity of 59-75 per cent and the characteristics[2] recorded at intervals of four days. Rate of respiration was determined by Petten Koffer method as suggested by Loomis and Shull[3]. Organoleptic evaluation was carried out by a panel of five judges.

After four days, all the fruits treated with ethrel (500 ppm and above) ripened. 83.3 per cent of fruits dipped in hot water containing ethrel (500 ppm) ripened followed by 75.5 per cent and 60 per cent of fruits treated by ethrel (250 ppm) and hot water alone respectively as against 33 per cent of the control fruits in accordance with the earlier 4-6 observations. Ethrel treated fruit (below 500 ppm) remained sound upto to 8 days while fruits treated at 750 ppm to 1000 ppm remained sound upto 6 days. The shelf life of fruits dipped in hot water containing ehtrel (500 ppm) was extended upto 9 days.

TABLE 1. MEAN VALUES OF PHYSICAL CHARACTERS OF MANGOES AFTER FOUR AND EIGHT DAYS OF STORAGE

Treatments	Physiological loss in wt (%)		Spoilage (%)		Ripe fruits (%)		Rating (%)
	4	8	4	8	4	8	4
Control	9.18	18.90	10.37	12.66	33.0	100	67
Hot water (53±1°C)	9.19	18.81	6.66	11.11	60.0	100	74
Ethrel 250 ppm	10.05	20.19	13.33	18.50	75.5	100	64
Ethrel 500 ppm	10.39	21.94	10.00	13.33	100.0	100	69
Ethrel 750 ppm	10.80	21.97	6.66	12.66	100.0	100	63
Ethrel 1000 ppm	11.34	22.62	6.66	20.00	100.00	100	34
Hot water+Ethrel (500 ppm)	10.36	21.00	—	—	83.3	100	56

Rating was done only after 4 days of storage

TABLE 2. MEAN VALUES OF CHEMICAL COMPOSITION OF MANGOES AFTER FOUR AND EIGHT DAYS OF STORAGE

Treatments	T.S.S. (°Brix)		pH		Ascorbic acid (mg/100g)		Acidity (%)		Reducing sugars (%)		Non-reducing sugars (%)		Total sugars (%)		Respiration rate (mg/ CO_2/kg/hr)	
	4	8	4	8	4	8	4	8	4	8	4	8	4	8	4	8
Control	22.50	20.0	3.99	4.75	48.85	34.24	0.93	0.76	2.10	2.18	9 .41	9.67	11.67	11.85	24.11	46.46
Hot water	23.50	19.7	4.00	4.85	43.44	34.24	0.74	0.68	2.55	2.70	10.28	11.01	12.83	12.71	33.54	66.02
Ethrel 250 ppm	23.50	22.2	3.80	5.50	47.48	33.68	0.55	0.49	2.88	3.11	11.11	11.43	13.99	14.54	26.62	60.36
Ethrel 500 ppm	24.75	21.5	3.90	4.75	45.68	34.80	0.49	0.22	2.16	3.01	12.06	12.23	14.62	15.24	27.18	62.46
Ethrel 750 ppm	24.90	23.0	4.00	5.0	44.80	33.60	0.49	0.12	2.63	2.80	11.13	12.00	13.77	14.80	27.63	36.42
Ethrel 1000 ppm	24.50	20.0	4.00	5.0	45.60	37.20	0.49	0.12	2.65	2.70	12.78	12.86	15.43	15.56	24.66	43.36
Hot water+ Ethrel (500ppm)	23.75	25.5	4.00	5.0	44.56	35.92	0.44	0.12	2.35	2.60	12.03	11.63	14.30	14.63	34.86	65.00

Ethrel treatment tended to increase the weight loss which was directly proportional to its concentration[7]. It was maximum (11.34 per cent) in fruits treated with ethrel 1000 ppm and minimum (9.18 per cent) in control at the end of four days storage.

There was no spoilage when fruits were dipped in hot water containing ethrel (500 ppm) even after eight days while more than 10 per cent spoilage was noticed in all the other treatments. Thus, application of ethrel (500 ppm) in combination with hot water appears to be effective in minmising the spoilage during storage.

Hot water treated mangoes were rated superior to the other fruits by securing maximum marks (74 per cent) in sensory evaluation followed by fruits treated with ethrel (500 ppm) securing 69 per cent marks. The fruits treated with maximum level of ethrel rated worst securing only 34 per cent marks after four days (Table 1).

No appreciable difference was observed in pH, ascorbic acid and total sugars among various treatment except for higher total sugar observed in fruits treated with hot water containing ethrel (Table 2). Ethrel (500 ppm) treated fruits had lower acidity (0.49 per cent) as controlled fruits had and hot water treated fruits (0.74 per cent) and ethrel (250 ppm) treated fruits had 0.55 per cent acidity after four days.

Maximum rate of respiration (34.86 mg CO_2/kg/hr) after 4 days was recorded in the fruits treated with ethrel (500 ppm) followed by hot water treatment (33.54 mg CO_2/kg/hr) as against minimum rate of respiration (24.11 mg CO_2/kg/hr) in control ir accordance with earlier[8,9] observations. Hot water treatment appears to enhance the respiratory activity[10,11].

References

1. Bhatnagar, H. C. and Subramanyam. H., Some aspects of preservation, processing and export of mango and its product. *Indian Fd Pckr.*, 1973, **27**(4), 33.
2. Mukherjee, P. K., *Studies* on *determination of right stage of maturity of mango for storage.* Annual Rep. Hort. Res. Institute, Saharanpur, 1960, 78.
3. Loomis, W. E. and Shull. C.A., *Methods in Plant Physiology*, McGraw Hill Book Co., New York, 1937.
4. Chandawat, B. S., Gupta, O. P. and Singh U. P., Effect of 2-chloroethyl phosphonic acid (ethrel) on ripening of mango (*Mangifera indica* L) fruits. *Haryana J. hort. Sci.*, 1973, **12** (3 & 4), 11.
5. Madhya Rao, O. N., Sanmugvelu, K. G., Srinivasan, C. and Padmanabhaiah, D.R., Studies on the effect of ethrel (2-Chloroethyl phosphonic acid on the ripening of fruits. *S. Indian Hort.*, 1971, **19**(1-4), 1.
6. Mann, S. S. and Dhillon, B.S., Effect of ethrel and calcium carbide on ripening of mango. *Prog. Hort.*, 1974, **6**, 59.
7. Singh, U. R., Singh, G. and Khan, A., Studies on the artificial ripening of banana cultivar Basrai dwarf. *Prog. Hort.*, 1977, **9**, 53.
8. Mathur, P.B. and Srivastava, H.C., Effect of skin coating on the storage behaviour of mango. *Fd Res.*, 1955, **20**, 559.
9. Mathur, P. B. and Subramanyam, H., Effect of a fungicidal wax-coating on the storage behaviour of mangoes. *J. Sci. Fd Agric.*, 1956, **7**, 673.
10. Desrosier, N. W. and Desrosier, J. N., *The Technology of Food Preservation*, The AVI Publishing Company, Inc., Wesport, Connecticut, 1977.
11. Ryall, A. L. and Lipton, W. J., *Handling, Transportation and Storage of Fruits and Vegetables.* The AVI publishing Company Inc., Wesport, Connecticut, 1972.

CHLORINATED PESTICIDE RESIDUES IN DESI GHEE

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Ghee (42 samples) collected from Lucknow and Sitapur districts of U.P. were analysed for organochlorine pesticide residues using GLC. All the samples were contaminated with HCH (Hexachiorocyclo hexane)and DDT (Dichloro diphenyl trichlorethane) residues. It was found that samples from Sitapur had higher levels of HCH and DDT as compared to that from Lucknow. Samples from Sitapur contained 4.00 ppm of HCH and 23.8 ppm of total DDT as against 1.88 ppm of HCH and 20.35 ppm of total DDT in Lucknow samples.

The tendency of tissues to accumulate organochlorine pesticides is now well recognised and warrants awareness among the masses with contaminated foods[1-3] like edible oils, eggs etc appear to be the major and potent source of residue-build up in body tissues. Desi ghee is a typical item of fat used by most of the Indian families either in cooking or consumed during meals. Forty two samples of ghee (clarified butter) collected from Sitapur (22) and from Lucknow (20) districts, both from household and from market were analysed for the degree of contamination.

Samples were stored at room temperature and analysed within 48 hr after collection. Reagents and chemicals used were of Analar grade unless otherwise indicated and checked against ECD (electron capture detector) contamination. Hexane used for extraction of pesticide) was purified and tested for any contamination. Other solvents used were double distilled. Special care was taken to avoid glassware contamination by pesticides, and so they were rinsed with alcohol followed by acetone.

The method used for extraction of pesticides was that of Mills[1] and described by Siddiqui *et al*[3].

Extracted samples were cleaned with distilled water (1 ml) in a clean testube, in a liquid air-methanol bath, to remove the traces of acetonitrile, if any. The unfrozen heaxne phase was further treated with concentrated H_2SO_4 (1 ml) three times, to remove the fat contents and the cleaned samples were analysed by GLC, Varian Aerograph Series "2400" with $3H^+$ detector, under the operating conditions described by Siddiqui *et al*[3]. Relative retention time (with respect to aldrin) is 0.47, 0.58, 0.60, 1.00, 1.93, 2.77 and 3.43 for α-BHC, γ-BHC β-BHC, aldrin, p,p'-DDE, p,p'-DDD and p,p'-DDT respectively. Detection limits for α, β-and γ-BHC and aldrin and p,p'-DDE is 1 ppb whereas for p,p'-DDD and p,p'-DDT it is 2 ppb.

Standards used were obtained from Poly Sciences Corporation, Illinois (USA).

Residue levels of HCH, DDT and its metabolites DDE and DDD detected in the samples are given in Table 1. Concentration of HCH comprised α, β and γ isomers, the main stereoisomers of hexachlorocyclohexane. Delta isomer was not found presumably because of being relatively labile to photo and biodegradation. Sample collected from Sitapur district showed as high as 4 ppm of HCH whereas the maximum contamination with HCH in sample collected from Lucknow market was 1.8 ppm.

Average level of total DDT equivalent which, in fact represents the complete exposure of Ghee samples from Sitapur buffaloes exposed to DDT through contaminated grasses and straw had double the quantity (9.86 ppm) of DDT compared to that of Ghee samples from Lucknow buffaloes (4.47 ppm). Ghee samples of Sitapur had the maximum of 28.85 ppm of total DDT while it was 20.35 ppm in samples from Lucknow. As low as 1.07 and 1.95 ppm of total DDT was observed in Sitapur and Lucknow samples respectively. In Sitapur the level of 9.86 ppm total DDT equivalent was contributed by 2.39 ppm of p,p'-DDT, the parent compound was about 50 per cent of total DDT equivalent (4.75 ppm). However in Lucknow, 4.47 ppm of total DDT equivalent was contributed by 1.27 ppm of p,p'-DDE and 1.17 ppm of p,p'-DDD, p,p'-DDT level was only 1.6 ppm.

Samples of ghee collected from houses in Sitapur were found to have significantly higher pesticide residues as compared with those found in samples from market of Sitapur. The range of higher value was detecetd in household samples which lead to a significantly higher

TABLE 1. ORGANOCHLORINE INSECTICIDE RESIDUE IN SAMPLES OF GHEE (PPM)

Pesticides detected	Sitapur Mean$\pm$S.E.	Lucknow Mean$\pm$S.E.
Total HCH	1.424$\pm$0.179	1.290$\pm$0.061
p, p'—DDE	2.293$\pm$0.212	1.274$\pm$0.162
p, p'—DDD	2.631$\pm$2.969	1.176$\pm$0.072
p, p'—DDT	4.75 $\pm$1.099	1.601$\pm$0.869
Σ DDT	9.86 $\pm$1.489	4.47 $\pm$1.506

Total HCH indicates the sum of α, β-and γ-isomer of HCH
Σ DDT=Total DDT equivalent.

mean level of total DDT equivalent in Sitapur samples as compared with the same in Lucknow. Comparatively lower contamination in market samples of Sitapur may possibly be attributed to the adulteration with some cheaper vegetable oils having less pesticide residues.

Thus ghee is a very potent source of residue build-up of tissues and fluids in persons consuming it regularly.

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References

1. Mills, P. A., Collaborative study of certain chlorinated pesticides in dairy products. *J. Ass. Off. agric. Chem.*, 1961, **44**, 171.
2. Siddiqui, M. K. J., Saxena, M. C., Mishra, U. K., Krishna Murti, C. R. and Nag, D., Long-term occupational exposure to DDT. *Int. Arch. Occup. Environ. Hlth*, 1981, **48**, 301.
3. Siddiqui, M. K. J., Saxena, M. C., Bhargava, A. K., Seth, T. D., Krishna Murthy, C. R. and Kutty, D., Agrochemicals in the material blood, milk and cord blood: A source of toxicants for prenates and necnates. *Environ. Res.*, 1981, **24**, 24.

DIETARY FIBRE CONTENT OF SOME FOOD MATERIALS

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Food materials selected were from different groups namely cerelas, legumes and vegetables. Detergent methods of Van Soest namely neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were used to estimate dietary fibre and its fractions along with conventional method of crude fibre (CF). The NDF content of all food materials were significantly higher than CF. Among the food materials analysed, cereals and legumes contained more amount of dietary fibre than vegetables (on fresh weight basis).

Dietary fibre, in some selected food articles belonging to three groups, namely, cereals, legumes and vegetables was estimated by Van Soest's neutral detergent fibre (NDF), acid detergent fibre (ADF) and Lignin methods along with the conventional crude fiber (CF) estimation[1-3]. The newer methods are found to be rapid and reliable[4-6]. The NDF content of all food materials were significantly higher than CF. Cereals and legumes contained more dietary fibre than fresh vegetables.

Materials used in this study are: bajra (*Pennisetum typhoideum*), jowar (*Sorgum vulgare*), maize (*Zea mays*), wheat (*Triticum aestivum*) flour whole, Bengal-gram (*Cicer arietinum*), blackgram (*Phaseolus mungo*) dhal, horsegram (*Dolichos biflorus*), Peas, (*Pisum sativum*) dried, redgram dhal (*Cajanus cajan*), fenugreek (*Trigonella foenumgraecum*) leaves, coriander (*Coriandrum sativum*) leaves, onion (*Allium cepa*), radish, white (*Raphanus sativus*), knol khol (*Brassica oleracea var. caulorapa*), clusterbeans (*Cyamopsis tetragonoloba*), lady's finger (*Abelmoschus esculentus*).

Samples in triplicate were purchased in the local market at three different seasons and duplicate samples were analysed. Cleaned cereals and legumes and edible portion of dried vegetables were powdered (40-50 mesh), and analysed for crude fibre, (CF)[7], neutral detergent fibre (NDF)[8], acid detergent fibre (ADF) and lignin[9]. The value for the cellulose fraction was taken as the difference between ADF and lignin values. The difference between NDF and ADF values was taken to indicate the hemicellulose content of the dietary fibre.

Foods with a high starch content, like cereals and legumes were found to give high NDF values, and therefore were treated with amyloglucosidase to hydrolyse their starch before determining NDF[10].

The values obtained (based on dry weights) are given in Table 1. The NDF values of all the foods were higher than CF values. Among cereals, jowar had the highest value of 10.9 per cent and bajra the lowest with 4.6 per cent. Among legumes, horsegram and peas had the highest and lowest NDF values respectively. Among vegetables, radish had the highest NDF value of 21.2 per cent and onion the lowest value of 11.1 per cent. However, the NDF content of fresh vegetables was calculated as such since all the vegetables contained less fibre than cereals and legumes and their moisture content ranged between 84 and 96 per cent in contrast to food grains which have a moisture content of 8-12 per cent.

