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Research Papers

Research Notes

Emulsification Efficiency of Various Extracts of Fish, Goat and Beef Proteins in Model Food Emulsions and Preparation of Sausage

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A method of determining emulsifying capacity has been described. The emulsification efficiency of fish and goat proteins in various extracts have been measured and compared with those of beef. The maximum phase volume ratio determined from salt extract, water extract, actomyosin and slurry, vary in the order beef>goat>fish. Emulsification efficiency of proteins in presence of mixed fat (extracted goat fat mixed with peanut oil in varying proportions) was also studied. Although the viscosity values for different systems change slightly, the maximum phase volume ratio for a particular protein source does not vary appreciably with change in composition of the mixed fat. The protein stabilised water-in-oil emulsions seemed to be less stable than oil-in-water emulsions. All fish, goat and beef sausages were found to be acceptable to a taste panel, although goat and beef sausages scored better than fish sausage.

Recently, attempts have been made to study the physico-chemical properties of model food emulsions because of the importance of such properties in the preparation of sausage. The efficiency of emulsification of proteins from different sources may vary with the source and composition of fat. A number of combinations of proteins and fats from different animal sources need to be studied to find a good sausage formulation. The literature reveals two distinct aspects of most studies, namely physico-chemical and preparative. Though the emulsifying properties of animal proteins have been studied[1-5], fish proteins have not been so extensively studied. The emulsifying properties of fish proteins have been demonstrated in some recent studies[6,7] in developing frankfurter type of products. But quantitative data on emulsifying capacity is still lacking. An attempt has been made in this paper to measure emulsifying efficiency of fish, goat and beef proteins quantitatively in terms of phase-volume ratio[8]. Fish sausage was prepared and its properties compared with those of goat and beef sausages.

Materials and Methods

Preparation of protein extracts and slurry: A 100 g sample of beef, goat or fish meat was blended with 650 ml of cold (5°C) water or 0.5 M sodium chloride solution in a blender for 5 min. The slurry was centrifuged at 5000 r.p.m. for 10 min and supernatant used for emulsification.

Preparation of actomyosin: Actomyosin was prepared following the method of Herring *et al.*[9]; it contained actin and myosin.

Extraction of goat fat: Goat fat was procured from local market and heated in a beaker until the fat melted and separated. The melted fat was then filtered through a piece of fine cloth and stored in a bottle at 5°C. The melting point of goat fat was 49°C and those of goat fat peanut oil mixtures (35:65), (50:50) and (60:40) were 31°C, 34°C and 36°C, respectively.

Determination of protein concentration: The protein concentration was determined by AOAC method[10].

Viscosity of emulsion: Apparent viscosity of the emulsions was measured in a Brookfield Synchro-

lectic Viscometer (LVT model). A known volume of aqueous solution of known protein concentration was placed in a beaker along with a known amount of oil so that the phase-volume ratio (Φ) could be calculated. The mixture was blended and apparent viscosity (η) of the resulting emulsion was measured at 30.0±0.1°C. The phase volume ratio of the emulsion was increased by stepwise addition of oil and η measured.

Determination of type of emulsion: The type of emulsion i.e., oil in water (O/W) or water in oil (W/O) was determined conductometrically according to the method of Bhatnagar[11].

Preparation of sausage: After several trials, the following recipe was found to be the best: meat 100 g, fat or fat-oil mixture 60 ml, ice cold water 20 ml, salt 5g, garlic 3g, black pepper 2g. Deboned meat was first passed through a meat mincer. The minced meat was placed in a waring blender, ice cold water and salt were added and mixed for 1 min; oil and spices were then added and mixed for 3 min. The resulting sausage was stuffed into sausage casing (salted, washed goat intestine) with a hand stuffer. The sausage was then cooked at 80°C for 30 min.

Sausages prepared from the meat of fish, goat and beef were served to a taste panel comprising five persons. Designation of fish sausage made with peanut oil is (A), with hydrogenated vegetable oil (Dalda, MP 34°C) is (B) and with molten goat fat and peanut oil (50:50) is (C). Sausages with goat and beef meat were prepared with 50:50 mixture of goat fat and peanut oil. For each variety of sausage, five independent batches were made and their scores for odour, flavour, texture and acceptability were rated by the members of the taste panel. Details regarding scores are given in Table 3.

Other ingredients: Double refined peanut oil was purchased from the market and used without further treatment. All salts were of analytical grade.

Statistical analysis: Analysis of variance[12] was used to analyse the organoleptic scores to find out the statistical significance of different parameters.

Results and Discussion

Emulsions were prepared with the various extracts of proteins from beef, goat and fish and apparent viscosity of the emulsions after each stage of oil addition was measured. Fig. 1 shows a plot of apparent viscosity as a function of phase volume ratio ($\Phi = \frac{Vo}{Vo+Vc}$, Vo= volume of oil, Vc=volume of continuous phase) of emulsions prepared with salt-extracts of beef, goat and fish proteins. The curves were obtained by two different ways; firstly by increasing the phase-volume ratio with gradual addition of oil and measuring the viscosity (forward curve, solid lines) secondly by decreasing the

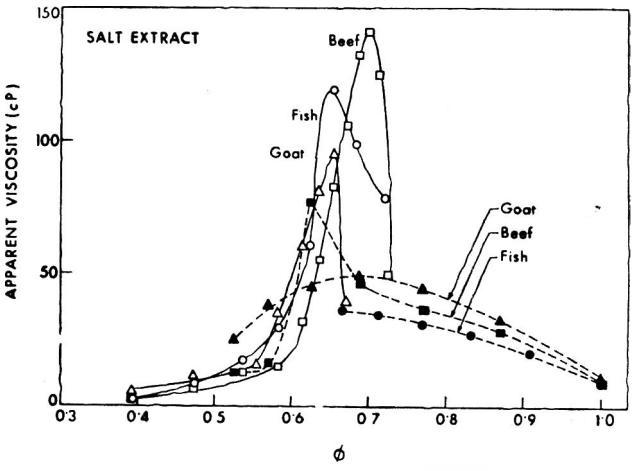

Fig. 1. Apparent viscosity against phase ratio of emulsions. Oil-water emulsion; – – – – – water-oil emulsion.

phase volume ratio with gradual addition of protein solution to the peanut oil (reverse curve, dashed line). Initially for the forward curve, the viscosity increased with increase in phase volume ratio, reaching a maximum at a certain phase volume ratio and then falling sharply. All the three emulsions-beef, goat and fish-show the same characteristics. The rise in viscosity is due to the excess population of emulsified droplets leading to a concentrate demulsion. The maximum viscosity represents a state of saturation with regard to the amount of emulsified oil in the protein solution. The sharp fall in viscosity is the result of coalescence of the emulsion droplets and subsequent breaking leading to the separation of oil phase. Thus, it is seen that the phase-volume ratio that gives the highest viscosity for a particular protein at a particular concentration may be a good index of emulsifying capacity. The end point is easily detectable and reproducible.

The average values of the emulsifying capacity of various extracts at different protein concentrations (Cp) as determined graphically have been presented in Table 1. It is seen that emulsifying ability of the three actomyosins are distinctly different. Beef actomyosin shows highest efficiency with maximum phase volume ratio (Φm) as high as 0.76. Fish actomyosin is the least efficient of the three having Φm as 0.64 only. Goat actomyosin has efficiency intermediate between the two having the maximum phase volume ratio of 0.70. It should be noted that a value of 0.74 for the Φm was attained theoretically if the emulsion droplets are of identical size[8]. The value of 0.76 for beef actomyosin probably indicates the theoretical saturation with a narrow distribution of particle size[8]. Fish and goat actomyosins are inefficient to reach the saturation. Although actomyosin acts as an emulsifier in all the three cases, the efficiency varies with the source. Chattoraj *et al.*[5] found during gel

TABLE 1. PEANUT OIL EMULSIFYING CAPACITY OF FISH, GOAT AND BEEF PROTEINS

Water extract		Salt extract		Actomycin		Slurry	
Protein concn. (mg/ml)	Φm	Protein concn. (mg/ml)	Φm	Protein concn. (mg/ml)	Φm	Protein concn. (mg/ml)	Φm
			Fish				
2.75	0.375±0.005	3.25	0.502±0.005	0.87	0.415±0.010	3.05	0.410±0.016
5.50	0.555±0.015	6.50	0.655±0.007	1.75	0.562±0.006	6.10	0.574±0.006
8.25	0.643±0.006	9.75	0.718±0.011	3.50	0.625±0.012	9.15	0.590±0.012
11.00	0.677±0.018	13.00	0.740±0.008	5.25	0.650±0.015	12.40	0.605±0.007
13.75	0.672±0.009	16.25	0.737±0.014			15.25	0.620±0.018
			Goat				
1.84	0.474±0.021	2.50	0.500±0.012	0.81	0.412±0.018	4.54	0.445±0.010
3.68	0.574±0.006	5.00	0.655±0.005	1.63	0.624±0.008	9.07	0.615±0.002
5.52	0.643±0.015	7.50	0.706±0.008	2.45	0.664±0.005	13.61	0.670±0.005
7.36	0.667±0.012	10.00	0.730±0.006	3.27	0.774±0.005	18.15	0.720±0.012
9.20	0.672±0.018	12.50	0.730±0.010			22.69	0.737±0.007
			Beef				
2.54	0.505±0.020	2.98	0.636±0.010	0.48	0.475±0.024	5.85	0.583±0.007
5.09	0.615±0.013	5.96	0.701±0.005	0.96	0.600±0.008	11.70	0.692±0.010
7.63	0.697±0.009	8.94	0.744±0.005	1.44	0.690±0.006	17.55	0.730±0.008
10.17	0.700±0.005	11.92	0.773±0.011	1.92	0.752±0.004	23.40	0.756±0.005
12.71	0.697±0.012	14.91	0.773±0.007	2.40	0.764±0.005	29.25	0.750±0.005

Mean ± SD of 3 determinations.

electrophoresis of actomyosins that number of protein bands differed depending upon the protein source. They also found that the mobilities of individual fractions of actomyosin from different sources were not exactly the same. This was attributed by them to the inherent small differences in the primary structure of the proteins. They further noticed that the colour intensities of the gel bands of the different fractions were also dependent on the source of actomyosin. These results may explain the differences in emulsifying capacity for various actomyosin preparations as being due to differences in the surface activity of the proteins. Schut and co-workers[4] suggested that one of the salt soluble proteins may become preferentially absorbed on the interface of the emulsion droplets so that the stability and efficiency of meat emulsion may be considerably increased.

From the data in Table 1, it is found that the relative order of effiicency of emulsification at a fixed protein concentration for water-extracts, salt extracts, slurries and actomyosins is the same i.e., beef>goat>fish. For a particular protein source, the efficiency of emulsification at a particular protein concentration varies in the order actomyosin>salt extract>water extract. The results of the study with mixed fat (goat fat mixed with peanut oil) are presented in Table 2 (Fig. 2). The fat was mixed in proportions of 35:65, 50:50 and 60:40 V/V with respect to goat fat and peanut oil. Results imply that for a mixed fat system at a particular protein concentration, the maximum phase volume ratio for fish, goat and beef protein emulsions is independent of the composition of mixed fat. A close comparison of the values in Table 1 and Table 2 reveals that Φm for emulsions of peanut oil only is always higher than that of emulsions of mixed fat at same protein concentrations;

TABLE 2. MIXED FAT EMULSIFYING CAPACITY OF FISH, GOAT AND BEEF SALT EXTRACTS AT 55°C

Goat fat: Peanut oil	Φm fish	Φm goat	Φm beef
35 : 65	0.520±0.008	0.610±0.015	0.550±0.006
50 : 50	0.525±0.012	0.615±0.010	0.550±0.004
60: 40	0.540±0.006	0.630±0.010	0.560±0.010

Concentration of proteins (mg/ml): fish, 3.25; goat, 5.00; beef, 2,98.