The NDF values of foods were higher than the ADF values. This is to be expected since ADF measures ligno-cellulose and NDF measures ligno-cellulose and hemicellulose.

The cellulose content of cereals ranges from 1.6 to 9.4 per cent and of legumes, from 1.9 to 9.4 per cent. In vegetables, the cellulose content varies from 3.7 per cent for onions to 11.4 per cent for radish. Among cereals, the hemicellulose content was highest in jowar and lowest in bajra. Horsegram had the highest and blackgram dhal the lowest hemicellulose content among the legumes. Lady's finger had the highest value of 5.4 per cent and onion had the lowest value of 2.8 per cent among vegetables. The lignin content ranged from 0.2 to 1.8 per cent in cereals and from 0.8 to 8.4 per cent in legumes. Coriander leaves and layd's finger had the highest

TABLE 1. DIETARY FIBRE CONTENT OF SELECTED FOODS

Food material	Crude fibre (g/100 g)	Neutral detergent fibre (g/100 g)	Acid detergent fibre (g/100 g)	Cellulose[a] (g/100 g)	Hemicellulose[b] (g/100 g)	Lignin (g/100 g)
Bajra	1.1±0.03	4.6±0.13	2.8	2.6	1.8	0.2
Jowar	1.6±0.01	10.9±0.40	5.0	3.2	5.9	1.8
Maize	2.7±0.03	6.8±0.13	3.1	1.6	3.7	1.5
Wheat flour	1.7±0.03	8.0±0.38	4.1	2.9	3.9	1.2
Bengalgram	3.3±0.05	14.2±0.25	10.7	9.4	3.5	1.3
Blackgram Dhal	0.7±0.03	7.9±0.03	5.3	4.5	2.6	0.8
Horsegram	5.3±0.05	19.6±4.65	10.8	2.4	8.8	8.4
Peas (dry)	4.5±0.03	6.4±1.30	3.7	2.0	2.7	1.7
Redgram Dhal	1.5±0.03	8.0±1.23	4.5	1.9	3.5	2.6
Fenugreek leaves	1.5±0.10 (0.10)	14.8±1.65 (1.8)	10.1	6.8	4.7	3.3
Coriander leaves	1.5±0.10 (0.15)	20.3±0.69 (2.0)	16.4	8.5	3.9	7.9
Onion	1.6±0.26 (0.26)	11.1±0.11 (1.8)	8.3	3.7	2.8	4.6
Radish	1.5±0.10 (0.06)	21.2±0.25 (0.9)	16.9	11.4	4.3	5.5
Knolkhol	1.9±0.12 (0.17)	17.0±0.05 (1.5)	11.9	7.9	5.1	4.0
Cauliflower	1.3±0.15 (0.20)	17.1±0.40 (2.6)	11.9	6.6	5.2	5.3
Cluster beans	4.1±0.02 (0.66)	17.5±0.70 (2.6)	13.1	10.1	4.4	3.0
Lady's finger	1.6±0.11 (0.16)	20.5±0.45 (2.1)	15.1	7.2	5.4	7.9

Values of vegetables are based on dry weight basis. Figures in parentheses are based on fresh weight basis. Values are mean of six determinations. SD was calculated on six determinations.

[a]Cellulose=Acid detergent fibre—lignin, [b]Hemicellulose=Neutral detergent fibre—Acid detergent fibre.

(7.9 per cent) and clusterbeans had the lowest (3.0 per cent) lignin content.

A knowledge of these individual components of dietary fibre in foods will be helpful in studying the specific metabolic effects in human beings. However, pectins and gums which may be present specially in higher quantities in certain fruits and vegetables are not measured by detergent methods. Suitable methods to measure all the components of fibre need therefore to be evolved. Information on dietary fibre content of foods is important to understand the physiological significance of various components.

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References

1. Van Soest, P. J. and McQueen R. W., The chemistry of estimation of fibre. *Proc. Nutr. Soc.*, 1973, **32**, 123.
2. Southgate, D.A.T., *Determination of Food Carbohydrates*, Applied Science Publishers Ltd., London, 1976.
3. Hellendoorn, E. W., Nordhoff, M. G. and Slagman J., Enzymatic determination of the indigestible residue (dietary fibre) content of foods. *J. Sci. Fd Agric.*, 1975, **26**, 61.
4. Asp, N. G., Critical evaluation of some suggested methods for assay of dietary fibre. *J. Plant Foods*, 1978, **3**, 21.

5. Saraswathi, G., Kanchana, S. and Shurpalekar, K.S., Estimation of dietary fibre of some selected Indian foods by different methods. *Indian J. med. Res.*, 1983, **77**, 833.
6. Kamath, M. V. and Belvady, B., Unavailable carbohydrates of commonly consumed Indian foods. *J. Sci. Fd Agric.*, 1980, **31**, 194.
7. *Official Methods of Analysis*, Association of official analytical chemists, Washington D. C., 1980.
8. Van Soest, P. J. and Wine, R. H., Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cellwall constituents. *J. Ass. Off. anal. Chem.*, 1967, **50**, 50.
9. Van Soest, P. J., Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fibre and lignin. *J. Ass. off. agric. Chem.*, 1963, **46**, 829.
10. Robertson, J. B., *Topics in Dietary Fibre Research* by Spiller, G. A. and Amen, R. J., Plenum Press, New York, 1976, 25.

A SCREW PRESS FOR FRACTIONATING GREEN VEGETATION

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A screw press for green vegetation crop fractionation, has been developed modifying indigenously available oil crushing equipment. It can handle about 100 kg vegetation per hour. Details of the modification done and the data on performance both in a two-step process, integrated with a vertical pulper, and in a single step extraction process are presented.

Steep rise in energy costs during the seventies, have led to a growing interest in green crop fractionation. The principle is; expression of juice from green vegetation leaving a press residue of disintegrated fibrous mass with a lower moisture than the original vegetation. The press residue can be used fresh as forage or dehydrated to obtain a meal. The juice can be directly used as a feed or heat/acid coagulated for the recovery of leaf protein concentrate (LPC).

Machinery development for green crop fractionation has followed different trends. Pirie and his coworkers have designed special equipment for the production of LPC, like belt press[1], IBP pulper[2] inovlving a two step extraction process, and a single step screw extractor[3]. The USDA group have tested the sugarcane rolls, the single and twin screw presses and the V-press[4]. With the object of utilizing indigenously available capability a heat coagulation unit[5] and a vertical pulper[6] for LPC production were developed by us earlier.

For expressing juice from pulped vegetation possibility of using the indigenously available oil screw presses, namely table model oil screw press, baby oil screw press No. 1 were tried. After trials, certain modifications were incorporated in the latter press which resulted in the development of a unit hereafter called green vegetation screw press, the performance of which is discussed here.

The green vegetation screw press adapted from the baby oil expeller No. 1 with a similar drive unit, but without the adjustable cone assembly and the heating kettle, is shown in Fig 1 and 2. The worm shaft bears only four worms five spacer collars, details of which are given in Fig 3. The feeder worm has a matching hopper and the expeller worms, alongwith spacers, are encased in a 45 cm long, 15 cm diameter cage assembly made of two spilt halves. The cage halves are lined with mild steel perforated sheet having 3 mm perforations and provided with three vertical plates. The cage is fastened with two bolt frames having two

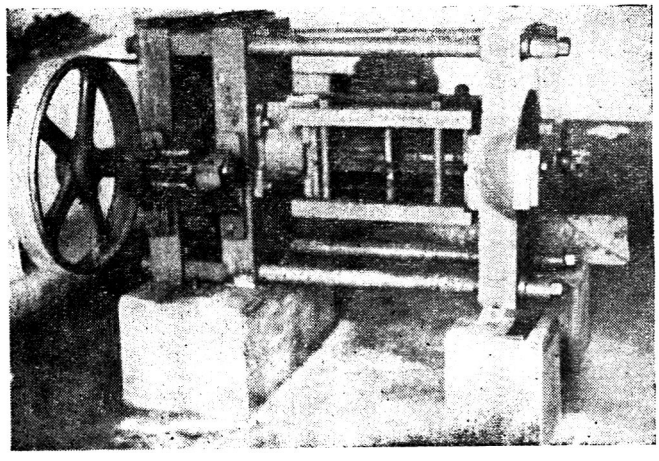

Fig. 1. Screw press for fractionation of green vegetation

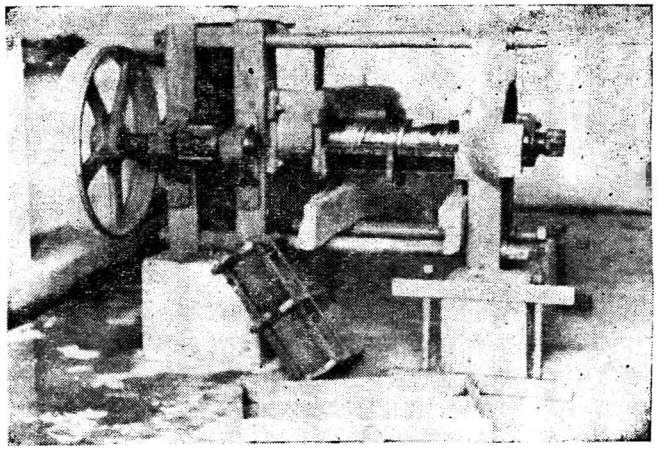

Fig. 2. Green vegetation screw press in dismantled condition

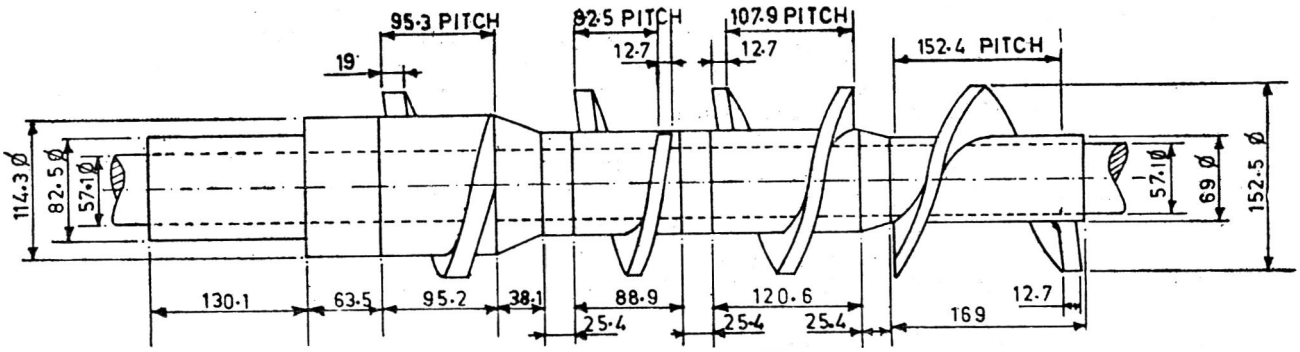

Fig 3. Details of worm assembly

bolts each. The unit is driven by a 3 HP motor through flat belt and cast iron gears to give a speed of 25 rpm to the worm shaft. The juice is collected in a galvanised iron tray placed under the cage assembly and the press residue falls at the outlet end.

Fresh lucerne (*Medicago sativa*) vegetation was purchased from the local market and used in the experiments. During testing and development of the press, vegetation pulped through the vertical pulper was used. Later the vegetation was directly fed to the expeller for single step extraction. Samples for dry matter (DM) and nitrogen (N) were drawn and estimations were made as per standard procedures. LPC from extracts was prepared as described elsewhere[7].

The drive was not altered but the kettle and the cone assembly were excluded. The number of worms and their diameters were adjusted to reduce the compression ratio to less than 3:1. By trials, we found the modified worm assembly, described earlier, most satisfactory. The changes from the original cage lined with bars to that of perfortated sheet was made to facilitate better cleaning. The readjusted worm assembly needed a shorter cage and in the process we also made it light and handy by reducing the number of vertical plates from ten to three and that of bolts from 9 to 2 in each of the bolt frames.

During the testing of the press, using pulped vegetation, we generally found the extractability of juice, DM and N in the range of 60-70, 24-38 and 45-60 per cent respectively. The yields of LPC dry matter were also about 1.8–2.5 per cent of fresh vegetation which are comparable with earlier studies[1,4,6].

In the course of our work, we thought of switching over from the two step processing, that is pulping and pressing in separate equipments, to a single step extraction in the press alone. This was done to reduce capital investment, and to give better roughage character to press residue than the highly compressed residue from pulped vegetation. The latter was considered unacceptable to cattle in India, with the practice of mechnically compressed feeds and fodders absent from the local scene. The results of typical trials comparing the extraction behaviour of two different lots of lucerne vegetation in single and two step processing are given in Table I.

The extractabilities of juice, DM and N are all reduced as also the yields of extracted LPC, when the vegetation was directly fed to the press. There is obvious lower-

TABLE 1. SINGLE / TWO STEP PROCESSING OF LUCERNE VEGETATION

Processing step	Extractability			Vegetation		Press residue		PLC yield
	Juice (%)	DM (%)	N (%)	DM (%)	N (%)	DM (%)	N (%)	(%)
				Trial with lot I				
Single step	54	27	39	15.5	3.3	27	2.7	1.3
Two step	60	31	53	15.4	3.7	31	2.6	1.8
				Trial with lot II				
Single step	50	20	32	11.6	3.5	24	2.8	—
Two step	65	29	58	12.6	3.4	31	2.0	—

ing of juice extraction because during pulping there is great disintegration of tissue which is more amenable to improved juice recovery. The press residue from two step processing has higher DM and less N than that from single step processing. Clearly the single step processing using the green vegetation screw press yields a residue of better forage quality fit for direct use as fodder. The extract may be directly used as fluid feed or subjected to protein precipitation for getting LPC for feed or food uses[7]. We are currently involved in collaboration with the University of Agricultural Sciences, Bangalore, for trials on the technical and economic feasibility of integrated fodder and LPC production.

The green vegetation screw press can process about 100 kg of lucerne vegetation per hour. The unit costs about Rs. 12,000 including the drive. For energy saving in fodder dehydration and/or for leaf protein as the main product, the press may be used in conjunction with the vertical pulper. However, for small scale farm based integrated production of fodder and leaf protein, we recommend single step extraction. We suggest further lines of work as follows: (*a*) Constant manual feeding of vegetation is a laborious task. Feeding of chopped vegetation is conveniant but reduces the capacity greatly. This aspect needs attention. (*b*) The drive and the frames derived from the oil expeller are heavy and bulky. The possibility of lighter assembly around the worm without affecting the operational efficiency have to be looked into. (*c*) Further improvements in capacity by increasing the diameter or by some other means needs to be investigated.

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References

1. Davys, M. N. G., and Pirie, N. W., A belt press for separating juices from fibrous pulp. *J. Agric. Engg.* Res., 1965, **10**, 142.

2. Davys, M. N. G., and Pirie, N. W., A laboratory scale pulper for leafy plant material. *Biotech. Bioengng.*, 1969, **11**, 517.

3. Pirie, N. W., A simple unit for extracting leaf protein in bulk. *Expl. Agric.*, 1977, **13**, 113.

4. Knuckles, B. E., Bickoff, E. M. and Kohler, G. O., PROXAN process: Methods for increasing protein recovery from Alfalfa. *J. Agric. Fd Chem.*, 1972, **20**, 1055.

5. Ahmed, S. Y., and Singh, N., A heat coagulation unit for leaf protein. *Indian J. Technol.*, 1969, **7**, 411.

6. Ahmed, S. Y., Harendranath, R. and Singh, N., A vertical pulper for leafy vegetations, *Res. Ind.*, 1979, **24**, 105.

7. Singh, N., Scope for leaf protein in rural areas. *Res. Ind.*, 1979, **24**, 149.

A SIMPLE METHOD FOR DETERMINING PROTEIN CONTENT IN FOOD STUFFS USING BIURET COLOUR REACTION

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The biuret colorimetric method has been suitably adapted for the assay of protein content. Protein content of 14 food stuffs were determined and the values compare favourably with those obtained by the standard Kjeldahl method.

In addition to, the well known Kjeldahl method for the quantitative determination of protein, simpler colorimetric methods employing the biuret colour or the phenol reagent have come into general use for the determination of protein content of serum and tissues[1,2]. The biuret colorimetric method was suitably adapted for the assay of protein content of foodstuffs and the values were checked by the micro Kjeldahl method.

The samples of food stuffs were purchased locally, cleaned, decorticated ground to a fine powder, sieved and stored in stoppered bottles at room temperature. These formed the protein source in the experimental diets.

The micro Kjeldahl method was done by standard procedure[3] using 100-200 mg of the powder The nitrogen content was converted to protein by multiplying by the factor 6.25 uniformly for all food stuffs[3].

For the biuret method 250 mg of the powder was extracted twice by shaking with 5 ml ether in a stoppered tube, the contents centrifuged at 1000 rpm and the supernatant was discarded after the extraction. The defatted sediment was stirred up with 5 ml 0.1N sodium hydroxide shaken for 10 min centrifuged at 2000 rpm for 10 min. Gornall's[3] modified biuret reagent (5 ml) was added to the supernatant (1 ml), incubated at 37°C for 10 min and the violet colour was read at 555 nm. In the case of milk powder, the defatted residue was shaken up with 5 ml of 0.1M acetate buffer pH 4.5, centrifuged to remove lactose and the residue was extracted as per the above and the estimation completed in the same way. This step was necessary to eliminate the effect of lactose on the colour reaction. The protein content was calculated from a calibration graph prepared with pure bovine serum albumin (Sigma) under the same conditions. The biuret colorimetric assays were done in triplicate and the Kjeldahl estimation in duplicate. The results are given in Table 1.

TABLE 1. PROTEIN CONTENT OF FOOD STUFFS AS ESTIMATED BY BIURET AND KJELDAHL MFTHOD

Foodstuff	Protein content (%)	
	Biuret method	Kjeldahl method
Rice	8.22	8.18
Wheat	13.89	13.14
Maize	10.16	10.52
Green gram	18.67	20.97
Red gram	20.40	21.26
Black gram	21.77	20.23
Soyabean	39.98	43.20
Lentil	24.44	25.46
Bengal gram	22.89	22.70
Skim milk powder	19.69	20.21
Whole milk powder	23.57	25.67
Low protein diet	5.86	5.29
High protein diet	9.91	9.85
Stock diet rats	19.25	19.66

In general the values obtained by the biuret method are in near agreement with the Kjeldahl figures, suggesting its suitability as an alternative method for the routine estimation of protein content of food stuffs.

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References

1. Lowry, O. H., Rosebrough, N. J., Farr, L. A. and Randall, R. J., Protein measurement with the folin phenol reagent. *J. biol. Chem.*, 1951, **193**, 265.

2. Gornall, A. G., Barda Will, C. J. and David, M. M., Determination of serum proteins by means of the biuret reaction. *J. biol. Chem.*, 1949, **177**, 751.

3. Oser, B. L. (ed), *Hawk's Physiological Chemistry*, McGraw Hill Book Co., New York, 14th ed., 1965, 1214, 1335.

A COMPARISON BETWEEN TOLUIDINE BLUE-DEOXYRIBONUCLEIC ACID AGAR PLATE METHOD AND TURBIDIMETRIC ASSAY FOR THE DETECTION OF STAPHYLOCOCCAL THERMOSTABLE DEOXYRIBONUCLEASE

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Toluidine blue—deoxyribonucleic acid agar plate method and turbidimetric assay were compared for the detection of thermostable deoxyribonuclease produced by enterotoxigenic cultures of *Staphylococcus aureus*. Three groups were formed based on the TDNase zone diameters (16-20, 21-25, 26-30 mm) and TDNase units/ml. (<1.0, 1.1-4.0, 4.1-6.0). Most of the cultures fell in the second or the third group. A close parallelism was observed between the two methods with respect to TDNase detection.

Production of thermostable deoxyribonuclease (TDNase) by *staphylococci* has been considered as a potential indicator of enterotoxigenicity. A high correlation between TDNase and enterotoxin production by staphylococci have been reported[1-5]. Several methods have been described[6-10] for the detection of TDNase, of which the toluidine blue-deoxyribonucleic acid (TB-DNA) agar plate method of Lachica *et al*[10]. has been widely used for its accuracy and time saving. Almost on similar lines another method namely, turbidimetric assay of Erickson and Deibel[11] has also been in use for the detection and estimation of TDNase. In the present study, an attempt has been made to compare agar plate method with that of turbidimetric assay, for the detection of TDNase elaborated by enterotoxigenic cultures of *Staphylococcus aureus* so as to find out whether there is any significant difference exists between the two methods.

The test cultures used in this study comprised of 64 'wild' isolates of enterotoxigenic *S. aureus* obtained from samples of market *Khoa*[12]. The distribution of enterotoxin(s) among the test cultures were 8 of A, 27 of B, 9 of C, 5 of AB, 2 each of E, AC, AE, ABDE and BC and one each of ABD, ABCD, ABCDE, BE and CE. In addition, the five standard strains of *S. aureus*—A_{100}, $B_{S\text{-}6}$, C_{137}, D_{472} and E_{326} producing their respective enterotoxins A, B, C, D and E were also used.

The test cultures were grown in requisite amounts of 3.7 per cent brain heart infusion (BHI) broth for 24 hr at 37°C, centrifuged at 3,354 RCF for 20 min and the supernatants were then steamed at 100°C for 15 min

TABLE 1. TDNASE PRODUCTION BY ENTEROTOXIGENIC *S. AUREUS* CULTURES AS EVIDENCED BY ZONE DIAMETERS AND NUCLEASE UNITS

Entrotoxigenic cultures	TDNase zone diameters* (mm)			Nuclease units/ml		
	16-20	21-25	26-30	<1.0	1.1-4.0	4.1-6.0
A (8)	1	7	nil	3	3	2
B (27)	5	14	8	6	9	12
C (9)	3	6	nil	6	3	nil
E (2)	nil	2	nil	nil	1	1
AB (5)	nil	3	2	nil	1	4
AC (2)	nil	2	nil	nil	1	1
AE (2)	nil	2	nil	nil	1	1
ABD (1)	1	nil	nil	nil	1	nil
ABDE (2)	nil	2	nil	nil	2	nil
ABCD (1)	nil	1	nil	nil	1	nil
ABCDE (1)	nil	nil	1	nil	nil	1
BC (2)	nil	nil	2	nil	nil	2
BE (1)	nil	1	nil	nil	1	nil
CE (1)	nil	1	nil	nil	1	nil
A_{100}, B_{s-6}, C_{137}	nil	nil	3	nil	nil	3
D_{472}, E_{326}	nil	2	nil	nil	2	nil

*Zone diameter includes the initial diameter of agar well (5 mm)

Figures in parantheses indicate the number of isolates producing enterotoxins.

cooled and then screened for TDNase by the two methods[10,11].

In TB-DNA agar plate method[10], positive reactions for TDNase was evidenced by the formation of pink zones around the agar wells and the diameter of the zones was measured. The nuclease (TNDase) activity by the Turbidimetric method was expressed as units/ml of the extract and calculated using the formula:

$$\text{Nuclease units/ml} = \frac{(\text{Substrate depolymerised/ml}) \times \text{Dilution factor}}{\text{Time of incubation (min)}}$$

It was observed (Table 1) that TDNase produced by the test cultures ranged from a minimum zone diameter of 16 mm to a maximum of 30 mm. In view of such variations among the test cultures for TDNase production, 3 groups were formed on the basis of zone diameters viz., (*i*) 16-20 mm, (*ii*) 21-25 mm and (*iii*) 26-30 mm.

Among the wild cultures of *Staphylococci*, 10 were in the first group, whereas 41 and 13 cultures, respectively were in the second and third groups indicating that TDNase zone diameters of 21-25 mm was more common among a large number of cultures. A definite relationship could not be drawn between the type of toxins produced and TDNase zone diameters. Most of the cultures which produced enterotoxins A, B, C and E singly and more, so, in combinations produced belonged to the second group. The three standard strains of *S. aureus* A_{100}, B_{s-6} and C_{137} produced TDNase zone diameters of 26-30 mm, while the other two strains D_{472} and E_{326} produced zones of 21-25 mm.

The distribution pattern of wild cultures was, however, slightly different in relation to the units of TDNase produced. Based on the TDNase units, wild cultures of *S. aureus* could be grouped as: (*i*) less than 1.0 unit, (*ii*) 1.1-4.0 units and (*iii*) 4.1-6.0 units/ml. Out of the 64 cultures, only 15 were in the first group, while the remaining cultures showed an equal distribution, between the second and third groups. The standard strains A_{100}, B_{s-6} and C_{137} fell into the third group whereas strains D_{472} and E_{326} belonged to the first group.

It was of interest to observe a correlation between TDNase zone diameters and units among staphylococcal cultures which produced enterotoxins in combinations

of 2 or more types, with the exception of cultures producing enterotoxin combinations of AB, AC and AE. On the basis of the above correlation, it would be appropriate to suggest that in cultures producing multiple enterotoxins, TDNase zone diameters of 21-25 mm and 26-30 mm corresponds to 1.1-4.0 and 4.1-6.0 units/ml, respectively. Similarly, such a clear correlation was also observed among the standard strains of *S. aureus*, wherein strains A_{100}, B_{3-6} and C_{137} which had produced zone diameter of 26-30 mm corresponded with 4.1-6.0 TDNase units/ml and those of strains D_{472} and E_{326} producing zone diameter of 21-25 mm corresponded with 1.1-4.0 units/ml.

However, a clear correlation was not figured out between the two methods where cultures elaborated single enterotoxins, though there exists a close parallelism. Out of eight cultures producing enterotoxin A, 7 were in the second group (21-25 mm zone diameter); there was almost an equal distribution among all the three groups in terms of TDNase units/ml. Out of 9 cultures elaborating enterotoxin C, 3 and 6 of them were in the first (16-20 mm) and second (21-25 mm) groups of zone diameters, respectively, whereas the reverse was true in terms of TDNase units/ml. However, in this case, 3 cultures each producing TDNase amount of less than 1.0 unit/ml and also in the range of 1.1-4.0 units/ml corresponded with zone diameters of 16–20 mm and 21-25 mm, respectively. Similarly, in the case of cultures producing enterotoxin B, out of 27 cultures, 5, 14 and 8 of them, respectively were grouped in first, second and third groups of TDNase zone diameter, while 6, 9 and 12 of them, respectively were grouped in first, second and third groups of TDNase units. In addition, it was observed, that 8 cultures producing TDNase zone diameter of 26-30 mm corresponded with 4.1-6.0 units/ml, while 9 cultures producing 1.1-4.0 TDNase units/ml corresponded with 21-25 mm zone diameter.

Among the cultures producing enterotoxin (s) E, AB, AC and AE, nothing definite could be stated, except that in these cultures, TDNase zone diameter of 21-25 mm would correspond either with second or third groups of TDNase units/ml. Similar to cultures producing multiple enterotoxins, even these cultures do not come under the first group of both the methods of TDNase detection.

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References

1. Lachica, R. V. F., Weiss, K. F. and Deibel, R. H., Relationships among coagulase, enterotoxin and heat-stable deoxyribonuclease production by *Staphylococcus aureus*. *Appl. Microbiol.*, 1969, **18**, 126.
2. Cords, B. R. and Tatini, S. R., Applicability of heat stable deoxyribonuclease assay for assessment of staphylococcal growth and the likely presence of enterotoxin in cheese. *J. Dairy Sci.*, 1973, **56**, 1512.
3. Rayman, M. K., Park, C. E., Philpott, J. and Todd, E. C. D., Reassessment of the coagulase and thermostable nuclease tests as means of identifying *Staphylococcus auerus*. *Appl. Microbiol.*, 1975, **29**, 451.
4. Tatini, S. R., Soo, H. M., Cords, B. R. and Bennett, R. W., Heat stable nuclease for assessment of staphylococcal growth and likely presence of enterotoxin in foods. *J. Fd Sci.*, 1975, **40**, 352.
5. Batish, V. K., Ghodekar, D. R. and Ranganathan, B., The thermostable deoxyribonuclease (DNase) test as a rapid screening method for the detection of staphylococcal enterotoxin in milk and milk products. *Microbiol. Immunol.*, 1978, **22**, 437.
6. Jeffries, C. D., Holtmans, D. F. and Guse, D. G., Rapid method for determining the activity of microorganisms on nucleic acids. *J. Bacteriol.*, 1957, **73**, 590.
7. Stretifeld, M. M., Hoffmann, E. M. and Janklow, H. M., Evaluation of extra cellular deoxyribonuclease activity in pseudomonads. *J. Bacteriol.*, 1962, **84**, 77.
8. Smith, P. B., Hancock, G. A. and Rhoden, D. L., Improved medium for detecting deoxyribonuclease producing bacteria, *Appl. Microbiol.*, 1969, **18**, 991.
9. Lachica, R. V. F. and Deibel, R. H., Detection of nuclease activity in semi-solid and broth cultures. *Appl. Micrbiol.*, 1969, **18**, 174.
10. Lachica, R. V. F., Genigeorgis, C. and Hoeprich, P. D., Metachromatic agar diffusion methods for detecting staphylococcal nuclease activity. *Appl. Microbiol.*, 1971, **21**, 585.
11. Erickson, A. and Deibel, R. H., Turbidimetric assay of staphylococcal nuclease. *Appl. Microbiol.*, 1973, **25**, 337.
12. Varadaraj, M. C. and Nambudripad, V. K. N., Staphylococcal incidence in market *Khoa* and their enterotoxins production. *J. Fd Sci. Technol*, 1982, **19**, 53.

SUITABILITY OF SOME ORGANIC AMINO COMPOUNDS IN CARAMEL PREPARATION

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Use of ammonia during caramel preparation is reported to produce 4-methyl imidazole, a toxic compound. When substitutes were tried; methyl-amine, diethylamine and t-butylamine gave good colour values but ethylene diamine was found to be the best substitute.

Caramel, an important food colour is generally prepared by heating carbohydrates, mainly corn syrup

or dextrose, in the presence of catalysts like acids (sulphuric, acetic), alkalis, (ammonium hydroxide, sodium hydroxide) and salts (ammonium sulphate and phosphate)[1]. When dextrose is heated with acid it polymerises to form caramel. Ammonia reacts with the aldehyde group of glucose, followed by condensation, rearrangement and polymerisation yielding caramel[2].

It has been reported that caramel prepared by this method contains 4-methylimidazole which has been shown to be toxic[3,4]. The formation of 4-methylimidazole is probably due to the condensation of ammonia, pyruvaldehyde and formaldehyde, the aldehydes being the pyrolysis products of glucose[4].

The present work was undertaken to try substitutes for ammonia with a view to reduce or prevent the formation of 4-methylimidazole without affecting the colour formation.