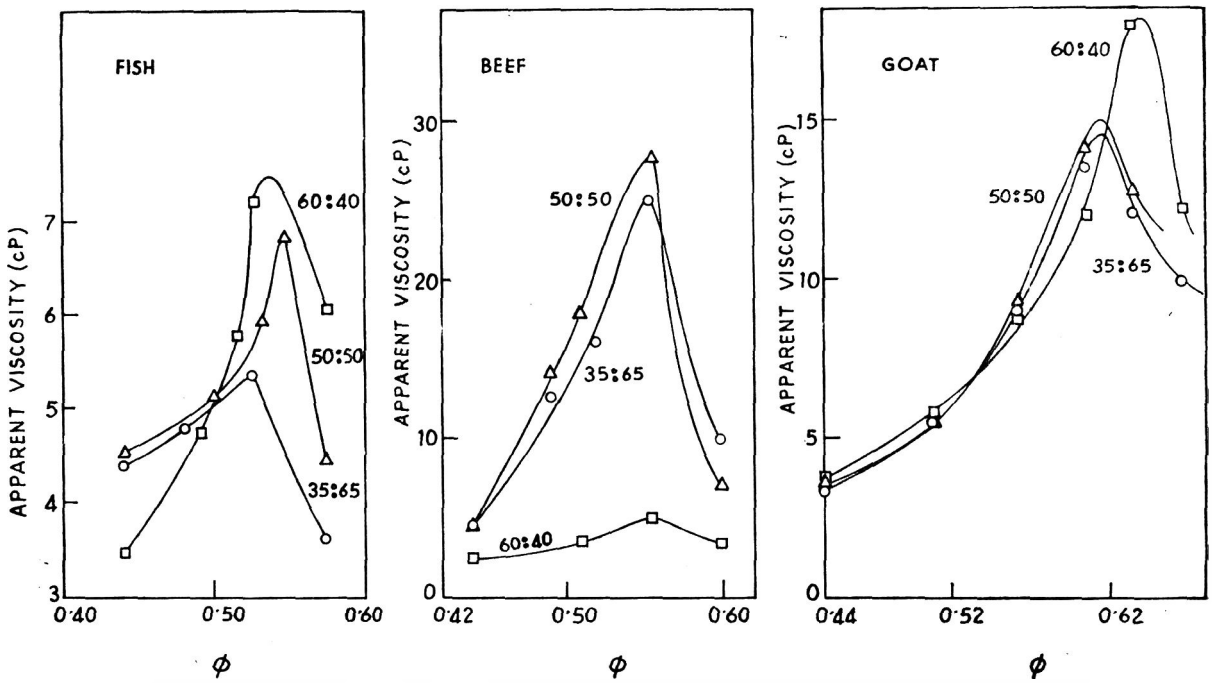

Fig. 2. Apparent viscosity against phase volume ratio of fish, goat and beef protein emulsions with goat fat: peanut oil in different ratios.

the difference may, however, be a result of different temperatures: 55°C for mixed fat and 30°C for peanut oil alone.

Analysis of the reverse curve of Fig. 1 reveals that initially viscosity increases gradually as we go from right to left. At a certain point, it cuts the forward curve and thereafter follows more or less the same trend as the forward curve. Initially, when the protein solutions were added to the large volume of oil, the emulsions were of W/O type[11]. At a certain stage, the viscosity begins to fall. This point was identified conductometrically as the phase inversion point changing from W/O to O/W

TABLE 3. SENSORY RATING OF COOKED FISH, GOAT AND BEEF SAUSAGES

Sample	Flavour	Odour	Texture	Overall acceptability	Average score
Fish A	4.0±0.3	4.0±0.0	3.0±0.2	3.5±0.2	3.63±0.40
Fish B	4.5±0.3	4.5±0.3	3.0±0.2	3.5±0.3	3.88±0.60
Fish C	5.0±0.0	5.0±0.0	3.5±0.3	4.0±0.1	4.38±0.60
Goat	5.0±0.0	5.0±0.0	4.5±0.3	4.5±0.3	4.75±0.25
Beef	5.0±0.0	5.0±0.0	5.0±0.0	5.0±0.0	5.00±0.00
Sig. diff. among 5 samples	0.178	0.126	0.509	0.193	
D. F. for error mean square	20	20	20	20	
S. E. of the mean	0.0419[a]	0.0297[b]	0.1200[c]	0.0456[d]	

Scoring

Flavour/Odour: Characteristic of fish or meat—5.0; slight fishy or meaty—4.0; just recognisable as fishy or meaty—3.0; no fishy or meaty—2.0; off flavour/odour—1.0.

Texture: Excellent (firm)—5; very good—4; good—3; fair—2; very soft—1.

Acceptability: Like extremely—5; like moderately—4; like slightly—3; neither like nor dislike—2; dislike—1.

system. Forward curves represent O/W emulsions and after reaching maximum viscosity do not generally invert to W/O system. This speaks in favour of greater stability of O/W emulsion than W/O emulsion with protein as emulsifier. It is also noted from the curve that it is often difficult to determine Φ m from such plots for W/O and O/W systems. So, in reporting the emulsifying capacity the type of emulsions formed need to be mentioned. The data in Tables 1 and 2 are for O/W emulsions. Analysis of variance shows that the differences in scores for each of the four parameters were highly significant ($P<0.01$). However, inspection of all differences between pair of mean square, employing Hantley's sequential variant of the Q method, originally due to Newman and Keuls as given by Snedecor and Cochran[12] showed that there was no difference in scores of fish C, goat or beef with regard to flavour and odour and with regard to texture and acceptability between Fish A and Fish B. In conclusion, with regard to flavour and odour Fish C compared well with beef and goat. But in texture and acceptability, none of the preparations compared well with beef.

Thus, although the texture of the fish and goat sausage was softer than beef sausage, and acceptability was not as high a popular fish sausage can be prepared.

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Enterotoxigenic *Staphylococcus aureus* in Infant Foods

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About 102 samples of infant foods were analysed for presence of enterotoxigenic staphylococci. A wide variation was observed in their incidence with a maximum average count of 3.5 per g. Staphylococci was present in 57.8% of the samples. Among the staphylococcal isolates, 30 per cent produced enterotoxins, mainly (90.7 per cent) of type B. Other enterotoxin types produced were A and C in combination with type B. Single radial immuno-diffusion was found to be less sensitive than rocket immunoelectrophoresis as these techniques detected 0.50 and 0.30 μg of enterotoxin, respectively. Other biochemical tests revealed that all enterotoxin producers were TDNase positive. However, only 84% of these produced coagulase. None of TDNase negative strains released enterotoxins, while 7.8% of coagulase negative strains produced enterotoxins.

Staphylococci constitute one of the most important group of pathogenic bacteria encountered in baby foods. These are widely distributed in nature. They gain easy access into milk and milk products and are responsible for various health hazards. A few workers have studied the incidence of pathogenic staphylococci in dried milk powder[1] and baby foods[2,3]. So far, no systematic attempt has been made to evaluate enterotoxin production by such strains. The present study was undertaken to investigate the pathogenicity of *S. aureus* strains isolated from baby foods by quantitatively evaluating their capability to produce different enterotoxins.

Materials and Methods

A total of 102 infant food samples were procured from different milk plants as well as from local market. These comprised different batches of 10 brands of infant milk food and 3 brands of infant cereal weaning food, designated as brand I to XIII. For microbiological analysis, samples were prepared as per the standard procedure recommended by Indian Standards Institution (ISI)[4].

Appropriate dilutions of the samples were spread over prepared plates of Baird-Parker (BP) medium using a hockey bat shaped bent glass rod[5]. A non-selective enrichment procedure[6] was followed using Tryptic Soy broth to enumerate low population of stressed cells of staphylococci. Suspected colonies from the BP medium were picked up and confirmed to be *S. aureus* using various biochemical tests such as sugar fermentation[7,8] catalase[9], phosphatase[10] and gelatinase[9] production and haemolysins[11] and salt tolerance (7.5 per cent). The enterotoxigenicity of *S. aureus* strains was initially checked by coagulase[12] and thermostable deoxyribonuclease (TDNase) production[13].

All the staphylococcal isolates were subsequently screened for enterotoxin production. A modified cellophane-over-agar procedure[14] was used to produce enterotoxins by individual strains. The identification of staphylococcal enterotoxins was done serologically, using microslide gel double diffusion test (MGDD)[15]. This test indicated the production of only three types of enterotoxins namely: A, B and C by the staphylococcal isolates. These enterotoxins were further quantified using single radial immuno-diffusion (SRID) technique[16] and rocket immunoelectrophoresis (RIE) technique[17]. Identification and quantification of staphylococcal enterotoxins was carried out using standard antisera A,B,C,D and E obtained from Dr. M. S. Bergdoll, FRI, Wisconsin, USA.

Results and Discussion

A wide variation was noted in staphylococcal counts in the different samples analysed. The average log counts ranged from 1.0 to 3.5 per gram of the sample. The distribution profile indicated the occurrence of staphylococci in 57.8 per cent of the samples (Table 1). A significant variation ($P<0.01$) with regard to the incidence of staphylococci was observed among and within different brands. The variation in incidence among different samples has also been reported by earlier workers[2,3]. One possible reason for such high incidence of staphylococci in infant foods may be due to their

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TABLE 1. DISTRIBUTION OF STAPHYLOCOCCI IN INFANT FOODS

Counts/g	Positive samples	Per cent
<10	18	30.5
10–100	13	22.0
101–500	22	37.3
501–1000	3	5.1
1001–5000	2	3.4
>5000	1	1.7

excessive load in raw milk[1]. The ISI[4] has not formulated any specific standards for staphylococci in infant foods. However, on comparing the present data with International Standards[18] it may be inferred that only 30.5 per cent of the positive samples can be considered to be of good quality as they contained less than 10 staphylococci per gram of sample.

The results obtained in relation to enterotoxigenicity indicated the production of enterotoxins by about 30 per cent of the isolates. These enterotoxins were produced either singly or in combination of more than one type. It may also be observed from Table 2 that 90.7 per cent of *S. aureus* isolates elaborated enterotoxin type B. Other types of enterotoxins produced were type A and C in combination with type B. The staphylococcal enterotoxins have been shown to be present in milk products including infant foods[2,3]. However, these authors indicated the predominance of enterotoxin A producers. Our observation of simultaneous production of more than one type of enterotoxin by single strain are in agreement with some of the recent reports[19,20]. Among isolates of *S. aureus* recovered during the present study, enterotoxin B producers predominated. Although this group of enterotoxins may not be as hazardous as type A or D, these have also been shown to cause food poisoning[21,22].

Higher sensitivity of RIE as compared to SRID is also evident, RIE and SRID detected a minimum of 0.3 and 0.5 μg enterotoxins, respectively. However, MGDD was found to be most suitable for qualitative purposes due to its higher sensitivity to detect a minimum of 0.25 μg. Majority of the cultures tested (72.1 per cent) produced enterotoxin less than 0.5 μg per ml.