All other chemicals and solvents used were of analytical reagent grade. Corn syrup used in this study had 85 °Brix

Caramel preparation: To 200g of corn syrup, 1.3 ml of 50 per cent sulphuric acid was added with constant agitation, and heated in steam at 1.1 kg/cm^2 pressure for one hour. After cooling 2 ml of liquor ammonia was added and the mixture was heated again for 3 hr at 1.20 kg/cm^2 pressure. The solution was then cooled, 3.5 g of dibasic ammonium phosphate was added and heated at 1.6 kg/cm^2 for 3 hr. It was then cooled and filtered. Similar procedure was adopted substituting ammonia by several compounds, listed in Table 1. The sample containing ethylene diamine was heated for just one hour instead of 3 hr at 1.6 kg/cm^2. Longer heating caused resinification of the caramel which would not form aqueous solution.

Colour intensities of 0.1 per cent solution was measured at 420 and 600 nm. Various other tests were carried out as mentioned in Table 2. 4-Methyl imidazole was isolated from caramel by using methylene chloride[5] and was analysed by TLC using iodine as colouring agent[6]. IR and UV spectral analyses were also carried out to detect the presence of 4-methylimidazole.

Colour intensities and Rf values of compounds found in caramel samples prepared by using ammonia and other compounds are given in Table 1.

Caramel prepared with alanine, t-ethyl amine, diethanol amine and dimethylamine did not give good colour values, while those prepared with methylamine, t-butylamine and diethylamine gave satisfactory colour values. The caramel prepared with ethylene diamine gave extremely good colour values comparable with commercial samples. Even liquor ammonia caramel gave satisfactory colour.

It can also be seen from Table 1, that each of the caramel sample prepared with substitutes characteristic compound with a different Rf value distinct from that of 4-methylimidazole. The UV and IR analyses also showed that these caramels did not contain 4-methylimidazole.

Table 2 shows various properties of the caramel prepared using ethylene diamine, in comparison with a commercial sample. It shows compatibility with alcohol and so can be used in alcoholic beverages. It also shows resistance towards various acids which are normally encountered in soft drinks. It has low isoelectric pH showing stability in high acid drinks in cola type beverages. These properties indicate its possible use in beverage industry.

TABLE 1. COLOUR INTENSITIES OF CARAMELS PREPARED WITH AMMONIA AND ORGANIC AMINO COMPOUNDS, COMMERCIAL SAMPLES AND THE Rf VALUES

Amino compounds	O.D. at 420 nm	O.D. at 600 nm	Rf
Alanine (1 g)	0.395	0.065	—
Ethylene diamine (2 ml)	1.12	0.155	0.727
t-butyl amine (2 ml)	0.54	0.075	0.468
Diethyl amine (2 ml)	0.54	0.090	0.583
t-ethyl amine (2 ml)	0.44	0.020	0.621
Diethanol amine (2 ml)	0.52	0.065	0.481
Methyl amine (2 ml)	0.61	0.080	0.496
Dimethyl amine (2 ml)	0.37	0.050	0.420
Liquor ammonia (2 ml)	0.86	0.160	0.865
Commerical samples	1.15	0.200	—
4-methyl imdiazole	—	—	0.870

TABLE 2.

PROPERTIES OF CARAMEL PRFPARED WITH ETHYLENE DIAMINE AND COMMERCIAL SAMPLE

Properties of caramel	Prepared with ethylene diamine	Commercial sample
pH (10% w/v soln.)	2.8	3.2
Compatibility with alcohol	Clear upto 60%	Clear upto 65%
1 g in 300 ml 2% citric acid solution	No haze	No haze
1 g in 300 ml 2% Na_2CO_3	Clear	Clear
Acid resistance 250 ml. 0.2% caramel + 7 ml conc. HCl, boil 5 min	Clear	Clear
0.5 ml phosphoric acid + 20 ml 5% caramel soln.	No ppt.	No ppt.
Isoelectric point	1.5 - 2	2 - 2.5

Ethylene diamine was found to be the best substitute for ammonia as it have caramel having very good colour intensity and properties making it suitable in beverage industry. Ethylene diamine is being used as pharmaceutic aid in injections and has very low toxicity, the LD_{50} being 1.16g/kg when fed orally to rats[7]. It could prove to be a promising catalyst in the preparation of caramel if the unidentified compound found in the ethylene diamine caramel were proved to be harmless.

References

1. Greenshields, R. N., Caramel 2. Manufacture, composition and properties, *Process Biochem.*, 1973, 8(4), 17.
2. Greenshields, R. N. and Macgillivray, A. W., Caramel. 1. Browning reactions, *Process Biochem.*, 1972, 7(12), 11, 16.
3. Nishie K., Waiss A. C. Jr. and Keyl, A.C., Toxicity of methylimidazoles. *Toxicology and Applied Pharmacology*, 1969, **14**, 301.
4. Shankaranarayana, M.L., Abraham, K.O., Raghavan, B. and Natarajan, C. P., Caramel colour. *Indian Fd Pckr*, 1977, **31** (2), 26.
5. Wilks R. A. Jr., Shingler A. J., Thurman L. S. and Warner, J. S., Isolation of 4-methylimidazole from caramel colour and its determination by thin layer and gas liquid chromatography. *J. Chromatogr.*, 1973, **87**, 411.
6. Wilks, R. A. Jr., Johnson, M. W. and Shingler, A. J., An improved method for the determination of 4-methylimidazole in caramel colour. *J. Agric. Fd Chem.*, 1977, **25**, 605.
7. Anon, *Merck Index, An Encyclopedia of Chemicals and Drugs*, 9th Edition, Publ. Rahway Merck and Co. Inc., 1976, 499.

POPPING QUALITY OF HALF-SIB FAMILIES OF POPCORN

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Seventy three half-sib families from 'Ludhiana popcorn' were evaluated for popping quality. Out of the 73 families, 54, 36 and 14 families were adjudged to be on par with the best performing families, on the basis of popping percentage, popped volume and popping index values, respectively. No Significant correlation of either popping percentage or the popped volume could be established with grain weight or grain volume. 'HS 23', 'HS 72' and HS 81' excelled all others in popping index, popped volume and popping percentage Irespectively. Popping index seems to be the best parameter for screening.

There is a growing demand for popcorn as snack food and Punjab Agricultural University is engaged in developing high yielding varieties suitable for popping. A variety, 'Ludhiana popcorn' which was obtained through random mating is being further improved through intrapopulation improvement for popping quality, grain yield and other agronomic traits. Varaiability for popping quality in that population has been studied and reported in this note.

A random sample of 73 half-sib families from the 1981 kharif harvest was evaluated in quadruplicate. All the samples kept in air tight container were equilibrated for moisture for a week. The test conditions were standardized using population bulk. Grain weight and bulk volume were recorded before popping. A 40 grain sample was popped in a revolving real baking oven at 246°C for 5 min. The grain sample was kept covered in 100 g loaf, baking pan[1]. The number of popped and unpopped kernels were counted and the volume measured. The popping index was calculated as the ratio of popped and unpopped bulk volumes. The results were evaluated statistically.

The popping percentage ranged from 67.5 to 98.8 (Table 1). Fifty four families with popping percentage

TABLE 1. POPPING CHARACTERISTICS OF PROMISING FAMILIES OF POPCORN

Family	Grain wt. (g)	Unpopped vol. (cc)	Popped vol. (cc)	Popping (%)	Popping index
HS-3	6.2	11.8	112.5	83.1	9.6
HS-5	6.9	10.0	124.0	88.8	12.9
HS-6	8.0	9.5	71.0	71.9	7.7
HS-8	7.6	8.1	117.5	76.9	14.5
HS-9	7.1	9.4	104.0	91.3	11.2
HS-12	6.6	9.8	124.5	86.9	12.3
HS-13	6.3	10.4	145.0	96.9	14.3
HS-16	6.7	10.8	125.5	92.5	10.9
HS-17	7.5	10.0	165.0	91.9	16.8
HS-18	7.1	9.6	112.5	91.3	11.8
HS-22	6.2	10.1	162.5	93.1	16.4
HS-23	7.1	8.4	167.5	90.0	20.6
HS-24	6.9	10.6	145.0	91.3	13.6
HS-26	5.7	10.4	125.0	93.8	12.2
HS-28	6.4	10.9	167.5	98.1	16.9
HS-30	7.5	9.0	111.5	78.8	13.1
HS-32	6.9	10.3	113.8	78.1	13.5
HS-34	6.8	9.5	105.5	92.5	11.4
HS-38	7.0	9.7	126.5	76.9	13.6
HS-39	6.2	8.4	149.5	96.3	18.0
HS-41	7.1	9.9	107.0	85.6	10.9
HS-44	6.9	9.5	142.3	91.3	15.3
HS-48	7.2	9.9	132.0	89.4	13.6
HS-49	6.3	8.9	122.0	91.3	14.0

(Contd.)

HS-51	8.2	10.9	133.0	91.9	12.2
HS-52	7.0	8.8	136.3	94.4	15.5
HS-53	6.9	9.4	149.0	93.8	17.3
HS-56	6.9	9.6	99.5	93.8	10.8
HS-58	6.9	10.0	124.0	94.4	12.8
HS-61	7.4	10.1	144.0	96.9	14.4
HS-63	7.6	10.6	128.0	92.5	12.2
HS-64	7.4	10.3	135.0	94.4	13.4
HS-69	6.6	9.1	165.0	98.1	18.0
HS-72	6.6	9.0	172.5	93.8	19.4
HS-75	7.1	10.1	125.5	93.1	12.7
HS-76	6.0	8.6	106.0	91.5	12.5
HS-79	6.4	9.0	93.0	91.9	10.6
HS-81	6.5	9.3	142.5	98.3	15.9
HS-83	7.1	9.9	137.5	95.6	14.0
HS-87	7.4	10.3	133.8	93.1	13.1
HS-88	7.2	10.1	136.0	94.4	13.8
HS-89	6.6	9.3	112.5	93.8	12.6
HS-91	7.2	10.4	119.5	88.8	12.0
HS-92	8.0	11.1	115.0	86.3	10.3
HS-94	7.5	10.3	127.5	80.0	12.3
HS-96	7.5	10.3	127.5	91.9	12.6
HS-98	7.5	10.5	115.5	85.6	10.9
HS-101	7.0	10.0	110.0	81.3	11.2
HS-106	6.4	8.9	117.5	85.0	13.9
HS-107	6.9	10.2	83.5	78.1	8.1
HS-109	7.9	11.0	102.3	67.5	12.0
HS-114	7.0	9.7	140.0	97.5	14.5
HS-115	6.6	9.5	135.0	93.1	14.3
HS-117	6.0	8.5	100.0	94.4	11.1
HS-119	6.6	9.8	133.5	96.9	14.0
HS-121	6.3	9.1	136.0	93.1	15.1
HS-122	6.8	9.5	81.0	95.0	9.0
HS-124	6.5	9.6	123.5	93.8	13.0
HS-125	7.4	9.1	130.5	96.3	14.4
HS-127	7.0	10.1	125.5	93.8	12.3
HS-129	7.1	10.1	123.5	95.0	12.3
HS-134	6.6	10.8	96.0	83.1	9.2
HS-135	6.9	9.8	117.5	91.3	12.3
HS-137	7.3	8.6	146.5	96.3	17.1
HS-138	6.7	9.4	122.0	89.4	13.2
HS-139	6.7	9.8	147.0	94.4	14.9
HS-141	6.6	9.3	115.5	90.0	12.1
HS-143	7.2	10.1	81.5	77.5	8.2
HS-146	7.2	10.0	114.5	87.5	11.4
HS-148	6.4	9.1	123.5	83.8	13.7
HS-150	6.6	8.9	107.5	80.0	12.2
HS-151	7.0	9.9	112.0	76.9	11.8
HS-154	7.5	10.4	95.0	81.9	10.7
Range	5.7-8.2	8.1-11.8	71.0-172.5	67.5-98.8	8.1-20.6
C.D.	—	—	48.4	12.9	5.8

of 85.9 and above were found to be at *par*. The highest percentage of 98.8 was obtained with 'HS-81' followed by 'HS-28' and 'HS-69' with 98.1 per cent popping. The popped volume ranged from 71.0 to 172.5 cc. The critical difference was 48.4 cc and as such 36 families were at *par*. The highest popped volume was obtained in the case of 'HS-72'. Based on popping percentage, 33 families were adjudged good.

The popping index value (v/v) ranged from 8.1 to 20.6, Fourteen families viz. 'HS-17', 'HS-22', 'HS-23', 'HS-28', 'HS-39', 'HS-44', 'HS-52', 'HS-53', 'HS-69', 'HS-72', 'HS-81', 'HS-121', 'HS-137' and 'HS-139' having popping index value of 14.8 to 20.6 were also adjudged best on the basis of both popping percentage and popping volume. In general, the average popping index values observed were on the lower side[2,3]. The popping index parameter can serve better for screening newer strains for popping quality as it brought out fewer strains at the top of performance compared with the popping percentage and the popped volume.

No significant correlation of the popping percentage or the popped volume with the initial weight or volume of the grain was found.

It was observed that there was significant variation in popping percentage, popped volume and popping index indicating the presence of genetic variability in the population. Thus, it should be possible to improve the popping quality of 'Ludhiana popcorn'.

References

1. *Approved Methods*, American Association of Cereal Chemists, St Paul, Minnesota, U.S.A., 1972.
2. Wallace, H. A. and Bressman, E. N., *Raising Popcorn*. In *Corn and Corn Growing*. John Wiley and Sons Inc. New York. 4th ed. 1937.
3. Alexander, D. E. and Creech, R. G., *Breeding special types of corn*. In *corn and corn Improvement*, Sprague, C. F. (Ed) American Society of Agronomy, Inc. Wisconsin, U.S.A., 1977.

NOTE ON LIPID COMPOSITION OF HYBRID PEARL MILLET VARIETIES

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Lipid content of five hybrid and one local variety of pearl millet ranged from 56.5 to 66.5 mg/g. The polar lipid contents varied from 12.0 to 20.0 mg/g but greater variation was noticed in nonpolar lipid contents. The ratio of nonpolar to polar lipids in hybrid varieties was ~ 3.0 and in the local variety ~ 4.5. Glycolipids were the major polar lipids and triglycerides and free fatty acids were the main nonpolar lipids. 'GHB-27' variety was more akin to S-207 local variety than the other hybrid varieties.

Improvement in the production of pearl millet (*Pennisetum typhoides*) through the introduction of high

yielding hybrid varieties has been considerable, but the grain flour has not found wide acceptance in diverse food preparations except for "roti" making among rural population, the limiting factor being its high lipid content which reduces its keeping quality[1]. Very little information is available on the lipid composition of newly developed hybrid pearl millet grown extensively in Gujarat.

Seeds of five hybrid varieties ('C.J.-104', 'B.J.-104', 'GHB-1399' and 'GHB-27') grown under uniform agro-climatic conditions at Millet Research Station, Jamnagar in summer 1981 and one local variety ('S-207') grown at Plant Breeding farm, Anand Campus, were milled to pass through 60 mesh sieve, and the lipids were extracted[2] thrice with chloroform: ethanol: water (200:95:5) using 20 volume solvent mixture and shaking for 30 min. The extractants were pooled, concentrated under reduced pressure at 40°C and separated into polar and nonpolar lipids by solvent partition method using n-hexane and 90 per cent methanol. Hexane fraction containing nonpolar lipid components were dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to make up to a known volume. An aliquot was taken in duplicate and evaporated to dryness to determine total content of nonpolar lipids. The remaining hexane extract was kept aside in the cold for TLC spotting. The methanol extract was made protein free[3] and an aliquot of it was used in duplicate to determine the total content of polar lipids. The remaining extract was used for TLC spotting. Individual nonpolar lipids were separated by TLC on silica gel G using the solvent system diethyl ether-toluene-ethanol-acetic acid (40:50:2:0.2) and diethyl ether-hexane (6:94) following a double development technique as recommended by Morrison *et al*[4]. Spots were visualized by spraying with 10 per cent sulphuric acid and charring the plate for 15 min at 110°C. Triolein, fatty acid and sterol were used as standards on the same plate. Spots not corresponding to the available standards used were tentatively identified from the lipid profile distribution under this solvent system as shown by Morrison *et al*[4]. Spot areas were scanned in a photovolt densitometer and relative per cent calculated. Such TLC-densitometric method of analysis of lipid components have been reported[5,6] and the relationship between area of the peak and quantity of lipid per spot has been found linear over a wide range. Downing[7] has reported very high recovery by this method. Individual polar lipids were separated by TLC on silica gel-G using the solvent system chloroform-methanol (80:25) by the procedure of Nichols[8]. Identity of constituent lipid fractions could not be confirmed for want of standards and therefore tentatively identified by comparision with the lipid profile distribution as recorded by Nichols[3], following similar TLC

TABLE 1. LIPID CONTENT (mg/g) IN PEARL MILLET FLOUR (AIR DRIED BASIS)

Variety	Total lipids	Polar lipids	Polar components lipids					Nonpolar lipids	Nonpolar component lipids						$\frac{NP}{P}$ Ratio
			Sulpho-lipids	Phospha-tidyl ethano-lamine	Unknown	Glyco-lipids	Neutral lipids		Unknown	Free fatty acids	Sterols	Diglycerides	Triglycerides	Unknown	Sterol esters	
C.J.104	56.5±0.55	20.0±0.61	3.6	5.5	—	9.2	1.7	36.5±0.50	—	16.9	—	3.6	14.9	—	1.2	1.8
B.J. 104	59.0±0.23	16.0±0.35	2.7	3.8	0.7	7.4	1.4	43.0±0.10	—	22.7	—	3.7	15.6	—	1.1	2.7
GHB 18	59.0±1.30	14.0±1.22	2.9	3.6	0.7	6.4	1.5	45.5±1.50	—	17.9	—	1.9	23.4	1.3	1.0	3.3
GHB 1399	64.6±0.48	17.8±0.21	3.8	2.6	1.5	8.3	1.8	46.8±0.75	0.9	24.8	—	2.3	16.9	—	1.7	2.6
S 207	66.5±1.27	11.7±0.55	2.4	4.9	1.0	2.2	1.3	54.5±2.00	—	4.0	3.1	3.5	33.6	—	1.6	3.2
GHB 27	60.0±0.18	14.2±0.10	3.8	7.2	1.2	1.1	0.8	45.8±0.25	—	8.9	2.8	4.9	35.4	—	3.1	4.6

NP—Nonpolar lipids; P—Polar lipids

operating conditions. As in the case of nonpolar lipids, relative composition of individual lipids were evaluated by TLC-densitometry. Table 1 shows the lipid composition of six varieties of pearl millet. Total lipid content of these varieties ranged from 56.5 to 66.5 mg/g. Ahuja *et al*[3]. have reported the combined bound and free lipid contents of Punjab pearl millet varieties to be in the range of 32.8-47.4 mg/g. Shah and Mehta[9] observed that the crude fat content (ether extractive) of Gujarat varieties ranged from 48.8 to 58.9 mg/g. The higher values in our experiment is due to better extraction by the solvent used. Variation between varieties is also noted. Total grain lipid was the highest (66.5 mg/g) in local 'S-207' and lowest (56.5 mg/g) in the hybrid 'CJ-104' variety.

Variations in polar lipid contents was narrow from 12.0 to 20.0 mg/g while there was relatively wide difference in nonpolar lipid content of different varieties (36.5-54.5 mg/g).

The ratio of NP/P in hybrid varieties were generally large as compared to local 'S-207'. The ratio of NP/P is generally regarded as a good index for assessing flour quality-lower the ratio, better the loaf volume and better bread making quality[10]. Polar lipids, particularly galactolipids, are generally considered to improve the loaf volume[11]. In this regard, hybrid varieties (except 'GHB-27') may have better flour characteristics as compared to local 'S-207' variety. Major polar component in these varieties was glycolipids which constituted about 10 to 16 per cent of total lipids. Similar proportions of glycolipids have been reported in finger millet by Mahadevappa and Raina[12]. Phospholipids and neutral lipids in all varieties ranged within a narrow limit of 1.9-3.8 mg/g and 0.9-1.8 mg/g respectively.

Free fatty acids and triglycerides were the predominant nonpolar lipids. Only two samples yielded sterols; in other samples sterols could not be separated from fatty acids, because of tailing effect of the latter, which also may enhance the fatty acid values. Sterol content when separated, was found to be low (3.0 mg/g only). Free fatty acids in pearl millet ranged from 4.0 to 24.9 mg/g and triglycerides from 14.5 to 35.4 mg/g. Except 'C.J.-104', all the varieties showed some relation with triglyceride content and free fatty acid content-higher amount of triglyceride accompanied by lower amount of free fatty acids. This indicates lipolysis of triglycerides. Similar observation was made by Ahuja *et al*[3]. in Punjab varieties. 'S-207 was characterised by highest content of total lipid, triglyceride, diglyceride, sterol ester and maximum NP/P ratio which makes it most unsuitable for bread making. 'GHB-27' hybrid was more akin to 'S-207' than the other hybrid varieties. Both these varieties had higher triglycerides, low free fatty acids and low glycolipid content. There were three unidentified compounds in these lipid fractions, however, their quantity was limited to 0.7-1.5 mg/g.

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References

1. Bapna, S. L. and Murty, B. R., in *Bajra Production: Problem and Aspects.*, I.C.S.S.R. Allied Publishers, 1976, 62.
2. Inkpen, J. A. and Quackenbush, F. W., Extractable and "Bound" fatty acids in wheat and wheat products. *Cereal Chem.*, 1969, **46**, 580.
3. Ahuja, K. L. Sekhon, K. S. and Sehgal, K. L., Lipid Composition of pearl millet flour. *J. Fd Sci. Technol.*, 1979, **16**, 32.
4. Morrison, W. R., Tan, S. L. and Harjin, K. D., Methods for the quantitative analysis of lipids in cereal grains and similar tissues. *J. Sci. Fd Agric.*, 1980, **31**, 329.
5. Blank, M. L., Schmit J. A. and Privett, O. S., Quantitative analysis of lipid by thin layer chromatography. *J. Am. Oil Chem. Soc.*, 1964, **41**, 371.
6. Barrett, C. B., Dallas, M. S. J. and Padley, F. B., The quantitative analysis of triglyceride mixtures by thin layer chromatography on silica impregnated with silver nitrate. *J. Am. Oil Chem. Soc.*, 1965, **42**, 580.
7. Dowing, D. T., Photodensitometry in the thin layer chromatographic analysis of neutral lipid. *J. Chromatogr.*, 1968, **38**, 91.
8. Nichols, B. W., in *New Biochemical Separation*, edited by A. T. James and L. J. Morris, Van Nostrand, New York, 1964, **3**, 24.
9. Shah, H. C. and Mehta, B. V., Magnesium—Phosphorus-crude fat interactions in the seed of pearl millet., *Soil Sci.*, 1959, **87**, 320.
10. Pomeranz, Y. Okkyung, C. and Robinson, R. J., Relation between chemical composition and bread making potentialities of wheat flour, *J. Ass. Off. Cereal Chem.*, 1968, **43**, 45.
11. Finlay MacRitchie, Flour lipids and their effects in baking. *J. Sci. Fd Agric.*, 1977, **28**, 53.
12. Mahadevappa, V. G. and Raina, P. L., Lipid profile and fatty acid composition of finger millet. *J. Fd Sci. Technol.*, 1978, **15**, 100.

INFLUENCE OF VEGETABLE PROTEIN MIXTURE ON SENSORY CHARACTERISTICS OF BEEF PATTIES

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Meat patties were prepared by replacing beef partly with mixtures of protein-rich plant products like defatted groundnut flour (DGF), maize protein concentrate (MPC) or maize flour (MF) in such a ratio that each component contributed 50 per cent of the protein in the blend. The vegetable mixtures had water absorption capacity of 1.5 ml/g. Beef patties containing vegetable protein mixtures upto 20 per cent had comparable protein level and other attributes as that of the control and also preferred by a sensory panel. Beef patties extended with MPC and DGF were superior in organoleptic quality to those extended with MF and DGF.

Animal products are not in a position to meet the ever increasing protein needs. The solution lies in the use of quality vegetable products also. It had been suggested that many developing countries may have to depend to a great extent on cereal grains for their protein requirement[1]. Protein-rich plant products like soybean, groundnut, and sunflower seed have been used in meat blends[2,3]. Often, traditional preference and economics reduce the use of these novel protein sources to mere laboratory exercises. The use of locally accepted raw materials is therefore advocated. The objective of this study was to evaluate the potential of maize and groundnut mixtures as beef patty extenders.

Flours: White variety of maize was purchased from a local market in Akure, cleaned crushed in a roller and sieved through 1 mm screen. The grit was later milled into flour to pass through a 0.5 mm mesh sieve. Defatted groundnut grits were obtained from Ejinaka and Thornber (Nigeria) Limited, Aba. The grits were ground into flour of same particle size as that of maize flour.

Maize protein concentrate: Maize protein concentrate was prepared from maize flour by the method of Wu and Sexson[4] with slight modifications. Maize flour was suspended in deionised water in the ratio of 1.3:1 (w:v) and the pH adjusted to 11.7 with 0.1N NaOH. The suspension was allowed to stand for 30 min with periodical stirring and screened through a sieve of 0.35 mm mesh to remove the solids. The liquid fraction was centrifuged for 20 min at a speed of 1100 rpm and decanted. The supernatant was then readjusted to pH 4.7 with 0.1N HCl to facilitate protein precipitation. The liquid fraction was decanted and the protein concentrate quantitatively transfered into dialysis bag. The protein was dialysed for 4 hr in tap water with two water changes. After dialysis, the protein concentrate was dried in an air oven at 40°C for 14 hr.

Patty preparation: The groundnut flour was blended with maize protein concentrate (MPC) or maize flour (MF) to form vegetable mixtures A and B, respectively. The cereal and legume mixtures were then blended in a ratio which ensured that each component contributed 50 per cent of the protein of the vegetable mixtures by analysis.

Beef used in this study was obtained from a local butcher and frozen before use. The frozen beef was slightly air-thawed at room temperature (24°C), sliced, and ground with a hand grinder. Beef patties containing 10, 20 and 30 per cent vegetable mixtures were fabricated after mixing ground beef, vegetable mixtures and water (1.5 ml/g of vegetable mixture) thoroughly in a bowl. Patties of approximately 15 cm diameter and 1.1 cm thick were moulded, wrapped with polyethylene, and frozen. The patties were air-thawed for 10 min and fried in a saucepan with vegetable oil and onions. Preliminary trial showed that frying each side for 12 min at 179°C resulted in satisfactorily fried patty. The fried patties were served warm for sensory evaluation.

Analyses and water absorption: Protein was determined by the Kjeldahl method[5] and ash, crude fat, and moisture by the methods of Pearson[6]. Total carbohydrate was calculated by difference. All analyses were done in triplicates.

Fifteen ml of tap water was added to 2.0 g vegetable mixture in a centrifuge tube and stirred periodically for 30 min. The liquid was decanted and measured. The quality of water remaining in the flour was calculated and defined as absorption capacity.

The patties were cooked and evaluated for colour, flavour, juiciness, and overall acceptability by a panel of 10 judges using a 7-point scale (where 7=excellent, 6=very good, 5=good, 4=fair, 3=poor, 2 very poor, 1=unacceptable). Results of sensory evaluation were statistically analysed[7].

Composition: The proximate composition of vegetable products, beef, and fabricated patties are presented in Table 1. The protein content of the maize flour and maize protein concentrate were 10.53 and 69.55 per cent respectively. Cereal: legume blends are reported to have desirable effect when half of the protein is contributed by each component. On this premise vegetable mixture

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TABLE 1. PROXIMATE COMPOSITION OF RAW MATERIALS USED AND PATTIES

	Moisture (%)	Carbohydrate (%)	Protein (%)	Ether extract (%)	Ash (%)
Maize flour (MF)	7.82	75.99	10.53	4.11	1.55
Maize protein concentrate (MPC)	2.70	22.25	69.55	0.50	5.00
Defatted groundnut flour (DGF)	7.05	24.49	57.16	5.20	6.10
Ground beef (100%)	48.20	1.41	18.09	31.21	1.09
Ground beef (90%) : MF + DGF (10%)	48.49	5.35	16.87	28.04	1.25
,, ,, (80%) : ,, + ,, (20%)	48.86	5.90	15.85	28.08	1.31
,, ,, (70%) : ,, + ,, (30%)	49.75	6.32	15.49	27.05	1.39
,, ,, (90%) : MPC + ,, (10%)	48.82	4.55	17.34	28.12	1.17
,, ,, (80%) : ,, + ,, (20%)	49.00	4.76	17.08	27.86	1.30
,, ,, (70%) : ,, + ,, (30%)	49.34	5.48	16.98	26.25	1.95

*Average of three determinations

B (maize flour and defatted groundnut flour) which had predominantly maize flour and vegetable mixture A (MPC and DGF) which contained more of groundnut flour were prepared.

Corbohydrate content of the maize flour was the highest but the ash content was low. Crude fat content of the maize protein concentrate was significantly lower. Addition of vegetable protein mixtures upto 30 per cent level did not significantly alter the composition, but there was substantial increase in the total carbohydrate content of the extended beef patties.

Water absorption capacity: Mixtures of plant proteinaceous material had water absoprtion capacity of 1.5 ml/g. The moisture content of the maize protein concentrate has little effect on the water absorption capacity of the vegetable mixture since defatted groudnut flour was the major component in the blend.

Sensory evaluation: Colour rating was higher for beef patties containing vegetable products (Table 2). The lower rating for all-beef patties may be due to the oxidation of muscle pigments. Extended beef patties were generally rated higher for flavour, probably because of the frying which imparted good flavour and aroma. Except for the 30 per cent beef replacement with mixture A, there was no significant difference in flavour ($P<0.05$). The low flavour rating for this sample was

TABLE 2. SENSORY EVALUATION OF PATTIES

Ground beef (%)	MF+DGF (%)	MPC+DGF (%)	Colour	Flavour	Juiciness	Overall acceptability
100	—	—	4.6	4.1	4.6	4.5
90	10	—	5.1	4.2	4.4	4.6
80	20	—	5.4	4.7	4.7	5.2
70	30	—	5.4	3.6[a]	4.6	4.5
90	—	10	5.0	4.5	4.7	4.7
80	—	20	4.9	5.4	5.4	5.5
70	—	30	4.7	4.8	4.8	4.5

MF =Maize Flour, MPC=Maize Protein Concentrate DGF=Defatted Groundnut Flour
*Mean of ten scores; [a]($P < 0.05$)

due to the strong maize flavour. There was no significant difference in the juiciness (as judged by mouth feel) samples.

The beef patties extended with vegetable mixtures were rated higher than the all-beef control in overall acceptability. Beef patties with 20 per cent vegetable mixtures were preferred. At this level, taste panel scores of mixtures A and B were highest for most attributes.