TABLE 2. ENTEROTOXIN PRODUCTION BY S. AUREUS ISOLATES

Isolates (No.)	Enterotoxin types	Per cent
39	B	90.7
2	AB	4.7
2	BC	4.7

TABLE 3. ENTEROTOXIN PRODUCTION AND OTHER CHARACTERISTICS OF S. AUREUS ISOLATES

Characteristics	Enterotoxin producers	
	Number	Per cent
Mannitol fermentation (anaerobic)	43	100.0
Phosphatase	42	97.6
Gelatinase	41	95.3
Salt tolerance (7.5%)	43	100.0
Haemolysins	18	41.8
Coagulase	36	83.7
TDNASE	43	100.0

No. of enterotoxin producers: 43

In view of the difficulties encountered during the extraction and assay of staphylococcal enterotoxins, production of various other metabolites by pathogenic strains were used to possibly correlate them with enterotoxin production. The relationship of enterotoxigenic strains with other characteristics of *S. aureus* is given in Table 3. It was noted that 69.3 per cent of coagulase positive strains elaborated enterotoxins. Interestingly, 7.8 per cent of the coagulase negative isolates also produced enterotoxins. Since, more emphasis is now given to the relationship of TDNase with enterotoxin production[23–26], it may be inferred from the present findings that all enterotoxin producers were TDNase positive, but the reverse is not true as about 20 per cent of TDNase positive cultures did not produce any enterotoxin.

It may further be observed that all the enterotoxin producers fermented mannitol anaerobically and were salt tolerant as well. However, about 84 per cent of such cultures produced coagulase, while phosphatase and gelatinase activities were recorded in 97.6 and 95.3 per cent of the isolates respectively (Table 3).

It is concluded that incidence of entertoxigenic *S. aureus* should be viewed with great concern, as infant foods are meant to be consumed by the most vulnerable of the population. No single characteristic can be considered as an absolute indicator of entero-toxigenicity of *S. aureus*. There is a need to introduce specific standards regarding the incidence of pathogens like *S. aureus* in high risk foods such as infant foods.

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A Thermostable β-Galactosidase from *Alternaria palmi*

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β-Galactosidase of *Alternaria palmi* was purified by acetone fractionation, ultrafiltration through amicon filter PM-30 and column chromatography on DEAE-Sephadex A-50. Its molecular weight was estimated to be about 160,000 daltons by SDS polyacrylamide gel electrophoresis. The enzyme was most active between pH 5.0 and 5.5 and at 65°C. Only 25% of its activity was lost when heated at 65°C for 2 hr. Km and Vmax with ortho-nitrophenyl β-galactopyranoside as the substrate were 0.8 mM and 0.14 μ moles/mg protein/min, respectively. With lactose as the substrate, the values for Km and Vmax were 263 mM and 0.21 μ moles/mg protein/min, respectively. Metal ions as well as group specific reagents had no effect on enzyme activity.

Lactose not only contributes to the nutritive value of milk and milk products, but also to the colour and flavour characteristics of certain dairy products. It, however, presents some problems during processing of milk into certain concentrated, frozen dairy products. Moreover, lactose intolerance is common in the majority of adults in the world. Hydrolysis of lactose in milk by the enzyme, β-galactosidase can be used to circumvent these difficulties. Extensive studies have been carried out on β-galactosidase from *Kluyveromyces fragilis* for this purpose and the enzyme, is now commercially available also. This enzyme, is, however, thermolabile and is inactivated at temperatures above 40°C. Agrawal *et al.*[1] screened fortyfive strains of molds to search for a thermostable β-galactosidase and reported that a strain of *Alternaria palmi* produces β-galactosidase which did not lose activity when heated at 65°C for 45 min. The present communication reports on the purification and properties of β-galactosidase from *A. palmi*.

Materials and Methods

Alternaria palmi was obtained from the culture collection centre of National Dairy Research Institute, Karnal and was maintained on Czapeck Dox agar containing lactose as the sole source of carbon[2]. Spore suspension of *A. palmi* (1×10^8 spores/ml) was inoculated into whey reconstituted to 6 per cent (W/V) total solids and supplemented with casein hydrolysate (2.5 per cent W/V) and corn steep liquor (2 per cent W/V) and adjusted to pH 4.0. Mold mycelia obtained after incubation for 9 days at 30°C were separated by filtering through Whatman No. 1 filter paper, washed twice with citrate buffer (0.05M, pH 5.0) and homogenised in the same buffer for preparation of cell free extract.

Cell free extract was prepared by disintegrating the mycelial suspension at 4°C by ultrasonic vibration at 60W in a B12 sonifier. Cell debris was removed by centrifugation (20 min × 20,000 g at 4°C) and cell free extract thus prepared was used for assaying β-galactosidase. All the assays were conducted in duplicate. β-Galactosidase activity was assayed according to Wendroff *et al.*[3] The cell free extract was incubated with 1 mM ortho-nitrophenyl-β-galactopyranoside (ONPG) in a total volume of 3 ml at 65°C for 10 min. After incubation, reaction was stopped by addition of 2 ml of 1.0 M sodium carbonate and colour developed was measured at 420 nm. A unit of enzyme activity was expressed as the amount of enzyme required to liberate 1 μ mole of ortho-nitrophenol in 1 min at 65°C under specified conditions. Protein was estimated according to the method of Lowry *et al.*[4]

Acetone precipitation: Two hundred and fifty ml of chilled acetone was added drop wise to the cell free extract (500 ml) at 4°C with constant stirring. The mixture was allowed to stand for 30 min and centrifuged (20 min × 500 g at 4°C) to remove the precipitated proteins. An incremental volume of chilled acetone was added to the supernate with constant stirring to get the cell free extract-acetone ratio to 1 : 1.5. The mixture was allowed to stand for 30 min and the precipitate formed was collected by centrifugation for 20 min at 5000 g. It was dissolved in 35 ml of phosphate buffer (0.02 M,

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pH 7.0) and dialyzed against the same buffer at 4°C for 12 hr. The dialyzate was concentrated using an ultrafiltration cell (Amicon filter PM-30).

DEAE-Sephadex A-50 column chromatography: DEAE-Sephadex A-50 was equilibrated with phosphate buffer (0.2 M, pH 7.0) and packed in a glass column (2.0 × 30 cm). The column was then charged with enzyme solution concentrated through the ultrafiltration cell and the chromatogram was developed with 0.1 M sodium chloride in phosphate buffer (0.02 M, pH 7.0). Five ml fractions were collected at a flow rate of 35 ml/hr and active fractions were pooled.

Determination of molecular weight: Apparent molecular weight of the purified enzyme was determined following the procedure of Weber and Osborn[5] using sodium dodecyl sulfate polyacrylamide gel electrophoresis at room temperature. Molecular weight was determined by comparing electrophoretic mobility (R_f) of the purified sample with known protein markers of molecular weight, 70,000, 140,000, 210,000 and 280,000.

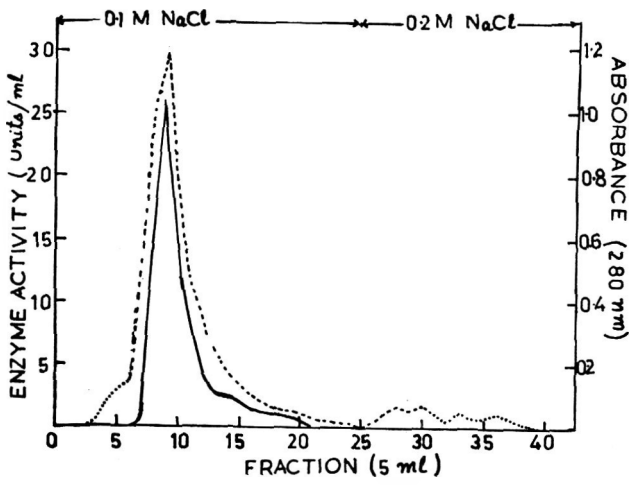

Fig. 1. DEAE-Sephadex A-50 column chromatography of β-galactosidase of *A. palmi*.
——, Enzyme activity...., protein.

Results and Discussion

Considerations of the growth temperature of microorganisms normally present in milk and milk products and the heat lability and optimum temperature of the lactose hydrolysing enzyme led several workers to search for a thermostable β-galactosidase preparation exhibiting an elevated optimum temperature[6]. This paper reports such a study on a thermostable β-galactosidase from *A. palmi*.

β-galactosidase from *A. palmi* was purified by acetone fractionation, ultra-filtration using Amicon filter PM-30, and DEAE sephadex A-50 column chromatography (Fig. 1.). Nearly 30-fold purification was achieved with an overall recovery of 60 per cent activity (Table 1).

Apparent molecular weight of the purified enzyme was observed to be approximately 160,000 daltons. Enzyme preparations from molds have been observed to have a molecular weight ranging from 1×10^5 to 5.7×10^5 daltons[6-11]. Various substrates viz., 0-nitrophenyl β-D-galactopyranoside, p-nitrophenyl β-D-galactopyranoside, p-nitrophenyl-α-D-galactopyranoside, p-nitrophenyl N-acetyl β-D-galactosamine, p-nitrophynyl α-D mannopyranoside, p-nitrophenyl α-L fucopyranoside, 0-nitrophenyl β-D-fucopyranoside, p-nitrophenyl β-D-glucopyranoside and 0-nitrophenyl β-D-xylopyranoside at a concentration of 10^{-3} M and lactose at a concentration of 10^{-1}M were examined to determine substrate specificity of the enzyme. The enzyme was observed to be highly specific, hydrolysing only β-D-galactopyranoside linkage. Strict specificity of β-galactosidase for β (1-4) linkage has been reported for the enzyme from other sources also[6,12-14].

Optimum temperature of incubation for β-galactosidase of *A. palmi* was found to be 65°C. A sharp decline in enzyme activity was observed at temperatures above 70°C. The enzyme was found to be quite stable at 65°C losing only 25 per cent of its activity on heating at this temperature for 2 hr. Heating at 70°C for 10 min caused a marked loss of enzyme activity (Fig.2). Sorensen and Crisan[15] reported that β-galactosidase of *Mucor*

Table 1. PURIFICATION OF β-GALACTOSIDASE OF A. PALMI

Step/Procedure	Volume (ml)	Enzyme activity (units/ml)	Protein (mg/ml)	Specific activity (units/mg)	Total activity (units)	Purification (fold)	Yield (%)
1. Cell free extract	500.0	0.15	1.00	0.15	75.0	1.0	100
2. Acetone fractionation	35.0	1.90	1.25	1.52	66.5	10.1	89
3. Ultrafiltration	3.5	18.50	7.00	2.64	64.7	17.6	86
4. DEAE-Sephadex A-50	20.0	2.35	0.50	4.70	47.0	31.3	63

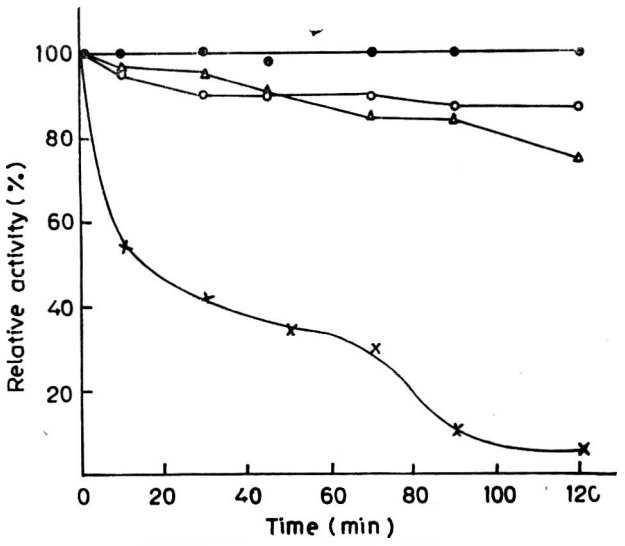

Fig. 2. Thermostability of *A. palmi* β-galactosidase. —●—, 55°C; —O—, 60°C; —△—, 65°C; —×—, 70°C.

pusillus exhibited an unusual thermostability having a half life of 10 min at 70°C. Griffiths and Muir[16] reported a thermostable β-galactosidase optimally active at 60°C, having a half life of 83 min at 65°C. The optimum temperature as well as stability at 65°C for β-galactosidase of *A. palmi* recorded in the present study would be important for commercial scale immobilized lactase reactor.

Optimum pH of the enzyme was recorded between 5.0 and 5.5; this would be suitable for the hydrolysis of lactose in acid products such as yoghurt or acid whey. The enzyme was stable on exposure to pH ranging from 1.0 to 7.0 overnight at 30°C. Stability over a wide pH range has been observed in β-galactosidase from molds by several workers[9,13,15,17,18].

The Michaelis constant (Km) and maximum velocity Vmax extrapolated from Lineweaver Burk plot were recorded as 8×10^{-4}M and 0.14 μ moles/mg protein/min, respectively with ONPG as the substrate. With lactose as the substrate Km and Vmax were found to be 0.263 M and 0.21 μ moles/mg protein/min, respectively.

There was little or no stimulation or inhibition of enzyme activity with 10^{-4} to 10^{-3}M concentration of metalions such as Pb^{++}, Ag^{+}, Hg^{++}, Ba^{++}, Co^{++}, Cu^{++} Ni^{++}, Mg^{++}, Mn^{++}, Fe^{++}, Li^{+}, K^{+} and Na^{+} and some group specific reagents viz., EDTA, 1, 10 phenanthroline α, α, dipyridyl, hydrazine sulfate, cysteine hydrochloride, 2-mercaptoethanol, N-ethyl maleimide, PCMB, thiomersal, iodoacetamide and ascorbic acid. Metal ions like Na^{+}, K^{+}, Mg^{++} and Mn^{++} have been reported to activate β-galactosidase preparations from yeast and bacteria[14,19,20]. However, other workers have reported that metal ions or certain group specific reagents did not stimulate or inhibit enzyme activity[11,18,21-23]. This enzyme would be important for commercial scale immobilized lactase reactor and might be used along with glucose isomerase in preparation of syrup from lactose and whey.

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Nitrosamines in Some Indian Brands of Soya Sauce Samples Treated with Nitrite

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Soya sauce on treatment with nitrite leads to the formation of at least three known carcinogenic nitrosamines, namely, nitrosodimethylamine (NDMA), nitrosodiethylamine (NDEA) and nitrosopyrrolidine (NPY). 1-Methyl-1, 2, 3, 4-tetrahydro-β-carboline-3-carboxylic acid (MTCCA) whose nitroso derivative is known to be mutagenic is also formed under these conditions. However, some antioxidant factors present in the food are likely to offer protection against nitrosamines in the diet containing soya sauce.

Diet and life-style related factors are responsible for more than 80 per cent of the cancers. Nitrosamines, present as such or formed by the interaction of food and tobacco amines with dietary and salivary nitrite are believed to subscribe to about 30 per cent of these cancers[1]. A number of processed foods have been examined for their nitrosamine content before and after treating with nitrite. Soya sauce is one of these as it is widely used for the seasoning of foods. In recent years, its use in India is on the increase with the popularity of Chinese foods and soya products. There are reports about the formation of nitrosamines in some samples of soya sauce of American and Japanese makes when treated with nitrite[2,3]. However, the nature and quantity of nitrosamines formed varies from product to product and it was therefore considered relevant to examine the nitrosamine content of the locally available soya sauce samples as such and after treatment with nitrite.

Materials and Methods

Three brands of soya sauce *a*, *b* and *c* were purchased from the local market. Sodium nitrite. 'Analar grade', was supplied by BDH (India). Standard nitrosamine samples were procured from Sigma Chemical Co. USA.

UV spectra were recorded with a Carl Zeiss Model RPQ 20A spectrophotometer. Gas chromatography was carried out on a Toshniwal gas chromatograph type RL04 equipped with a hydrogen flame ionization detector. The column was a 10 per cent silar 7C glass column. The operating conditions were: Nitrogen flow rate, 40 ml/min; injection temp, 240°C; column temp., 140/180°C. For HPLC, liquid chromatograph model ALC/GPC-244 equipped with model 6000 A solvent delivery system and model U6K injection system supplied by M/s Waters Associates, Milford, USA was used. The HPLC column was a μ Bondapak C_{18} column. The operating conditions were: Solvent system, H_2O: CH_3OH (90:10 v/v); flow rate 1 ml/min; chart speed 0.2 in./min; detector UV at 254 nm.

Isolation and identification of volatile nitrosamines in soya sauce as such and after treatment with nitrite: One litre of soya sauce was first defatted with petroleum ether

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(b.p. 60-80°, 3×500 ml) and extracted with methylene chloride (3×500 ml) to isolate preformed nitrosamines, if any. Both the extracts were concentrated and then examined for Griess-reagent[4] positive compounds by thin layer chromatography (TLC) (Silica Gel G plates; hexane: ethyl acetate: methylene chloride, 5:2:2 v/v).

The defatted soya sauce (as above) was treated with 2 g (2000 p.p.m.) of sodium nitrite, acidified to pH 3.5 with HCl (1:1) and stirred at room temperature for 2 hr. The mixture was then steam-distilled[5] and the distillate extracted with methylene chloride (3×500 ml). The extract was washed with water, dried over anhydrous sodium sulphate and concentrated to 1 ml. The concentrate was examined by TLC as above. Preparative TLC was carried out to separate the various products (silica gel G plates; hexane: ethyl acetate: methylene chloride 5:2:2 v/v). Compounds corresponding to Griess-reagent positive areas were extracted with methylene chloride and then examined by gas liquid chromatography (GLC) and co-chromatographed with authentic standards.

Isolation of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3 carboxylic acid (MTCCA) from soya sauce samples: The carboline derivative was isolated by partial modification of the procedure of Wakabayashi *et al.*[3]

Hundred ml of soya sauce was first defatted by extraction with petroleum ether (b.p. 60-80°, 2×50 ml). It was diluted 20 times with water and passed through a column of activated charcoal (4×16 cm). The column was washed with water and the material eluted with (*i*) methanol, (*ii*) methanol-1M acetic acid, (*iii*) methanol followed by (*iv*) methanol-1N NH_4OH and finally (*v*) with methanol again as described by Wakabayashi *et al*[3]. The fractions eluted with methanol, 80 per cent methanol-1M acetic acid and methanol were pooled and evaporated to dryness and the dry material (4 g) was dissolved in water (90 ml) and loaded on an Amberlite XAD-7 column (4×20 cm). The column was washed with water and the material eluted with methanol. The eluate was again evaporated to dryness and dissolved in benzene. This solution was loaded onto a silica gel column (250 g, 5×35 cm) and eluted with benzene-methanol (100:0 to 0:100 v/v) gradient. Most of MTCCA was eluted with 80:20 benzene, methanol: The fraction containing MTCCA was concentrated and purified by preparative TLC (silica gel G, methanol: benzene 40:60 v/v). The compound so obtained gave a single spot when rechromatographed.

Results and Discussion

The nitrosamines characterized in the steam distillate nitrite-treated sauce were nitrosodimethylamine (NDMA), nitrosodiethylamine (NDEA) and nitrosopyrrolidine (NPY) (Fig. 1). The nitrite-treated soya sauce

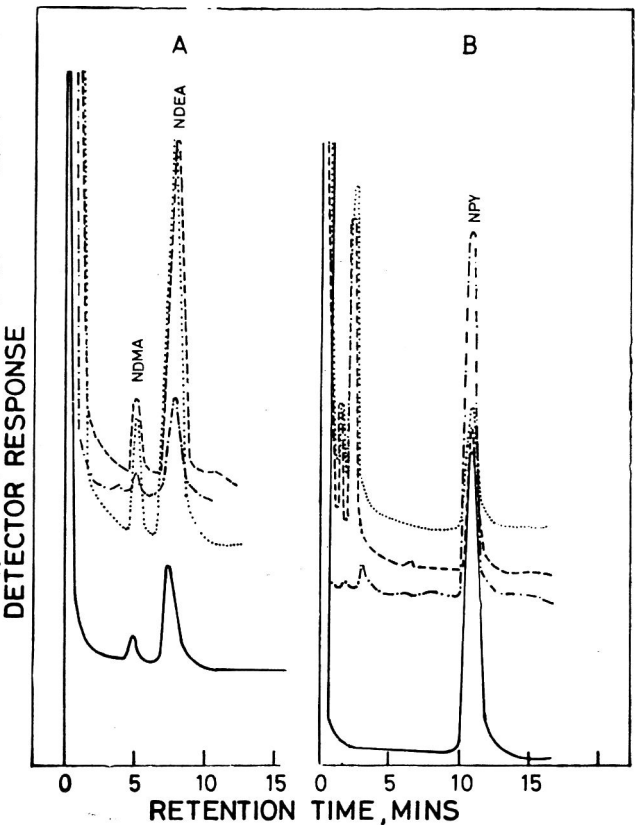

Fig. 1. GLC profile of soya sauce samples showing the presence of nitrosodimethylamine (NDMA), nitrosodiethylamine (NDEA) and nitrosopyrrolidine (NPY) (A–column temp 140°C, B–column temp 180°C).

mother liquor left in the flask after steam distillation was extracted and examined for the presence of any other nitroso derivative, but no detectable amounts of any nitrosamines could beobserved.

1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCCA) is known to be present in some soya sauce samples of Japanese and American brands[3]. In the present study, only one isomer of MTCCA could be isolated. On nitrosation, it gave a single spot on TLC. This was further characterized by HPLC of the nitroso derivative obtained by preparative TLC. This nitroso derivative was compared with the two derivatives obtained synthetically (Salvi and Choughuley, unpublished work)[6] for TLC behaviour as well as for retention time on HPLC column. This comparison indicated that the isomer present in soya sauce is probably (–) – (1 S, 3 S)-MTCCA. The mass spectrum of MTCCA isolated from soya sauce was identical to the mass spectrum of synthetically prepared sample (M^+ m/e 230). The UV absorption maxima in methanol were observed at 221, 271, 278 and 288 nm. In Fig. 2 is shown the HPLC profile of synthetic MTCCA and also of the compound obtained from soya sauce samples *a*, *b* and *c*.

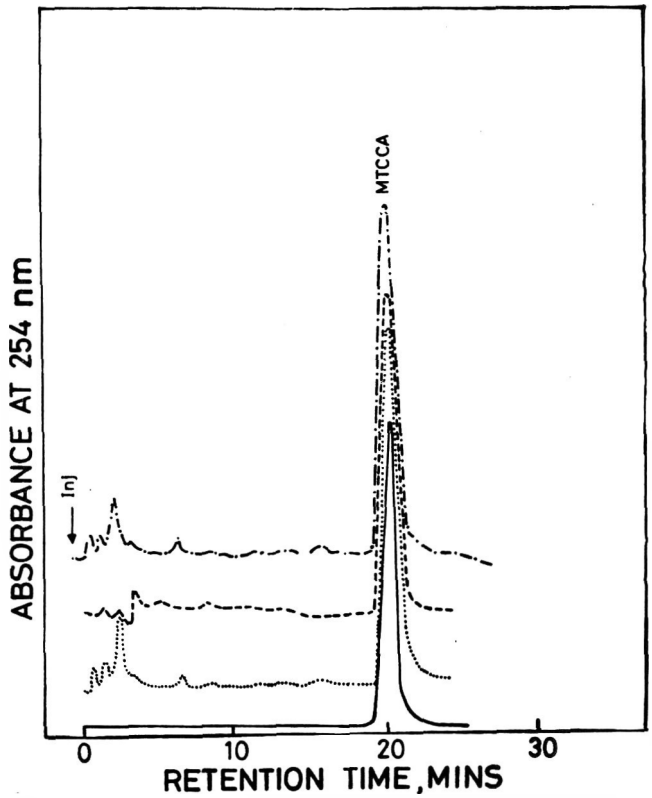

Fig. 2. HPLC profile of soya sauce samples showing the presence of 1-methyl-1, 2, 3, 4-tetra hydro-β-carboline-3-carboxylic acid.