References

1. Shukla, T. P., Cereal proteins: Chemistry and food applications. *Crit. Rev. Fd Sci. Nutr.*, 1975, **6**, 1.
2. Lachance, P. A., Meat extenders and analogs in child feeding program. *Proc. Meat Ind. Res. Conf.*, 1972, **24**, 97.
3. Wills, R. B. H. and Kabirullah, M., Use of sunflower protein in sausages. *J. Fd Sci.*, 1981, **46**, 1657.
4. Wu, Y. A. and Sexson, K. R., Protein concentrate from normal and high lysine corns: Preparation. *J. Fd Sci.*, 1976, **41**, 512.
5. *Official Methods of Analysis*, Ass. Offic. Anal. Chem., Washington, D. C., 11th Ed. 1970.
6. Pearson, D., *The Chemical Analysis of Foods*, Churchill Livingstone Co., New York, 1976.
7. Snedecor, G. W. and Cochran, W. G., *Statistical Methods*. Iowa State Univ. Press, Ames., Iowa1, 967.
8. Oke, O. L., A method for assessing optimum supplementation of a cereal-based diet with grain legumes. *Nutr. Rep. Intl.*, 1975, **11**, 313.

USE OF SOME VEGETABLE OILS, PLANT EXTRACTS AND SYNTHETIC PRODUCTS AS PROTECTANTS FROM PULSE BEETLE, *CALLOSOBRUCHUS MACULATUS* FABR IN STORED GRAM

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Effect of certain plant extracts, vegetable oils and organic compounds on the pulse beetle (*Callosobruchus maculatus* Fabr.), infesting gram was studied. Thirty three days after treatment, it was revealed that *Ratanjyoti* oil at 0.1, 0.2 and 0.3 per cent levels (v/w), Neem oil at 0.2 and 0.3 per cent levels and *Bit* as well as *Undi* oil at 0.3 per cent level significantly inhibited the emergence of adult beetles. They further exhibited hundred per cent mortality of eggs and controlled the beetles upto a period of 33 days. The germination of the seed was, however not affected.

Legume is one of the main sources of protein and pulse beetle, *Callosobruchus maculatus* Fabr. is a serious pest of pulses in storage. Bags are treated either with insecticides or fumigants to check damage during storage. However, chemicals, used are hazardous to living man and animals and hence the present study was undertaken to find out an effective, cheaper and non-hazardous plant product to control *C. maculatus*.

Ten products Bit (benzyl isothocyanate), allitin (diallyl disulphide) Ratanjyothi oil (*Jatropha curcas* L), neem oil (*Azadirachta indica* J., undi oil (*Callophyllum inphyllum*), Mahwa oil (*Basia latifolia*), Dodonaea extract (*Dodonaea viscosa* L) Nirgundi extract (*Vitex negunda*) coconut (*Cocos nucifera*) shell extract and clove oil were tested for their effectiveness against *C. maculatus*. These products were either obtained from out side agency or extracted in laboratory. Bit and *Ratanjyoti*, Neem, *Undi* and Mohwa oil were received from the Biological Group, Bhabha Atomic Research Centre, Bombay and the Directorate of Non-edible oils and Soap Industry, Pune respectively. Rest of the products were extracted in the laboratory except clove oil. These products were dissolved in petroleum ether and gram seeds were treated at 0.1, 0.2 and 0.3 per cent volume by weight of seed and kept in perforated polythene bags. 100 seeds were treated for each treatment, a petroleum ether control and control seeds were also maintained. The effect of products was studied at start and after a period of 33 days.

Efficacy of vegetable oils/extracts was tested at start and after 33 days, by introducing 5 pairs of 0.24 hr old beetles into each petridish. Adult mortality, if any, was recorded after 24 hr and percentage of adult mortality was calculated. The percentage of eggs hatched was recorded. The adult emergence was recorded from 27th day onwards after release of beetles till the emergence was completely stopped. The percentage of adult emergence was calculated on the basis of the number of eggs hatched and the number of adults emerged. The data were analysed statistically.

At the end of the experiment (2 months after treatment) germination test was carried out at 27 ± 2°C and 65-70 per cent RH.

The data presented in Table 1 indicate that Bit and Allitin, both synthetic compounds showed cent per cent adult mortality even at the lowest concentration of 0.1 per cent and proved to be the most effective against pulse beetle. No eggs laying was observed in Bit and Allitin treated seed. Amongst the oils and plant extracts *Ratanjyoti* oil and Dodonaea extract at 0.3 per cent level exhibited significantly lesser number of eggs laid by *C. maculatus* than in other treatments. The average per centage of eggs hatched ranged from 0.0 to 98.74. All the test samples were significantly superior to control in reducing egg hatching at all the levels, except clove oil and Dodonae extract at 0.1, 0.2 and 0.3 per cent level

TABLE 1. EFFECT OF DIFFERENT PRODUCTS ON THE GROWTH AND DEVELOPMENT OF *C. MACULATUS* FABR. AT START

Protectant	Concn. % (v/w)	% mortality of beetles (Av.)	Number of eggs laid (Av.)	Av. eggs hatched (%)	Av. % of adults emerged
Bit	0.1	100.0	0.00	0.00(0.00)	0.00(0.00)
	0.2	100.0	0.00	0.00(0.00)	0.00(0.00)
	0.3	100.0	0.00	0.00(0.00)	0.00(0.00)
Allitin	0.1	100.0	0.00	0.00(0.00)	0.00(0.00)
	0.2	100.0	0.00	0.00(0.00)	0.00(0.00)
	0.3	100.0	0.00	0.00(0.00)	0.00(0.00)
Ratanjyoti oil	0.1	0.0	14.00	0.00(0.00)	0.00(0.00)
	0.2	0.0	12.33	0.00(0.00)	0.00(0.00)
	0.3	0.0	4.66	0.00(0.00)	0.00(0.00)
Neem oil	0.1	0.0	58.00	1.71(6.01)	0.00(0.00)
	0.2	0.0	53.33	0.64(2.64)	0.00(0.00)
	0.3	0.0	27.33	0.00(0.00)	0.00(0.00)
Undi oil	0.1	0.0	86.00	5.39(13.43)	19.44(21.74)
	0.2	0.0	51.66	0.00(0.00)	0.00(0.00)
	0.3	0.0	19.66	0.00(0.00)	0.00(0.00)
Mohwa oil	0.1	0.0	88.00	10.73(18.95)	19.44(25.54)
	0.2	0.0	84.66	0.39(2.09)	0.00(0.00)
	0.3	0.0	72.33	0.00(0.00)	0.00(0.00)
Dodonaea extract	0.1	0.0	29.66	95.40(77.76)	36.98(37.45)
	0.2	0.0	16.33	90.29(72.19)	33.92(35.59)
	0.3	0.0	8.33	10.00(15.00)	0.00(0.00)
Nirgudi extract	0.1	0.0	98.66	90.55(72.19)	42.82(40.86)
	0.2	0.0	77.00	86.62(68.60)	37.57(37.78)
	0.3	0.0	49.66	83.84(66.44)	27.93(31.89)
Coconut shell extract	0.1	0.0	101.66	94.41(76.32)	36.82(37.76)
	0.2	0.0	94.66	90.84(72.45)	34.11(35.76)
	0.3	0.0	90.66	65.78(54.19)	32.93(35.01)
Clove oil	0.1	0.0	91.66	96.98(80.18)	41.55(40.11)
	0.2	0.0	90.00	96.30(79.37)	40.02(39.24)
	0.3	0.0	77.00	92.21(73.91)	39.39(38.85)
Petroleum ether (control)	0.0	0.0	105.00	98.41(82.82)	73.57(59.02)
Control	—	0.0	106.66	98.74(83.66)	73.72(59.24)
S.E. ±		—	2.04	1.92	2.23
C.D. at 5%		—	6.04	5.66	6.59

Figures in parentheses are the transformed arcsin values.

TABLE 2. EFFECT OF DIFFERENT PRODUCTS ON GROWTH AND DEVELOPMENT OF *C. MACULATUS* FABR*

Protectant	Concn (%) (v/w)	% mortality of beetles	No. of eggs laid	Eggs hatched (%)	Adults emerged (%)
Bit	0.1	0.0	62.00	95.64(78.01)	79.64(63.21)
	0.2	0.0	44.33	7.12(12.12)	36.66(31.92)
	0.3	0.0	32.66	0.00(00.00)	0.00(0.00)
Allitin	0.1	0.0	66.33	95.50(77.89)	75.79(60.53)
	0.2	0.0	62.33	95.20(77.68)	72.50(58.41)
	0.3	0.0	60.00	94.92(77.33)	71.44(57.69)
Ratanjyoti Oil	0.1	0.0	56.00	1.85(11.76)	0.00(0.00)
	0.2	0.0	52.00	0.00(0.00)	0.00(0.00)
	0.3	0.0	47.33	0.00(0.00)	0.00(0.00)
Neem Oil	0.1	0.0	59.66	17.80(24.86)	38.33(38.16)
	0.2	0.0	53.00	0.00(0.00)	0.00(0.00)
	0.3	0.0	42.00	0.00(0.00)	0.00(0.00)
Undi Oil	0.1	0.0	90.66	48.57(44.19)	60.84(51.27)
	0.2	0.0	86.66	31.95(34.40)	43.43(41.18)
	0.3	0.0	71.66	0.00(0.00)	0.00(0.00)
Mohwa Oil	0.1	0.0	93.33	51.47(45.86)	65.96(54.31)
	0.2	0.0	85.00	25.45(30.28)	41.65(40.20)
	0.3	0.0	84.33	7.90(16.20)	35.55(36.57)
Dodonaea extract	0.1	0.0	72.33	93.99(76.11)	64.19(53.25)
	0.2	0.0	39.33	91.57(73.25)	52.92(46.68)
	0.3	0.0	24.00	81.15(65.07)	49.38(44.65)
Nirgudi extract	0.1	0.0	99.66	96.31(78.98)	71.51(57.73)
	0.2	0.0	92.66	99.98(72.55)	70.75(57.30)
	0.3	0.0	71.33	86.78(68.81)	69.24(56.32)
Coconut Shell extract	0.1	0.0	103.33	95.80(78.28)	74.02(59.50)
	0.2	0.0	96.66	94.45(76.42)	73.49(59.08)
	0.3	0.0	92.33	87.20(69.36)	72.21(58.19)
Clove Oil	0.1	0.0	100.00	96.04(78.85)	76.63(58.34)
	0.2	0.0	97.33	95.19(77.32)	72.32(58.27)
	0.3	0.0	91.00	93 04(74.76)	72.04(58.08)
Petroleum ether (control)	0.0	0.0	107.66	96.28(78.96)	78.77(62.57)
Control	—	0.0	108.33	96.31(79.01)	78.59(62.45)
S.E.	—	—	±1.48	±1.77	±3.16
C.D. at 5%	—	—	2.38	5.22	9.37

Figures in parentheses are the transformed arcsin values.
*33 days after treatment.

respectively. Cent per cent mortality of eggs was noticed in *Ratanjyoti* oil at all the levels, *Undi* oil at 0.2 and 0.3 per cent and Neem, Mohwa oil at 0.3 per cent level. This might be due to inhibition of spreading of binding material of egg on the grain surface. The percentage of adult emerged in different treatments ranged from 0.0 to 73.72, Neem oil, Bit, Allitin and *Ratanjyoti* oil at 0.1, 0.2 and 0.3 per cent levels, Mohwa and *Undi* oil at 0.2 and 0.3 per cent levels completely inhibited adult emergence.

The data given in Table 2 (33 days after treatment) indicate that none of the treatments had any effect on the adult mortality of *C. maculatus*. The average number of eggs laid in different treatments ranged between 24.00 and 108.33. All the products were significantly effective in reducing the average number of eggs laid. The treatments with neem oil and *Ratanjyoti* oil at 0.2 and 0.3 per cent, Bit and *Undi* oil at 0.3 per cent level were significantly more effective in inhibiting the hatching of the eggs than the rest of the treatments. *Ratanjyoti* oil at 0.1, 0.2 and 0.3 per cent, Neem oil at 0.2 and 0.3 per cent and Bit and *Undi* oil at 0.3 per cent level were significantly superior to the rest of the treatments in reducing the per cent adult emergence. The average percentage of germination ranged between 86.00 and 87.66 which is statistically not significant. Therefore it could be concluded that the protectants tested can safely be used in storing pulses for prolonged period. Mammigatti and Raghunathan[2] have observed that coating of green gram with castor, gingely and mustard oils at 0.3 per cent and coconut or groundnut oils at 0.5 per cent level inhibited the multiplication of *C. chinensis* for 45 days. Similarly Schoonhoven[3] has reported the use of groundnut oil against *C. maculatus*. Naik[4] also reported neem oil and karanj oil at 0.5 per cent level protected cowpea seed from *C. maculatus* damage for about 6 months. Cowpea seed was protected from the attack of *C. maculatus* if treated with neem kernel oil at 8 ml/kg seed which exhibited reduction in egg laying, ovicidal and larvicidal activity[5].

References

1. Prabhu V.K.K., John M. and Ambikamma, B., Juvenile hormone activity in some south Indian plants. *Curr. Sci.*, 1973, **42**, 725.
2. Mammigatti, S. G. and Raghunathan, A. N., Inhibition of multiplication of *Callosobruchus chinensis* L. by vegetable oils. *J. Fd Sci. Technol.*, 1977, **14**, 184.
3. Schoonhoven, A. V., Use of vegetable oil to protect stored bean from bruchid attack. *J. econ. Ent.*, 1978, **71**, 254.
4. Naik, R. L., *Efficacy of some vegetable oils as surface ptotectants for stored cowpea seeds against pulse beetle*, M.Sc. (Agri.) Thesis, Konkan Krishi Vidyapeeth, Dapoli,, Ratnagiri, India, 1980.
5. Pereira, J. P., The effectiveness of six vegetable oils as protectants of cowpeas and bambara groundnuts against infestation by *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *J. Stored Prod. Res.*, 1983, **19**, 57.

FATE OF QUINALPHOS AND MONOCROTOPHOS RESIDUES ON BITTER GOURD FRUITS

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Persistance of quinalphos and monocrotophos resisdues on bittergourd fruits following foliar spray each at 0.05 and 0.1 percent concentrations at fruiting stage was evaluated. Quinalphos treatments resulted in comparatively very low deposits but its residues were found to persist for one to two weeks above the maximum permissible limit of 0.25 ppm whereas monocrotophos degraded at a faster rate from 7.19 and 11.4 ppm to non-detectable limits within 10 and 11 days respectively.

Bitter gourd fruits punctured by fruitfly infestation degrade the market value of the fruits as well as adversely affect the economic cultivation of the crop due to heavy damages. The timely insecticidal sprays of quinalphos (0, 0-diethyl 0-quinoxalin-2-yl phosphorothioate) and monocrotophos (dimethyl (E)-1-methyl-2-methyl carbamoyl, vinyl) were found effective in controlling the damage to the crop[1,2]. These insecticidal treatments while protecting the crop from insects also leave their residues. Since no information is available on the residues of these insecticides on bitter gourd, it was thought fit to collect data on the build-up of the residues of these two insecticides.

Bittergourd, *Momordica charantia*, (cv. Arka Harit) was raised in $3 \times 3 m^2$ plots at Horticultural Experimental Farm of the Indian Institute of Horticultural Research, Bangalore during winter season of 1981 (December-February). The crop was sprayed with quinalphos (Kotophos 25 EC supplied by SEARLE India Ltd.) and monocrotophos (Nuvacron 40 EC supplied by CIBA-GEIGY India Ltd.) each at 0.05 and 0.1 per cent concentrations at fruit formation stage. Experiment was laid out in randomised block design, keeping three replications. The fruit samples from treated plots were collected after 1 hr of treatment for estimating initial residue levels and thereafter 1, 3, 5, 7, 10, 15 and 21 days after treatment.

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The fruit samples were cut into small pieces, 50g of these were blended with 100 ml chloroform in a Waring blender. Extraction of insecticide residues was repeated thrice with further aliquots of chloroform. The combined extracts were concentrated in an evaporator and passed through chromatographic glass columns containing activated adsorbent mixture of charcoal: Cellite 545: neutral alumina (2:2:1 w/w) for cleanup of insecticide residues from other co-extractives of plant material. The columns were eluted with chloroform. The chloroform extracts containing cleaned up insecticide residues were concentrated to a known volume under reduced pressure. A 5 ml aliquot representing 5g plant material was used to estimate the residues of quinalphos and monocrotophos colorimetrically by Getz and Watts[3] method. In order to know the efficiency of analytical techniques, recovery experiments were conducted by fortifying control samples of bittergourd fruits with known quantities of the two insecticides and residues were determined. The average recoveries of quinalphos varied from 85.0 to 87.5 per cent and monocrotophos from 80.0 to 85.0 per cent.

The initial residues of 1.92 and 3.3 ppm quinalphos in bitter gourd fruits of the crop sprayed at 0.05 and 0.1 per cent spray concentrations were found to degrade to non-detectable levels by 7 and 15 days respectively (Table 1). The rate of degradation of quinalphos residues from either of the treatments was slow at initial stages of spray treatment. This could be due to low wind velocity and temperature of the environment during this period which have adversely affected the evapotranspiration of the chemical from the fruit surface. The successive reduction in residues continued at 35.4 and 41.8 per cent within 3 days; 71.3 and 54.2 per cent in 7 days, 100.0 and 90.9 per cent in 10 days from 0.05 and 0.10 per cent applications respectively. The persistence of residues from higher dose (0.10 per cent) for longer period against lower dose (0.05 percent) indicates that the decay of chemical was dominated by chemical forces only and hence the degradation of residues followed the first order reaction of chemical decay. In addition, the grooved surface of the bitter gourd prevented the physical forces of insecticide degradation and allowed the residues to persist for longer period. In another study Prasad Rao[4] worked out 9 days waiting period for quinalphos on cole crops. This observation is almost similar to the present investigation.

Monocrotophos spray at 0.05 and 0.1 per cent concentrations were found to result in 7.19 and 11.4 ppm initial residues which were comparatively higher than from quinalphos treatments, applied at the same rate (Table 1). This difference in the build up of initial residues from the two different insecticides can be attributed to the purity of their respective formulations only. Monocrotophos, relatively a highly polar chemical persisted for 10 and 15 days after spray treatment at 0.05 and 0.1 per cent concentrations respectively. Comparatively increased loss in monocrotophos residues was observed just after 3 days of spraying. This could be due to the monocrotophos, being systemic and highly water soluble, finds a ready access to inner tissues allowing the chemical to degrade by biochemical processes. The degradation rate of monocrotophos residues on bittergourd fruits was comparatively higher than that of quinalphos and therefore the residues reduced down the tolerance limit of 0.2 ppm by 7 and 10 days almost same as that of quinalphos. While working on cucurbits, Prasad Rao[4] recommended a lapse of 9 days and Awasthi *et al*[5]. worked out 11 to 15 days for monocrotophos to degrade on cowpea pods. Awasthi *et al.*[6] have further confirmed the high persistence of monocrotophos residues in soybean by suggesting a waiting period of 13.1 days. Present investigations are also similar to the above findings. However, due to differences in texture, composition and growth rate of different vegetable crops, variation in the behaviour of pesticide residue degradation is quite obvious.

TABLE 1. RESIDUES (PPM) OF QUINALPHOS AND MONOCROTOPHOS ON BITTER GOURD FRUITS

Days after treatment	Quinalphos at concn of		Monocrotophos at concn of	
	0.05%	0.10%	0.05%	0.10%
0	1.92	3.3	7.19	11.4
1	1.51 (21.3)	2.72 (17.5)	5.82 (19.05)	8.28 (27.3)
3	1.24 (35.4)	1.92 (41.8)	2.97 (58.6)	5.78 (49.2)
5	0.55 (71.3)	1.51 (54.2)	2.03 (71.7)	3.44 (69.8)
7	N.D. (100.0)	0.96 (70.9)	0.62 (91.3)	2.03 (82.1)
10	—	0.41 (87.5)	N.D. (100.0)	0.78 (93.1)
15	—	N.D. (100.00)	—	N.D. (100.0)
21	—	—	—	—

The values are averages of three replications.
N.D.=Not detectable.
The figures in parentheses denote per cent reduction.

Authors wish to sincerely thank Dr K. L. Chadha, Director, Indian Institute of Horticultural Research, Bangalore for his keen interest and facilities provided for the present studies.

References

1. Man, G. S., Darsan Singh and Bindra O. S., Comparative efficacy of some newer insecticides against cucurbit fruit fly infesting spongy gourd. *Indian J. Pl. Prot.*, 1975, **4**, 110.
2. Gupta, S. M. and Verma, A. N., Relative efficacy of insecticides and contact poison to the adult of melon fruit fly. *Dacus cucurbitae*. *Indian J. Ent.*, 1979, **41**, 117.
3. Getz, M. E. and Watts, R. R., Application of 4-(P-nitrobenzyl) pyridine as a rapid quantitative reagent for organophosphate pesticides. *J. Ass. off. agric. Chem.*, 1964, **47**, 1074.
4. Prasada Rao, V. L., *Studies on the effect of certain organophosphatic insecticides on the important pests of cruciferous vegetables and on the residues of selected pesticides.* Ph. D. thesis 1976, Univ. Agric. Sci., Bangalore.
5. Awasthi, M. D., Dixit, A. K., Verma, S., Handa, S. K. and Dewan, R. S., Fate of monocrotophos and carbaryl on cowpea. *Indian J. Pl. Prot.*, 1977, **5**, 55.
6. Awasthi, M. D., Handa, S. K., Dixit, A. K. and Bhattacharya N. S., Dissipation of Metasystox and monocrotophos in or on soybean crop. *Indian J. Agric. Sci.*, 1978, **48**, 245.

BOOK REVIEWS

Advances in Biochemical Engineering. Vol—23: Edited by A. Fiechter, Springer-Verlag, Rezensionen 4023, Post fach, Heidelberger Platz, West Germany; 1982; Pp. 194.; Price: $ 39.10.

This volume has four review articles relating to different areas. The first review by Doell and his colleagues refers to the regulation of glucose metabolism in bacteria. Our current knowledge of glucose metabolism in bacteria is dominated by two major phenomena namely the Pasteur Effect and the Crabtree Effect. This review brings out clearly the role of various phosphofrctokinases and shows that this enzyme has perhaps little to do in this phenomenon. With regard to the Crabtree Effect the review shows that this process may not even be functional in bacteria and brings out the dangers in generalization with regard to regulation of glucose metabolism and shows that this may be different from that reported in yeast.

The second article by Rogers and his colleagues reviews the status of ethanol production and metabolism in Zymomonas. It covers all the known literature about this organism and the various conditions that have been developed to test its usefulness for alcohol production. It also brings out the future areas of work that need to be done specially keeping in view the high productivities this organisms can generate and the possibilities of genetic manipulations. The authors correctly conclude that the laboratory observations are extremely promising but challenges exists at the pilot plant level. The third article by Prof. Aiba on the growth of photosynthetic organisms, deals with the conditions that stimulate growth in eutrophic environments. The review also brings out the fact that photosynthetic bacteria and algae can not be treated in the same category as fat as the effect of either carbondioxide or oxygen is concerned. The last article by Fan and his colleagues is on the nature lignocellulose materials and their pretreatment for enzymatic hydrolysis. The various methods relevant for treating lignocelluloses have been compared but nothing appears cheaper than the caustic soda treatment. Although economical, this process needs specialised equipment.

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Advances in Biochemical Engineering/Biotechnology: Vol. 26, Edited by A. Fiechter, Springer-Verlag Berlin, Heidelberg, New York, 1983, Pp. 209, Price, US $ 38.

The latest volume in this popular series carries four excellent review articles, the first two of which are directly in keeping with the theme title "Downstream processing" while the other two pertain to Molecular cloning and Amino acid Fermentation. A welcome change has been introduced in renaming the series as Advances in Biochemical Engineering/Biotechnology in recognition of the value of this rapid developing technology to "progress in scientific developments and their practical applications".

As in the earlier volumes, the classification of the subject matter in each review article is excellent and helps at a glance to perceive the scope and depth of the topic discussed. Bell, Hoare and Dunnill have presented a comprehensive review on the formation of protein precipitation and their centrifugal recovery in which a vast area ranging from basic aspects of protein chemistry to the principles of centrifugal separation and modifi-

cation of industrial centrifuges is dealt with in detail. Technological processes involving protein precipitation and separation are also briefly discussed. Flaschel, Wandrey and Kula discuss ultrafiltration for the separation of Biocatalysts in the second review article in the book. Ultrafiltration as a separation process as well as its application to membrane reactions is analysed along with discussions on theory of ultrafiltration, process design and its utility for isolation of biocatalysts.

Esser and Hinrichs outline the basic strategies and limits to molecular cloning in heterologous systems. Discussing the stability and expression of foreign DNA, the review discusses results of recombinant DNA with biotechnological relevance at present and those which may have future perspectives in applied research. The "Conclusions and Perspectives" section of the Chapter presents a balanced and realistic apprisal on the state of the art of molecular cloning with respect to bacterial, yeast and higher plant systems. Emphasis is laid also on the more stable DNA recombinants obtained through the easier and less elaborate "classical" methods of sexual or parasexual cycle.

The last chapter on production of L-tryptophan by Microbial processes by Nyeste, Pecs, Sevella and Hollo' gives brief discussion on L. Tryptophan biosynthesis and its regulation, methods of production including preparation of substituted tryptophan derivatives and some data on the economics of tryptophan production and its potential applications.

The Chapters written in this volume give a wealth of information for those engaged in biotechnological research in academic as well as industrial areas. Tehy would form valuable reference material for many years to come and may deservedly receive repeated citation in future biotechnological literature.

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Computer Aided Techniques in Food Technology: Edited by Israel Saguy, Marcel Dekker, INC, 270, Madison Avenue, New York-10016, Pp. 494; $ 69.75.

Computer—aided techniques in the current decade has pervaded in almost all the spheres of human activity, and has almost saturated in certain fields of science. The author has rightly pointed out that the computer-aided techniques in Food technology is in its teething stage, and its application in Food Science is yet to be accomplished.

This volume almost fulfils the author's objectives, namely, to use this book as a reference literature for professional food engineers, food technologists, food scientists and Industrial personel, to expand and up date their knowledge on computer aided methods and techniques.

This book has tried to connect the circuit comprising of the components like computers, fundamental programming numerical techniques, Model building followed by various applications in Food Science, operational research, design problems, data processing, proces control, production control, rheology studies, optimization, PERT (Programme evaluation and review techniques). Quantitative analysis and simultation of food quality loses during processing and storage and transportation, finally new approaches in computer system developments, interface and sub-routines and professional responsibility all in correct sequence. To achieve this, the editor has chosen a multiauthor contribution.

The editor discusses the pros and cons of multiauthored collection. This approach may result in different philosophies, uneven courage, unwarrented duplication and inadvertant omission of important material. A few papers related to computer aided techniques in food science have been elaborately illustrated. To identify one of them i.e., "Computer analysis of Food chromatographic and Electrophoretic data of protein' the following details have been given: Introduction, Data Acquisition and smoothing, peak analysis, slope analysis, peak detection and baseline correction, moment analysis, protein characterisation, gel chromatography, molecular weight distribution analysis, Trans Electrophoresis and Estimation of molecular weights and diffusion coefficients.

In a similar way, the vast vacancy still existing in the field of computer-aided techniques in food science can be filled one by one. To mention a few of the areas to be occupied are anlytical field comprising of analysis of food nutrients, quantitative analysis of uxygen, nitrogen, carbon & sulphur, identification of the quantitative existence of Zn, Mn, Pb, etc., in food stuffs, technologies like Fermentation, Baking, Flavour, Microbiology, Packaging, Pesticide control, etc., product development, process control, planning monitering, evaluation services and anlytical quality control are the fields still left open for intensive exploration. This book will play a vital part in this heavy unending task.

VENKATAKUPPAIAH
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Food Protein Deterioration Mechanisms and Functionality: Edited by J. P. Cherry, A. C. S. Symposium Series, 206, Amrican Chemical Society, Washington D. C., 1982; pp. 444; US and Canada: $ 47.95, Export $ 57.95.

The major importance of proteins in food is due to its functional and nutritional properties. Various factors influence these properties either favourably or adversely. Under the auspices of the Agricultural Food Chemistry Section of the American Chemical Society a symposium on deterioration of food proteins was held in New York in August 1981 and the proceedings of this symposium have been published in the form of this monograph. The monograph has fifteen articles and is a mixed bag of diverse topics.

From the point of view of a food technologist, functional properties of a protein mean some characteristics like solubility, dispersibility, foam capacity, emulsification, gelation properties etc. These are intrinsically decided by the structure of the protein and interaction with other components in the food system. The intrinsic structure of a protein is essentially governed by its amino acid sequence which is decided by the genes which code for it. In the present monograph Asim Even deals with the genetic mechanisms which influence protein properties specially of plant proteins. Deterioration of food proteins can be due to either chemical, physical or microbiological factors.

Chemical factors like lipid perox dation, interaction with carbohydrates, phenolic compounds or strong alkali, deliberate chemical modification (such as acylation or reduction of disulfide bridges) which influence protein quality and functionality have been examined by specialists in these areas in six articles. Protein functionality as affected by physical factors like temperature, ionic environment etc. have been dealt with in four articles. The influence of endogenous and exogenous proteolytic activity on proteins have been discussed in one article. Some types of oriental food (Miso, Shoya, Tempeh, Ontom) are made from proteinaneous material through the deliberate intervention of microorganims and the processes affect protein quality. Improper storage of proteinaceous food material can lead to damage by fungi resulting in the formation of toxins. These aspects have been considered in an article by Cherry. The nutritional consequences of protein modification have been examined in another article.

This monograph is really meant for the specialist in the area of protein foods.

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'Phal Tatha Tarkari Parirakshan Proudyogiki' (Hindi): Sadasivan Nair S. and Harischandra Sharma, Rajasthan Hindi Grantha Academy, Jaipur; 1981; Pp. XXI+544; Price Rs. 48.00.

The authors have put lot of efforts in bringing out the present publication. Claimed to be the first university level book on the subject in Hindi, it attempts to present a comprehensive account of theoritical and practical aspects of the subject.

This book is divided into two parts.

Part I — *Pariraksan siddhant* ('Principles of preservation): Provides basic information on fruit and vegetable preservation in 7 chapters. First chapter '*Parirakshan-ek-parichay*' introduces the concept of preservation with a brief history. Principles of preservation are discussed under 'temporary' and 'permanent' preservation in the second chapter. Chapter on temporary preservation disucsses the principles, advantages and conditions for preservation raw fruits and vegetables by 'asepsis', 'low temperature', 'exclusion'/'retention' of moisture, waxing, 'exclusion' of air and by using mild antiseptics. Various 'non-heat' and 'heat' processing techniques are briefly discussed under 'permanent preservation'. Third chapter deals with the role of microoragnisms in food deterioration and fourth chapter is on the nutritive value of fruits and vegetables and effect of various preservation techniques on them. The fifth chapter on food additives is a compilation of exhaustive information on food additives and preservatives, specifications, etc. Sixth chapter is devoted to containers used in the fruit and vegetable preservation industry.