The amounts of nitrosamines detected in steam-volatile fraction of nitrite treated soya sauce are given in Table 1. The quantity of MTCCA isolated from each sample of soya sauce was *a*, 110; *b*, 405; and *c*, 387 p.p.m. It would be observed that in sample *c* volatile nitrosamines are slightly less than those in samples *a* and *b*. However, the amount of MTCCA is less in sample *a* than in samples *b* and *c*.

Of the three soya sauces analysed, two were prepared by fermentation process and the third, presumably so. The constituents of the sauce as reported by the manufacturers are: fermented soya beans, sugar, salt, vinegar and spices.

Nitrosamines were not detected in the three soya sauce samples prior to nitrite treatment. However, they were detected in nitrite-treated samples. This has relevance, as soya sauce used for seasoning, etc. comes in contact with salivary nitrite and nitrate[7,8] and is most likely to increase the total body burden of nitrosamines in the stomach.*

However, it is also possible that ascorbic acid, phenolics, sulphydryl compounds and several other antioxidants may offer protection against nitrosamine formation in the stomach. This aspect has been well studied with respect to NDMA, NDEA, NPY and other aliphatic nitrosamines[9-11]. The nitrosation of MTCCA and its inhibition by diet-related factors has been studied in our laboratory (Salvi and Choughuley, unpublished results)[3]. It has been observed that ascorbic acid and a number of phenolics do inhibit nitrosamine formation and consequently, it can be reasonably expected that the nitrosation of MTCCA in the stomach would also be controlled by various antioxidant factors present in the diet.

Table 1. NITROSAMINES IN STEAM-VOLATILE FRACTIONS OF NITRITE TREATED SOYA SAUCE SAMPLES

Soya sauce	NDMA (ppm)	NDEA (ppm)	NPY (ppm)
a	1.2	18	23
b	8.0	24	67
c	3.1	6	18

The data are the average of three experiments.
NDMA: Nitroso dimethylamine.
NDEA: Nitroso diethylamine.
NPY: Nitroso pyrrolidine.

*The levels of nitrate and nitrite on an average are 14 mg of nitrate as KNO_3 and 17 mg of nitrite as $NaNO_2$. perlitre of saliva, After a mea consisting of nitrate-rich vegetables such as celery, etc,. the salivary nitrite concentration can go as high as 500 mg/l. The nitrite is formed by the reduction of nitrate by microflora in the mouth. The ductal saliva does not contain any nitrite[7].

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Study of the Physico-chemical, Rheological, Baking and Noodle Quality of Improved Durum and Bread Wheat Cultivars

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Physico-chemical, rheological and end use properties of 10 improved cultivars of durum and two of bread wheats were investigated. Durum flours were characterised by significantly higher pigments (β-carotene), protein, damaged starch and diastatic activity. Rheologically, durum cultivars showed dough development times of 0.9–2.1 min as compared to 2.4–2.8 min of bread wheats. Their water absorptions were significantly higher and were attributed to the higher damaged starch content. The loaf volumes of durum flours were less than those of bread wheat flours. Among durum cultivars 'HD 4571', 'Bittern S', 'DWL 5061', 'DWL 5023', 'DWL 5009' and 'HI 8137' were outstanding in respect of noodle quality, characterised by pigment content and gruel clarity.

Durum wheat production in India is estimated at about 3 million tonnes. This is likely to increase as about 10 per cent of the cropped wheat area in Punjab, is expected to be covered by the newly evolved durum varieties which are more resistant to Karnal bunt disease than bread wheat cultivars[1,2]. Durums are preferred for pasta production[3]. Bread wheats are more extensively grown in different parts of the world including India[4-7]. The technological implications of likely increased production of durum wheat by Punjab, a surplus bread wheat producing state, have several implications for the cereal processing industry and the consumer. A number of durum cultivars are undergoing field tests. Information regarding their technological suitability is lacking. Results of physico-chemical, rheological and end use properties of promising durum cultivars are presented in this paper.

Materials and Methods

Representative samples of ten varieties of durum, 'DWL 5023', 'DWL 5009', 'DWL 5031', 'MPO 456', 'Bittern 'S', 'HI 8137', 'DWL 5061', 'HD 4571', 'Raj 2413', 'DWL 5045' and of two bread wheats, 'WL 1562' and 'WL 711' were obtained from the 1980–81 crop grown at the experimental farms of the Punjab Agricultural University. 'DWL 5023' durum is now grown extensively in the Punjab.

The samples were cleaned and milled into flour in the Buhler Pneumatic Laboratory Flour Mill (ML 202). The durum varieties were conditioned to 16.0 per cent moisture and bread wheats to 15.5 per cent moisture, respectively. A feed rate of 100g/min facilitated satisfactory milling. Bran and shorts were dusted in the bran finisher to recover adhering fractions of flour and homogeneously mixed with the break and reduction flours, kept in air-tight containers and used for different studies.

Mixing properties of the doughs were evaluated using the Brabender farinograph, according to the AACC methods[8]. Extensibility of the dough was determined by the alveograph method. The dough was prepared as described by Kent-Jones and Amos[9]. The curves were interpreted for extensibility (l, cm), tenacity (T, curve height, cm) and area (S, cm^2). Moisture, protein ($N \times 5.7$), gluten, diastatic activity, damaged starch, ash, sugars and pigments were determined according to the AACC methods[8].

Noodles: Flour (30g) was made into a stiff dough by hand kneading until smooth, rolled to a uniform 9-inch diameter disc using a wooden rolling pin. Strips of uniform size of 1 mm width were cut and dried under ambient conditions. The noodles were evaluated for cracks, appearance and cooking quality. The gruel of the cooked noodles was evaluated for iodine blue (IB) values using the method of Batchel *et al*[10].

Baking: Straight dough procedure of Irvine and McMullan[11] was followed without the remixing step. The baking formula consisted of 100g flour, 2.25g yeast, 1.5g salt, 2.0g sugar, 10 ppm potassium bromate and optimum water absorption. Loaf volume was measured by the rapeseed displacement method.

The results have been expressed on 14 per cent moisture basis and statistically examined for the varietal

TABLE 1. YIELD AND COMPOSITION OF DURUM AND BREAD WHEAT FLOURS

Yield/composition	Durum flours			Bread wheat flours	
	Mean	Range	C. V. (%)	Mean	Range
Flour yield (%)	60.6	54.1–66.9	8.2	71.1	70.9–72.2
Ash (%)	0.65	0.59–0.74	7.6	0.45	0.43–0.48
Dry gluten (%)	11.0	9.6–12.7	8.2	9.8	9.6–10.1
Pigments as β-carotene (ppm)	3.9	3.0–4.8	15.8	1.8	1.2–2.3
Reducing sugars (%)	0.36	0.3–0.4	7.9	0.275	0.27–0.28
Non-reducing sugars (%)	1.8	1.5–2.2	12.0	1.5	1.3–1.6
Damaged starch (%)	14.6	10.8–24.5	14.8	9.2	8.9–9.5

C.V.: Coefficient of variation

differences by Thomas and Hills[12] method. The coefficient of variation (C.V.) was also calculated.

Results and Discussion

The durum cultivars gave lower yields of straight-grade flour than bread wheats. Their ash content was higher than that of bread wheat flours (Table 1). Higher ash contents in durum flour have also been reported by other workers[13–15]. A durum semolina of 65 per cent extraction was reported to have an ash content of 0.55 to 0.75 per cent[15]. The ash content depended on the variety and milling efficiency.

The protein contents of durum flours were generally higher than those of bread wheat flours. Bread wheat glutens were stronger and more elastic than those of durums. However, glutens of 'MPO 456' and 'DWL 5023' were generally more elastic but not as strong. The pigments in durum flours were distinctly higher than in bread flours. 'HI 8137' flour had maximum yellow pigments among the durum cultivars. The higher pigments in the durum flours were also reflected in their gluten pigments which were distinctly higher than pigments in bread wheat glutens which were whitish looking.

Reducing sugars were maximum in the flours of 'Bittern 'S' and 'DWL 5009' varieties, whereas 'HD 4571' had the lowest value; no marked differences were observed among the flours of different varieties. Unlike reducing sugars, the non-reducing sugars predominated in the flours of durum varieties as compared to those of bread wheat flours.

Farinograph curve characteristics: Durum flours invariably showed higher absorption in the range of 66.0 to 81.0 per cent for centering the curves on the 500 B.U. line as compared to 64.8 and 66.2 per cent absorption of 'WL 1562' and 'WL 711' bread wheat flours,

TABLE 2. FARINOGRAPH AND ALVEOGRAPH CURVE CHARACTERISTICS OF DURUM AND BREAD WHEAT FLOURS

Rheological parameter	Durum flours			Bread wheat flours	
	Mean	Range	C.V. (%)	Mean	Range
		Farinograph curve			
Farinograph water absorption (%)	72.3	66.0–81.0	6.9	65.5	64.0–66.2
Dough development time (min)	1.7	0.9–2.1	21.2	2.6	2.4–2.8
Mixing tolerance index (B.U.)	100	65–140	23.0	10.5	10.0–11.0
Stability (min)	3.4	1.8–7.7	54.1	14.1	13.9–14.3
Softening (B.U.)	89.5	50–130	34.0	9.3	4.0–14.6
		Alveograph curves			
Resistance to deformation (cm)	12.1	7.0–15.0	22.3	11.6	10.2–13.0
Extensibility (cm)	4.1	2.7–5.7	29.3	5.9	5.3–6.4
Curves area (cm^2)	32.7	10.0–47.0	39.4	34.0	33–35
Burette water level (ml)	12.5	9.0–15.5	16.8	16.0	15.5–16.5

respectively (Table 2). The higher absorption was the artifact of the distinctly higher damaged starch in the durum flours. The dough development time of durum flours varied slighty as compared to bread wheat flours. Dough softening was less in 'DWL 5009', 'HI 8137' and 'DWL 5061' cultivars but was quite pronounced in the doughs of the other durums. 'DWL 5061' and 'DWL 5045' had higher dough stability than the doughs from the other varieties. The faster dough development of durum flour/semolina is considered an advantage. Further, lower water absorption, stability of the dough and resistance to softening are additional economic and quality factors which determine the suitability of a durum variety for making macaroni. Rahim *et al*[14]. reported higher range of dough development times i.e. 2.08 to 8.0 min for some Indian durums. Flours of varieties, 'DWL 5045' and 'Bittern 'S' ' showed high water absorption which was attributed to their higher damaged starch content. This caused pronounced softening of dough during mixing in the farinograph test.

Alveograph curve characteristics: After several preliminary tests, a system consisting of 50g flour plus 16 ml of 2.5 per cent salt solution was adopted for developing the alveograms (Table 2). The differences in the physical properties of the doughs of durum varieties were marked. The resistance to deformation and extensibility were less than those of bread wheats despite increased absorption. Minimal resistance to extension as shown by the height of the curve and an extensibility of 2.8 and 3.0 cm were shown by the flours of 'DWL 5031' and 'Raj 2413' respectively as compared to the maximum values for 'DWL 5023'. These differences were also reflected in the values for curve areas. The alveograph has been employed extensively to evaluate the strength of bread wheat flours[9]. Its use for evaluating the strength of durum flours is also emphasized.

Breadmaking properties: Durum flour doughs became very sticky on remixing before sheeting and moulding. Therefore, the remixing step was eliminated. In addition, water absorption was considerably reduced to obtain doughs amenable to satisfactory handling. The reduced absorption for durum flours averaged 60.1 per cent (Table 3) which was considerably below the water absorption shown by the farinograph test (Table 2) The bread wheat flours of 'WL 1562' and 'WL 711' also took 7.8 and 6.4 per cent less water respectively than that indicated by the farinograph. Deviations in the baking absorption from farinograph absorption have been reported for flours of Indian bread wheats[17-18]. Higher damaged starch, typical of indigenous bread wheats, was also observed in durum flours (Table 1). Such doughs become sticky as fermentation proceeds due to susceptibility of damaged starch to alpha-amylase action. Durum flours made unsatisfactory loaves, which were compact, with coarse and yellowish crumb as compared to those of bread wheats.

TABLE 3. BREADMAKING QUALITY OF FLOURS OF DURUM AND BREAD WHEAT CULTIVARS

Wheat	Baking absorption (%)	Mixing time (min)	Dough handling	Loaf vol. (ml)	Crust colour	Crumb grain
Durum cultivars						
DWL 5023	59.5	3.5	Sticky	260	PB	C
DWL 5009	59.8	3.5	,,	315	DB	C
MPO 456	57.9	3.5	,,	280	PB	C
Bittern 'S'	64.1	3.0	,,	300	DB	C
HI 8137	63.2	3.5	,,	360	DB	M
DWL 5061	62.0	3.5	,,	335	DB	C
HD 4571	59.9	3.5	Stiff	295	LB	C
DWL 5031	54.8	2.5	,,	280	B	C
Raj 2413	55.7	3.5	,,	305	B	C
DWL 5045	64.2	4.0	,,	270	B	C
Mean	60.1	3.4	—	300	—	—
S.D.(±)	3.3	0.39	—	30.6	—	—
C.V. (%)	5.5	11.5	—	10.2	—	—
Bread wheat cultivars						
WL 1562	57.0	3.5	Sticky	415	DB	M
WL 711	59.8	3.0	,,	480	DB	M

P : Pale C : Coarse B : Brown
M : Medium D : Dark F : Fine
L : Light

Noodles: Noodle doughs prepared by using appropriately reduced absorption handled well and were amenable to rolling. No cracks were seen in noodles whether prepared from durum or bread wheat flours. However, distinctive difference in their appearance was perceived visually (Table 4). Noodles of 'MPO 456' seemed chalky white as compared to the clear yellowish noodles of 'Bittern 'S', 'HI 8137', 'HD 4571' and 'DWL 5031' flours. Noodles made from the remaining durum varieties and bread wheat flours had creamyish colour. Loss of pigments was primarily responsible for the poor colour of noodles (Table 5). Durum variety 'MPO 456', initially had maximum pigment but its noodles were graded chalky white. It suffered a maximum pigment loss of 60 per cent during noodle preparation. Minimal loss of pigments was observed in the noodles of 'HD 4571' which along with 'Bittern 'S', 'HI 8137' and 'DWL 5031' suffered pigment losses of 7.7, 33.3,

TABLE 4. COOKING QUALITY OF NOODLES PREPARED FROM DURUM AND BREAD WHEAT FLOURS

Wheat	Water uptake (ml/g)	Gruel solids (%)	IB values (O.D.)	Score	Remarks
Durum cultivars					
DWL 5023	4.0	0.82	0.45	7	G
DWL 5009	4.3	0.74	0.39	7	G
MPO 456	5.2	0.68	0.58	5	G
Bittern 'S'	4.4	1.00	0.56	8	VG
HI 8137	4.9	0.87	0.50	7	G
DWL 5061	4.4	0.93	0.44	8	VG
HD 4571	3.5	0.58	0.28	10	E
DWL 5031	3.7	0.59	0.36	5	A
Raj 2413	3.8	0.56	0.44	5	A
DWL 5045	3.6	0.59	0.45	5	A
Mean	4.2	0.74	0.44	—	—
S. D. (±)	0.56	0.16	0.08	—	—
C. V. (%)	13.4	21.6	18.4	—	—
Bread wheat cultivars					
WL 1562	3.9	0.98	0.36	5	A
WL 711	4.4	0.63	0.35	3	P

G : Good A : Acceptable VG : Very good
P : Poor E : Excellent

TABLE 5. PIGMENT LOSSES IN THE PREPARATION OF NOODLES FROM DURUM AND BREAD WHEAT FLOURS

Wheat	Pigment as β-carotene (ppm)		Pigment loss (%)	Appearance
	Flour	Noodles		
Durum cultivars				
DWL 5023	3.3	2.1	36.4	Creamy
DWL 5009	3.3	2.4	27.3	Creamy
MPO 456	4.5	1.8	60.0	Chalky
Bittern 'S'	4.2	2.8	33.3	Yellowish
HI 8137	4.2	3.3	21.4	Yellowish
DWL 5061	3.3	2.1	36.3	Creamy
HD 4571	3.9	3.6	7.7	Yellowish
DWL 5031	4.2	3.2	23.8	Yellowish
Raj 2413	4.2	3.4	19.1	Creamy
DWL 5045	3.0	2.1	30.0	Creamy
Mean	3.8	2.7	29.8	—
S. D. (±)	0.53	0.66	13.8	—
C. V. (%)	13.9	24.5	46.2	—
Bread wheat cultivars				
WL 1562	2.1	1.8	14.3	White
WL 711	1.5	1.5	0.0	,,

21.4 and 53.8 per cent, respectively. However, they were visually judged as yellowish.

Variation in gruel loss was more than that of the water uptake of the noodles during cooking. Noodles of durum were less sticky than those of bread wheat. Consistency was relatively better for the noodles of 'DWL 5031', 'Raj 2413', 'DWL 5045', 'MPO 456', 'Bittern 'S'' and 'HI 8137' varieties as well as of the bread wheats. Eating quality of noodles from all the varieties was rated high with the exception of 'WL 711' noodles which were softer to bite. The noodles of 'HD 4571' variety were rated high closely followed by those of 'Bittern 'S'', 'DWL 5061', 'DWL 5023,' 'DWL 5009' and 'HI 8137'.

Though durum cultivars are less satisfactory for breadmaking they were more suitable for macaroni products. It may be important to develop new varieties by crossing bread wheat and durum wheat genotypes to combine resistance to diseases, at the same time ensuring suitability for baking.

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Effect of Traditional Processing on the Functional Properties of Cowpea (*Vigna catjang*) Flour

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The functional properties i.e. nitrogen solubility (NS) water absorption capacity (WAC), fat absorption capacity (FAC) bulk density (BD), foaming capacity (FC), foam stability (FS) and emulsification capacity of raw and processed (fermented, germinated, boiled, pressure cooked, puffed and roasted) cowpea flours were investigated. Heat treatments reduced the nitrogen solubility of cowpea flour. All the processing treatments increased the WAC of cowpea flour. Heat treatments increased the BD of cowpea flour, whereas it was reduced by germination. Germination increased FAC, while other processing treatments did not have marked effect. Processed cowpea flour had a lower foaming and emulsifying capacity.

Pulses play an important role in the acceptability of monotonous diets in many parts of the world[1]. Their role as protein supplements in the diets based on cereals and millets is well recognized[1]. Recently, attention has also been directed towards increasing the utilization of these protein sources for food use. The ultimate success of utilizing plant proteins in food formulations depends largely upon the functional attributes[2]. It is well known that processing conditions, particularly heating improves protein quality by destroying anti-nutritional factors but concomitantly may alter the functionality[3,4]. Cowpea (*Vigna catjang*) is an important food legume of many tropical countries. It is used as a fresh vegetable and also as reconstituted dry bean in many of the traditional food preparations in India. It is reported that only small amounts of cowpea can be used replacing portions of wheat flour and milk in bread and biscuits[2,5,6]. Therefore, an attempt was made to study the functional properties of cowpea flours subjected to various traditional processing conditions such as heating wet-boiling and pressure cooking, dry-puffing and roasting, fermentation and germination.

Materials and Methods

Cowpea (*Vigna catjung*) of C-120 variety was obtained in bulk from the Karnataka State Seed Corporation, cleaned and stored. Cowpea seeds in 1-kg lots were subjected to the following processing conditions.

(*a*) Dehusked seeds were soaked in water (1500 ml) for 8 hr. The soaked cowpea was then ground into a paste and was allowed to ferment for 16 hr at room temperature ($\sim$ 28°C) and sun-dried.

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(*b*) Seeds were soaked for 8 hr in water (1500 ml). After draining the water, it was allowed to germinate for 72 hr at room temperature (~28°C) and then sun-dried.

(*c*) Seeds were soaked in water (1500 ml) for 30 min, boiled with sufficient water (2850 ml) for 25 min in an open vessel till they became tender. Excess water was drained (50 ml) and sun-dried.

(*d*) Pre-soaked cowpea (as mentioned above (*c*) was pressure cooked with sufficient water (820 ml) at 15 lbs pressure for 7 min, the pressure cooker was immediately cooled under running tap water. Excess water (condensed steam due to immediate cooling-100 ml) was drained and the material sun-dried.

(*e*) Seeds were soaked in water for 3 hr. It was then puffed over hot sand at 250°C. Puffed cowpea was cleaned to make it free of sand particles.

(*f*) Cowpea was roasted over hot sand at 240°C and passed through a sieve to remove sand particles.

Raw and processed cowpea except the fermented sample were dehusked. All the samples were powdered and passed through a 60 mesh (British standard mesh) screen. Moisture, protein (N×6.25), crude fat and total ash were determined by the AOAC method[7] in triplicate.

Nitrogen solubility (NS): Two g of flour was shaken with 20 ml of water and the suspension was adjusted to the desired pH (in the range 2-10) by the addition of 2N HCl or 2 N NaOH. The suspension was shaken mechanically at room temperature (~ 28°C) for 1 hr, centrifuged at 4500 r.p.m. for 20 min and the supernatants were taken for nitrogen estimation by micro Kjeldahl method. The solubilized nitrogen was expressed as per cent of total nitrogen.

Bulk density (BD): The method of Wang and Kinsella[8] was used to measure the bulk density of the powdered samples.

Water absorption capacity (WAC): This was determined by the method of Sosulski[9] using 5g flour sample at room temperature.

Fat absorption capacity (FAC): This was determined by the method of Sosulski *et al*[10]. using 4 g flour sample and refined groundnut oil at room temperature. The values are expressed as g of oil absorbed per g flour or protein.

Foam capacity (FC) and foam stability (FS): Two g sample of flour was blended with 100 ml water in a Waring Blender. The suspension was whipped at 1600 rpm for 5 min. The mixture was poured into a 250 ml measuring cylinder and the volume was recorded after 30 sec. FC is expressed as per cent increase in volume using the formula[11]

$$\text{Foam capacity} = \frac{\text{Volume after whipping - Volume before whipping}}{\text{Volume before whipping}} \times 100$$

The foam volume was recorded at 15,30,60 and 120 min after whipping to determine FS according to Ahmed and Schmidt[12].

$$\text{Foam stability} = \frac{\text{Foam volume after time 't'}}{\text{Initial foam volume}} \times 100$$

Emulsification capacity (EC): The method of Beuchat *et al*[13]. was used. A 2 g flour sample and 20 ml water were blended at room temperature for 30 sec in a Waring Blender at 1600 rpm. After complete dispersion, refined groundnut oil was added continuously from a burette and blending continued until there was a phase separation. This was observed visually. Emulsification capacity is expressed as ml of oil emulsified per g of flour/protein.

Results and Discussion

As shown in Table 1, processing did not have any marked effect on the proximate composition of cowpea flour. Since wet heat processing involved draining of water, protein contents of boiled (21.06 per cent) and pressure cooked (20.67 per cent) cowpea flour were lower than that of unprocessed cowpea flour (24.50 per cent).