Last chapter of the part I gives the potential food manufacturers-some entrepreneurial guidelines.

Part II—though titled '*Alpa tap parirakshan*' (Low temperature preservation) deals in detail with preservation techniques including heat processing. First two chapters are on refrigeration, cold storage and freezing. The controversial technique of preservation by irradiation is briefly covered in the 3rd chapter. Fourth chapter on preservation by fermentation includes methods ranging from domestic pickling and vinegar preparation to industrial fermentation. Unit operations, raw materials, equipment detail for making fruit juices and beverages is the topic of fifth chapter. Chapter 6, 7 and 8 on commercial canning, canning of fruits and of vegetables respectively are complementary to each other. Subtitling of the 9th chapter categorieses sundrying as drying process and other methods as dehydration process. 10th chapter is all about jams, jellies, marmalades,

murabbas etc. followed by another chapter on concentrates with special emphasis on tomato products.

The text concludes with a small but useful chapter on byproduct utilization followed by glossary, bibliography and errata.

The second part could have been bifurcated into 'low temperature' and 'high temperature' preservation. Interchanging of certain chapters, clubbing of some sub divisions would have helped to maintain the sequence in text and avoided repetitions. Limiatation of type faces and sizes might have resulted in erratic and sometimes misleading subtitles. This could be overcome by adopting decimal system which is more scientific and useful to the students who heavily rely on captions and titles for last minute references and revision. A few illustrations in part I are wrongly placed. Spelling mistakes are much more than those listed in errata. Some factual errors seem to have escaped authors attention like inclusion of B and C vitamins under fat soluble group, giving tomato the honour of being the only one among fruits and vegetable to have acid content, stressing cough and cold as Vitamin D deficiency symptoms, omission of role of sunlight in Vitamin D synthesis and so on.

Since technical terminology in Hindi is still in the process of standardization, science writers have the task of discrete and careful handling of the same. The authors' aware of these and sincere efforts are made to tackle them. Influence of english has affected the flow of thought resulting in ambiguity in some places. Simple and straight narration would have been more appropriate. True and unimaginative translation of English terms on one side and use of English terms when much, popular and standardised Hindi terms are available, use of one word to convey more than one technical concept and vice verse, all these facts make a strong case for urgent standardization and popularization of the technical terminology. Different connotations of similar words, nuances of synonyms could have been taken advantge of.

Considering the comprehensive coverage of the subject '*Phal tatha tarkari parirakshan proudhyogiki*' is very useful as a source book to the students.

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

Elements of Food Borne Diseases: by J. A. Awan The Institute of Management and Technology, P.M.B. 01079, Enuger, Nigeria; pp. 156; Price: $ 12.00.

Food is the most important biological necessity and has to be consumed in sufficient quantity and should also supply all the ingredients necessary for the metabolic activity of the organism. There is competition for food from different living beings like man, animals including insects, plants and microorganisms and each one of them try to utilize it for its growth and development. Once the food is attacked by an agent it may be modified in such a manner that it becomes deleterious to other organisms. In addition it is also made unwholesome by extraneous chemical intentionally used by man to preserve crop plants, food articles and in keeping the environment 'clean'. The above mentioned book has been brought out by the author to dissiminate information on the agents responsible for food poisoning, food contamination and health hazards for students of food science and technology, catering establishments, nutritionists etc., who are interested in disease prevention.

There are nine chapters dealing with food poisoning caused by intrinsic chemicals toxicity by extraneous chemicals, fungal food poisoning, bacterial food intoxication, bacterial food infections, potozoen infestations, worm infestations and diseases transmitted by insects and rodents.

Some common elements, their sources and possible hazardous effects due to their excess in the diet have been listed. Some intrinsic material that may decrease the nutritive availability of certain food constitutents are presented in a tabular form. Many food articles that are items of diet like cassava brassicas, potatoes, bananas and certain vegetabls, when eaten in excess may prove toxic due to the presence of certain chemicals like hydrocyanic acid, oxalic acid, alkaloids etc. Certain precautions to be observed are evermented. Certain toxins of animal origin also have been mentioned.

Toxicity of agricultural chemicals like fertilizers, fungicids, herbicides, insecticides, rodenticides and molluscides have been discussed. Deliberate addition of adultrants to food articles, which are toxic have also received attention. Food additives which are not of edible grade, are commonly used in food processing that may be hazardous to health.

One of the important area, which is receiving worldwide attention by food technologists and nutritionists is fungal food poisoning. The author has described food poisoning caused by direct consumption of certain mushrooms as also other mycotoxins. The next chapter deals with bacterial food intoxication with emphasis on botalism *Bacillus cereus* and *Staphylococcal* toxins. The fifth chapter is devoted to bacterial food infections. Which causes illness when food containing living bacterial cells are infested. The organism listed are. *Brucella abortus, Campylobacter jejuni, Clostridium perfungens, Escherichia Coli, Salmonella* spp, *Shiegella* spp *Streptococcus faecalis, Vibrio cholerae, V. parahaemolyticus,* and *Yer inie faecalis.* Diseases caused by protozoans

and helminths are discussed in the next two chapters. The final chapter is devoted to food borne diseases spread through the agency of rodents and insects. The author also has given a list of titles of publications for further reading, followed by a glossary of terms relevent to the text of the book and an index.

The book provides information on food-borne diseases and will be a useful reference book for students of Food Science and Technology and other disciplenes concerned with food preservation and processing.

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

The Maillard Reaction in Foods ana Nutrition: Edited by George R. Waller and Milton S. Feather, ACS Symposium Series 215, American Chemical Society, Washington D. C. 1983; Pp. 585 Price: US & Canada $ 59.95 Export $ 71.95.

The book is based on a symposium sponsered by the Divisions of Agricultural and Food Chemistry and Carbohydrate Chemistry at the 183rd Meeting of the American Chemical Society, Lass Vegas, Nevada, from March 28 to April 2, 1982.

Louis Camille Maillard, a French Scientist, first reported his outstanding observation on the condensation of amino acids and sugars 72 years ago, and since 1950, the reaction is named after him. The reaction occurs between sugars and amino acids, polypeptides or polysaccharides and proteins, and gives rise to undesirable flavours and colours in foods during processing and storage. The reaction transaforms many foods, bland and unplesant tasting in their natural form, into most desirable ones as in coffee, bread, roast beef, toasted nuts etc., and has been deliberately used to flavour the engineered foods, so much so, during the last 20 years, thousand patents have been granted. Although many of them may be redundant, at last 45 patents specify mixing one or more amino acids with one or more carbonyl compounds and heating. The reaction produces mutagens some of which are carcinogens, affect nutrition and health, and also produce chemicals which have antioxidant and therapeutic values. These aspects are covered in 29 papers divided into seven sections.

The history of the Maillard reaction, written by, Kawamura, comprises the first section of this book.

The second section relates to the mechanism of the Maillard reaction and the identification of these products. The mechanism proposed by Hodge in 1953 involving Amadori rearrangement as a key step, has been accepted over a quarter of a century as most apt description. Namiki and Hayashi, now, propose a mechanism involving cleavge of the sugar molecule to yield a highly reactive two-carbon fragment prior to Amadori rearrangement. Nyhammar *et al.* discuss Strecker degradation products from glucose-glycine reaction. Russell discusses reaction of Amadori compounds and their rearrangement products with nitrite to form nitrosamines having genotoxic effect. Change of colour in cane sugar during storage has been shown by Cheng *et al.* to be due to the formation of humic acids, caramel, 5-hydroxymethylfurfural, and melanodins. Coloured compounds formed by the interaction of glycine and xylose reported by Nursten and O Reilly include 4-hydroxy-3 methyl-3 (2H) furanone, and 2-furfurylidene-4-hydroxy-5 methyl-3 (2H) furnanone. Lipid-oxidative and sugar-derived fluorescences have been proposed by Porter *et al.* for measuring deterioration during storage.

The various flavours, tastes and odours produced by the Maillard reaction are detailed in the third rection. Included in this section are a variety of odours produced in more than 400 model systems drawn from 21 amino acids and sugars studied by Lane and Nursten: mutagenetic and antioxidative properties of the roller-dried soya flour, glucose, and lysine by Dworscha'k *et al.*, formation of desirable meat flavour by reaction between amino acids and carbohydrates written by Bailey; and a survey of sensory properties of nearly 450 Maillard reaction products by Susan Fors.

Chapters 14 to 16 relate to the application of Maillard reaction in food processing. Danehy and Wolnak have discussed the formation of desirable flavours in bland raw-materials like chocolate, cocoa and meats during processing and exploitation of Maillard reaction to produce desirable flavours. Bichner and Wolf describe the use of Amadori compounds as a means to optimize the drying and storage conditions of dehydrated carrot. Lingnert *et al.* by dialysis and electrophoretic fractionation of the histidine-glucose reaction product and electron paramagnetic resonance studies of the fractions, demonstrate the presence of free radicals responsible for the antioxidative property of the Maillard reaction product.

The effect of browning on nutrition is discussed in the fifth section. Included in this section are the report by Johnson *et al.* that consumption of toasted and browned foods like cornflakes reduced zinc retention; the urinary zinc is bound to heavier compounds; and the urine of such subjects contain higher-molecular-weight amino substances than subjects eating corn grits. Knipfel *et al.* discuss the naturally occurring Maillard reaction in cereal grains with particular reference to rye, and the effect of heat treatment of alfalfa which results in protein increase of the fibre fraction and decrease in the *in vitro* digestibility of organic matter. Tsen *et al.* report the loss in the nutritive value during conventional

baking of bread, an excellent staple, although, Maillard reaction plays an important role in producing the unique golden brown crust essential to the development of full flavour and aroma. Also, included in this section are losses of available lysine in protein in Maillard reaction systems by Tucker *et al.*; effect of glucose and lysine reaction mixture on protein and carbohydrate digestion and absorption by Oste *et al.*; and determination of available lysine by various procedures in Maillard type products by Erbersdobler and Anderson.

The discovery, in the past decade, that Amadori products form readily in *vivo*, and that the levels of glycosylated hemoglobins reflect the glycemia over several weeks has greatly stimulated the interest in the Maillard reaction *in vivo*. Monnier and Cerami review the reports of nonenzymatic glycosylation causing acute or chronic molecular changes observed in diabetes and involvement of Maillard browning in general aging process. Therapeutic effects of Maillard reaction products have come to be well established. Mester *et al.* in their review report on stimulation of *Lactobacilli* and *Aspergilli* growths by N-glucosylglycine, and the usefulness of deoxyfructoserotonin in the treatment of leprosy.

The final section is devoted to the possible toxicological effects of the Maillard reaction. Pintauro *et al.* report based on long-term feeding of Maillard browned proteins to rats find no toxic effect. Barnes *et al.* Jagerstad *et al.* and Nagao *et al.* report that cooking processes such as frying, grilling and broiling of meat and fish, and roasting of coffee beans, involve Maillard reaction which gives rise to mutagens some of which are carcinogens. A ray of hope amidst these disturbing findings is the observation that addition of soya protein or antioxidants inhibit the formation of mutagens. Unlike the products formed during reaction at high temperatures, Omura *et al.* report the formation of weak mutagens by Maillard reaction at 100°C. Principal mutagens in such reactions include 5-hydroxymethyl furfural, (2-formy.-5-(hydroxymethyl) pyrrol-1-yl)-norleucine, 2-methyl-thiazolidine and reaction products of troise reductone with amino acids or nucleic acid related compounds.

Research on Maillard reaction and its product will continue. It is a 'Pandora Box' with good as well as evil phases. The literature reviews and the papers provide an up-to-date wealth of information to students, research workers, processors, nutritionists and toxicologists, and is a valuable publication.

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

ASSOCIATION NEWS

Hyderabad Chapter

The Annual General body meeting of the above Chapter was held in Hyderabad. The Secretary presented his report for the year 1983. The highlights of his reports, included the increase in membership to about 150, organization of seminar on "The role of food industries in Andhra Pradesh—constraints for Development, in collaboration with SISI and Food Craft Institute, arranging visits to BHEL, Hyderabad, Vinedale Breweries and Distilleries and arranging of seminars by experts on specialised topics. This was followed by the presentation of report by the Treasurer.

The nominations for the office of AFST(I) Hyderabad Chapter for the year 1984 were called but there was no response. Hence the Executive Committee met on February 4, 1984 and nominated the following for the different offices. President—P.G. Shrotreya, Vice-President Mrs. Yamuna Ranga Rao, Secretary—Mr. B. D. Tripathi, Joint Secretary—Mr. N. Mallikarjuna Rao, Treasurer—Mr. P. S. V. Ramana Rao.

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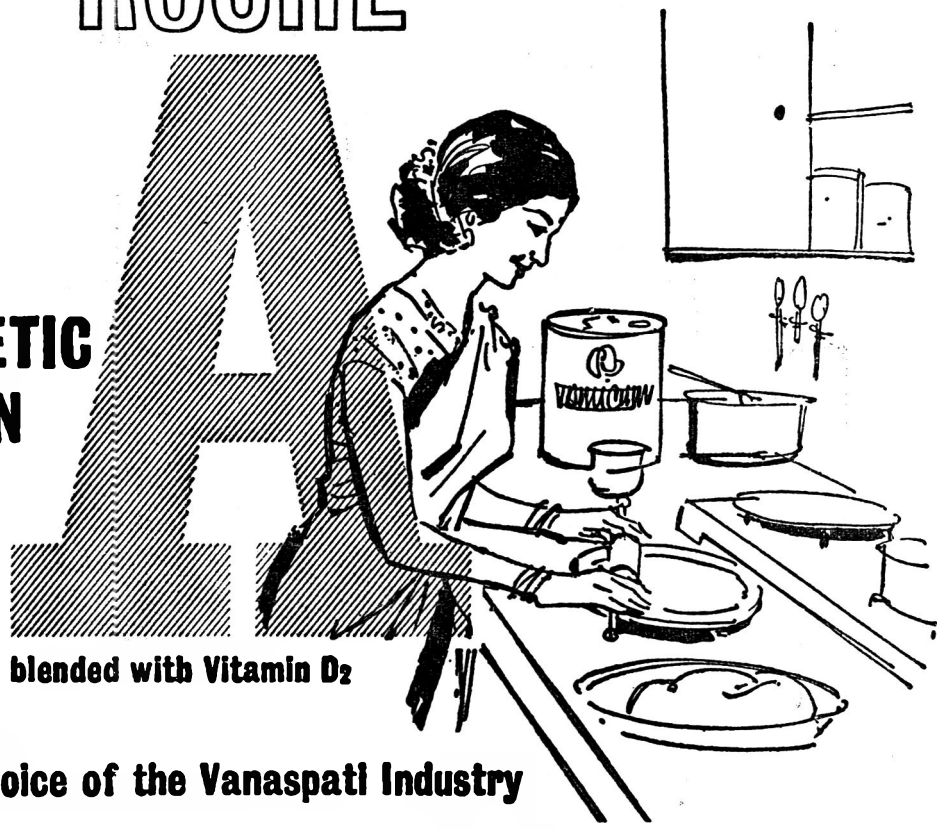

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INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (*in triplicate*) should be typewritten in double space on one side of bond paper. 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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Research Papers

Research Notes

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