Nitrogen solubility (NS): The effect of processing on the nitrogen solubility as a function of pH is shown

TABLE 1. PROXIMATE COMPOSITION OF RAW AND PROCESSED COWPEA FLOUR

Parameter	Raw flour	Fermented	Germinated	Boiled	Pressure cooked	Puffed	Roasted
Moisture (%)	8.60	8.00	8.00	7.60	7.20	6.00	6.80
Fat (%)	0.83	1.17	1.09	0.63	0.50	0.85	0.75
Protein (Nx6.25) (%)	24.50	26.69	25.94	21.06	20.67	26.74	26.69
Total ash (%)	1.53	1.90	1.52	1.71	1.96	1.44	1.98
Carbohydrate (by diff.)	67.24	62.24	63.45	69.0	69.67	64.97	63.78

*Expressed on dry basis. Mean of three replications.

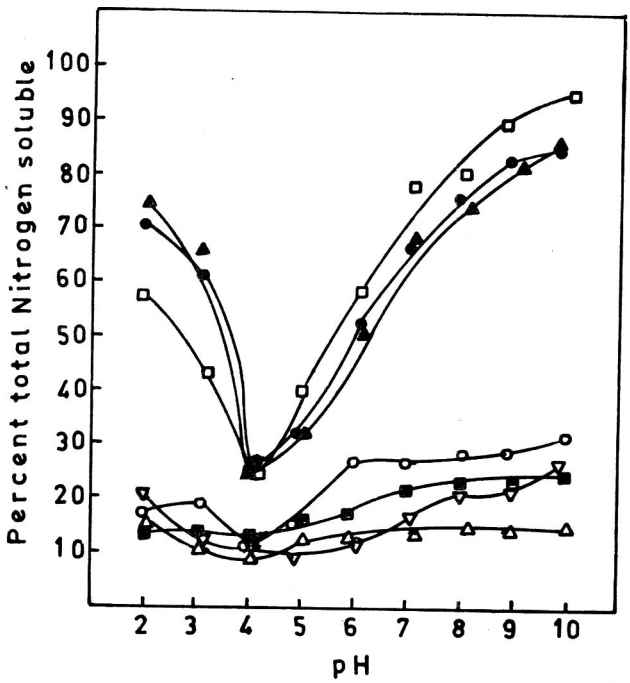

Fig. 1: Nitrogen solubility index of raw and processed cowpea flour

□ Raw; ▲ Fermented; ● Germinated; ■ Roasted; O Puffed; △ Boiled; ▽ Pressure cooked.

in Fig 1. Raw cowpea flour gave a U shaped curve in the pH range 2-10 with minimum solubility (26 per cent) at pH 4 and this increased considerably at acidic (58 per cent) and alkaline (96 per cent) pH. This is similar to the solubility profile reported by Okaka and Potter[6] for raw cowpea flour. While germination and fermentation did not have significant effect, heat treatments (roasting, puffing, boiling and pressure cooking) significantly reduced the solubility of cowpea proteins; solubility was low in the range of 2 to 10 pH. It has been reported that water soluble cowpea proteins are particularly sensitive to heat and undergo thermal dissociation and unfolding at temperatures lower than that generally observed for plant proteins[14]. Probably, heat treatments denatured the cowpea proteins and reduced their solubility. In case of heat processed sunflower seed, rapeseed, groundnut and soya bean proteins also, reduction in NS has been reported[10,15,16].

Water absorption capacity: The WAC values are given in Table 2. Raw cowpea flour had a WAC of 1.6g/g flour. All the processing conditions increased WAC. Germinated seeds had the lowest (1.9g/g) whereas puffed seeds had the highest (4.5g/g) WAC. It is known that polar amino acids of a protein have an affinity for water and denatured proteins are reported to bind more water. Polar amino acid content of cowpea is 25-65 g/100g protein[17]. The enhanced WAC of processed cowpea may be due to the denaturation of protein which facilitates water binding. Hermanssen[18] pointed out that proteins can increase their water holding capacity when their swelling capacity is increased. It is also known that polysaccharides which are hydrophilic greatly affect water absorption[19]. The higher WAC seen in case of puffed, pressure cooked and boiled samples may also be due to gelatinization of starch and swelling of crude fibre. WAC is considered a critical function of protein in viscous foods like soups, gravies, doughs, baked products, etc[10]. Hence, increased WAC observed due to processing can be made use of in these preparations.

Bulk density (BD): Bulk density of raw, fermented and roasted cowpea flour were similar (Table 2). Other heat treatments increased bulk density while germination lowered it. Higher BD is desirable since it helps to reduce the paste thickness. This is an important factor in convalescent and child feeding.

Fat absorption capacity (FAC): FAC has been

TABLE 2. BULK DENSITY, WATER ABSORPTION AND FAT ABSORPTION CAPACITY OF RAW AND PROCESSED COWPEA FLOUR

Type of sample	Bulk density* (g/ml)	Protein (%)	Water absorption*		Fat absorption	
			g/g flour	g/g protein	g/g flour	g/g protein
Raw	0.888	24.5	1.6	6.8	0.5	2.06
Fermented	0.885	26.7	2.4	9.2	0.6	2.25
Germinated	0.775	25.9	1.9	7.3	1.0	3.86
Boiled	0.940	21.1	2.3	10.9	0.7	3.32
Pressure cooked	0.940	20.7	2.4	11.6	0.7	3.38
Puffed	0.940	26.7	4.5	16.6	0.6	2.25
Roasted	0.890	26.7	2.2	6.0	0.6	2.25

*Mean of three replications.

TABLE 3. EMULSIFICATION CAPACITY, FOAM CAPACITY AND STABILITY OF RAW AND PROCESSED COWPEA FLOUR

Type of sample	Emulsifying capacity*		Foam vol* increase (%)	Foam stability+ (%)
	ml/g protein	ml/g flour		
Raw	35.0	85.0	20	91
Fermented	35.5	95.0	11	99
Germinated	27.5	70.5	15	97
Boiled	19.0	40.0	5	97
Pressure cooked	19.0	39.0	5	97
Puffed	31.0	83.0	8	97
Roasted	25.7	69.0	3	97

*Mean of three replications; +after 120 min.

attributed to the physical entrapment of oil. This is important since fat acts as flavour retainer and increases the mouth feel of foods[8]. FAC of raw cowpea was 0.5g/g flour (Table 2). Germination increased the FAC of cowpea flour, while other processing conditions did not bring about a significant increase. Fat absorption is primarily attributed to the protein and is reported to be affected by temperature, size of ingredient particles and degree of denaturation of protein[21]. From the reported amino acid composition data, apolar amino acid content of cowpea is 40.57g/100 g protein[17]. Generally, more hydrophobic proteins show superior binding of lipids[10], implying a direct correlation between FAC and apolar amino acids. However, no such relationship was observed in the present study between FAC and apolar amino acid content. FAC of raw cowpea was lower than that of soy flour (1.2g/g) which is reported to have an apolar amino acid content of 30.7g/100 g protein, a value lower than that of cowpea flour[8].

FAC is reported to have an inverse correlation with the bulk density. However, FAC of raw and processed cowpea flour did not follow this pattern. This is in conformity with the observation of Okaka and Potter[6] who reported that FAC of raw and blanched cowpea flour did not consistently follow this pattern.

Foam capacity (FC) and foam stability (FS): Though considerable decrease in FC was observed in all the processed samples, it was more pronounced in heat processed cowpea flour, indicating that heat treatment had lowered FC, probably by denaturing protein (Table 3). It is reported that mild heat treatment causes surface denaturation of proteins and yet keeps it in solution to result in better foaming properties[20,21]. This shows that heat treatments used in the present study were drastic enough to precipitate the proteins. It has been stated that FC is also dependent on nitrogen solubility[21]. No such relationship between FC and NS was seen in case of raw, fermented and germinated cowpea flour as these samples also showed lower FC. However, foam stability was not different between raw and processed cowpea flours.

Emulsifying capacity (EC): The ability of a protein to aid the formation and stabilization of emulsion is critical for its application in preparations like batters, milk and frozen desserts[22]. EC of the processed cowpea flours except that of fermented flour was lower than that of the raw cowpea flour (Table 3). Even the EC of raw cowpea flour (35 ml/g protein) was much lower than that reported for raw soy flour (70 ml/g protein). This observation is similar to the findings of Okaka and Potter[6] who showed that the EC of raw cowpea flour was lower than that of soy flour. The EC of raw cowpea flour is consistent with the findings of Mcwaters and Cherry[22] who reported a EC of 36.4 ml/g protein for cowpea flour. Though effect of pH and salt solutions on EC has not been studied in the present study, it is reported that EC is higher when cowpea flour is dispersed in water at neutral pH than in salt solutions at different pH.

It may be concluded that processing treatments increased the WAC, BD and FAC of cowpea flour but lowered FC and EC. It may be suggested that cowpea flour may be suitable in viscous foods like gravies, soups, baked products where good protein-water interactions are required, but will be unsuitable for foods like *idli* and *dosai* where good foaming properties are essential.

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Inhibitory Effect of Mercuric Compounds and Dyes on Decay and Sprouting of Seed Potato Tubers During Storage

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Mercurial compounds are commonly used to reduce the spoilage of seed potatoes during storage. To aid in differentiating the treated seed potatoes from the untreated, 13 synthetic dyes were assessed for colouring the potatoes as also in inhibiting the growth of *Fusarium oxysporum* and *Erwinia carotovora*, the two microbes causing spoilage of potatoes during storage. The ED_{50} values indicated that brilliant green and malachite green were equally effective against both the microbes compared to mercuric chloride. Treating seed potatoes with these two dyes in combination with mercuric chloride was more effective than when used individually in reducing the microbial spoilage. Sprouting was also significantly reduced by the combination treatments. Treated seed potato tubers, when planted at the end of storage, in pots as well as in field, did not show any inhibitory action on plant growth and the yield of tubers was at par with that of untreated tubers. The mercury residue in harvested tubers obtained from treated seed tubers was 0.025 μg/kg fresh weight which is well within the permissible limit. It is concluded that, it would be better to use mercurial salts in combination with an antimicrobial dye for the control of decay of seed potatoes.

In Karnataka, during storage of seed potatoes under ambient conditions (21-35°C, RH 40-75 per cent) upto periods of 2 to 3 months, 20-70 per cent losses occur due to microbial spoilage, sprouting and shrivelling. Microbial spoilage is mainly due to the fungus, *Fusarium oxysporum* (Schl. ex. fr) emend which causes 'dry rot of potatoes' and the bacterium, *Erwinia carotovora* (Jones) Dye causing 'soft rot of potatoes'. Mercurial compounds are being used to control the microbial spoilage[1-4]. Since these toxic mercurial compounds are colourless, it is necessary to differentiate the treated seed potatoes from the untreated in order to prevent accidental consumption. This difficulty could be overcome by colouring the tubers with synthetic dyes; some of them are also known to have antimicrobial activity[5-10]. In this context, the effect of 13 dyes on the growth of *F. oxysporum* and *E. carotovora* was studied.

Materials and Methods

In vitro *studies:* The following dyes, namely basic fuschin, brilliant green, cotton blue, eosin, gentian violet, janus green, malachite green, methyl red, methylene blue, nigrosin, rhodamine b, safranin and victoria blue were screened for their antimicrobial activity against *F. oxysporum* and *E. carotovora*. Analytical grade mercuric chloride was used.

In vitro screening and assay of the antimicrobial activity of chemicals was done by the agar well method, food poison technique[11] and serial dilution technique. In the agar well method, used normally for screening the compounds, the antimicrobial property is expressed as diameter of the inhibitory zone, while in the food poison technique (for fungi) and serial dilution technique (for bacteria), the per cent inhibition calculated using Vincent's formula[12], enables to quantify precisely the extent of antimicrobial action of test compounds. ED_{50} (concentration required to bring 50 per cent inhibition) of the test organisms, *F. oxysporum* and *E. carotovora* was determined by food poison technique[13].

In vivo *studies:* Washed, sound potato seed tubers of variety 'Kufri Jyoti' procured from Devanahalli (Bangalore District) were used. The seed tubers were dipped in solutions at various concentrations of test chemicals individually as well as in various combinations for 5 min. Tubers dipped in water for 5 min served as controls. The dipped tubers were surface dried and then packed in gunny bags (40×25 cm). Each treatment consisted of five replicates, each of 60 medium size tubers weighing 50 to 70 g. During storage, the untreated tubers were observed at intervals for (*i*) microbial spoilage and (*ii*) sprouting behaviour.

Pot experiments: To test the sproutability of treated and untreated tubers, tuber pieces having eyes were planted in pots (37.5 cm) having sandy soil. Five replicates each of 25 cut pieces were planted in a pot. Later, they were transplanted individually into a number of pots provided with nutrients and the pots were watered regularly. At weekly intervals, length of plants, number of tuber pieces germinated and those that were spoiled were recorded. At the end of the growth period, fresh weights of root, shoot and tuber yield were determined.

Field experiments: Field performance tests on the yield of the treated and untreated tubers were done at Devanahalli, Bangalore district. Completely randomised design was employed for raising seed tubers, under 'seed plot technique'. Plots of 3m×3m (9 m^2) were used as replicates. About 5-8 replicates were maintained for each treatment in the different experiments. Both the laboratory and field experiments were repeated 2 to 3 times during 1982–84.

Mercury residues in the tubers obtained by planting treated seed tubers were estimated by AOAC method[14] as modified by Vibhakar et al[15].

Results and Discussion

Out of the 13 dyes studied, malachite green and brilliant green were inhibitory to the growth of both the

TABLE 1. INHIBITORY EFFECT** OF SOME DYES ON F. OXYSPORUM AND E. CAROTOVORA BY AGAR CUP METHOD

Dye+	Inhibition zone (diam. in cm) at indicated concn (μg/ml)				
	100	500	1,000	10,000	ED_{50}
		F. oxysporum			
Brilliant green	2.4 ±0.5	2.6 ±0.05	3.6 ±0.2	3.8 ±0.1	0.63
Gentian violet	0	0	0	0	34.67
Malachite green	1.5 ±0.8	2.2 ±0.6	2.3 ±0.1	2.8 ±0.3	0.95
Mercuric chloride	1.1*±0.5	1.4*±0.1	1.8 ±0.2	2.6 ±0.1	9.12
		E. carotovora			
Brilliant green	1.6 ±0.8	1.7 ±0.5	2.5 ±0.2	3.3 ±0.1	8.03
Gentian violet	1.1 ±0.3	2.2 ±0.2	2.7 ±0.1	3.2 ±0.5	13.18
Malachite green	1.5 ±0.7	1.9 ±0.2	2.3 ±0.5	2.6 ±0.05	9.88
Mercuric chloride	0	0.9*±0.05	1.7*±0.04	2.1*±0.2	4.12

** Inhibition zone recorded on the third day of incubation at 28±2°C.

+ Other dyes viz. Basic fuschin, Cotton blue, Eosin, Janus green, Methyl red, Methylene blue, Nigrosin, Rhodamine b, Safranin, Victoria blue—did not show antimicrobial activity.

* Inhibitory activity was lost after 3 days.

Mean ± S.D.

test organisms (Table 1). Though gentian violet exhibited antimicrobial activity even at 100 μg/ml against *E. carotovora*, it failed to exhibit any inhibitory effect against *F. oxysporum*, even at a concentration as high as 10 mg/ml. Mercuric chloride was inhibitory against both the organisms, but after 3 days of incubation they invaded the inhibitory zone. In the case of dyes, the inhibitory zone was not overgrown by the test organisms upto the observed period of 15 days.

The dyes which proved effective were studied further to determine the ED_{50} values for both the test organisms. ED_{50} values of brilliant green and malachite green against *F. oxysporum* were far less than mercuric chloride. However, the ED_{50} values of these two dyes against *E. carotovora* were about two times that of mercuric chloride (Table 2). At a concentration of 9.12 μg/ml, mercuric chloride was effective in controlling both the microbes. At the same concentration, either of the two dyes was also equally effective in the control of both the microbes. Further, non-toxicity and ability to impart colour to tubers that helps to differentiate the treated seed potatoes are the advantages of using the dyes. However, gentian violet was far less effective than the above two dyes or mercuric chloride.

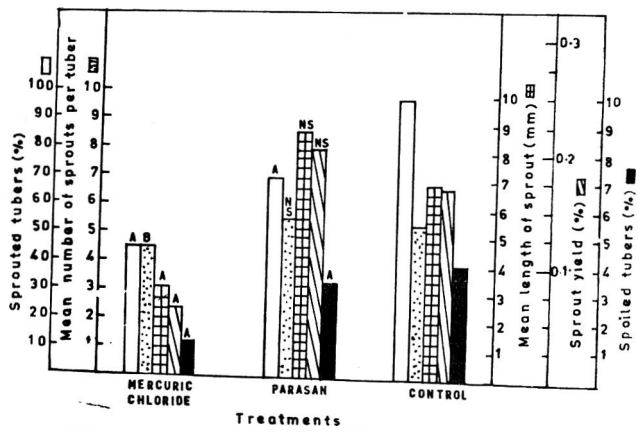

Fig. 1. Effect of mercuric chloride and parasan (at 1% level) on sprouting and spoilage behaviour of seed potato tubers stored for 30 days at ambient conditions. A & B denote significance at $P \leq 0.001$ and 0.005 respectively; NS—not significant.

TABLE 2. EFFECT OF MERCURIC CHLORIDE ON THE CONTROL OF SPROUTING AND SPOILAGE OF SEED POTATOES STORED FOR 45 DAYS AT AMBIENT CONDITIONS

Concn. of mercuric chloride (%)	Spoiled tubers (%)	Sprouted tubers (%)	Sprout yield (g/100 tubers)
0	21.0±8.2	97.5±5.6	6.9±0.7
0.1	20.0±4.5	75.1±6.6	1.4*±0.7
0.5	18.0±4.3	62.0±5.2	0.4*±0.03
1.0	13.0*±2.5	56.0±6.5	0.3*±0.03
2.5	25.4^{ns}±6.0 (Slight pitting)	87.0±5.6	1.8*±0.03
5.0	Severe pitting		

*denotes significant at $P \leq 0.001$
ns—not significant
Mean ± S.D.

The effectiveness of parasan as an antimicrobial agent was next to that of mercuric chloride (Fig. 1). The data show that at the end of 15 days of storage (this being a confirmatory experiment of the work done by Kapur et al[16], potatoes at sprout emergence stage were used and hence it was possible to record sprouting within 15 days), mercuric chloride exhibited statistically significant antisprouting activity. These findings on the antimicrobial and antisprouting activities of mercuric chloride corroborate with those of Kapur *et al*[16].

Mercuric chloride (1 per cent) was found optimal for reducing microbial spoilage as well as sprouting (Table 3).

TABLE 3. EFFICACY OF MERCURIC CHLORIDE ALONE AND IN COMBINATION WITH BRILLIANT GREEN ON THE CONTROL OF SPROUTING AND SPOILAGE OF SEED TUBERS STORED (FOR 150 DAYS) AT LOW TEMPERATURE (2±1°C)

Treatment	Concn. (ppm)	Spoiled tubers (%)	Sprouted tubers (%)	Sprout yield (g/100 tubers)
Mercuric chloride	10,000	0*	72.9*±2.8	135.9^{ns}±17.0
Brilliant green	500	2.4*±0.9	88.9*±3.7	62.7*±16.7
Mercuric chloride + Brilliant green	10,000 + 500	0*	72.6*±3.5	32.5*±11.6
Control	—	4.1 ±0.2	100.0 ±0.0	109.1 ±16.5

*denotes significant at $P \leq 0,001$
ns—not significant.
Mean ± S.D.

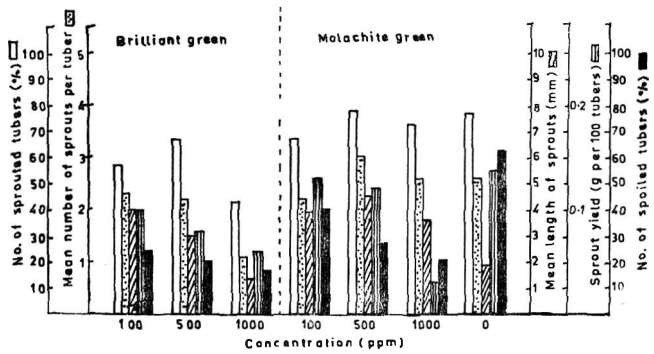

Fig. 2. Effect of dyes (Brilliant green and Malachite green) on sprouting and spoilage behaviour of seed potato tubers stored for 30 days at ambient conditions.

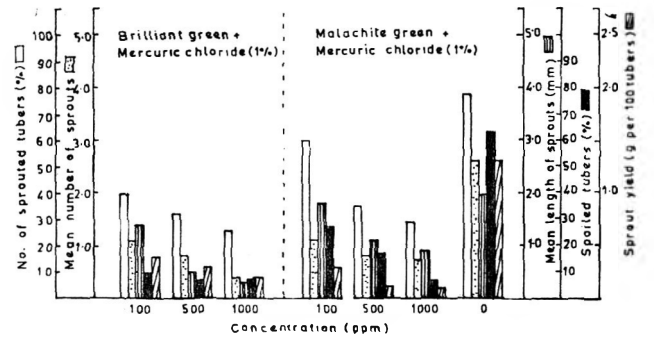

Fig. 3. Effect of dyes (Brilliant green and Malachite green) at various concentrations in combination with mercuric chloride (1%) on the control of sprouting and spoilage of seed potato tubers stored for 30 days at ambient conditions.

At concentrations above this, there was reduced spoilage but it was accompanied with pitting which is an undesirable feature. At lower concentrations, its efficacy in controlling the microbial spoilage and sprouting was less.

Brilliant green was more effective in reducing microbial spoilage than malachite green at all the concentrations studied (Fig. 2). The ability of these two dyes to reduce sprouting was directly proportional to their concentrations (Fig. 2).

Brilliant green or malachite green in combination with mercuric chloride (1 per cent) caused further significant reduction in microbial spoilage at all the levels studied (Fig. 3) as compared to the dye or mercuric chloride alone (Fig. 2 and Table 3). Hence, in all further experiments, mercuric chloride and brilliant green at 1 and 0.05 per cent respectively were used in combination treatments. Even though 0.05 per cent brilliant green is higher than the minimum effective concentration viz. 0.01 per cent, in order to colour the potatoes distinctly, this concentration was used. The effects of mercuric chloride (1 per cent) and brilliant green (0.05 per cent) either alone or in combination on the spoilage and sprouting were further confirmed in a subsequent experiment (data not shown). Similar trends in results were obtained when tubers were stored at 2 ± 1 °C for a period of 5 months (Table 3).

A study on the effect of time lag after harvest on the efficacy of mercuric chloride and brilliant green in controlling spoilage and sprouting at ambient conditions indicated that treating the tubers on the 5th day after

TABLE 4. EFFECT OF TIME LAG AFTER HARVEST ON THE EFFICACY OF MERCURIC CHLORIDE IN COMBINATION WITH BRILLIANT GREEN ON THE CONTROL OF SPROUTING AND SPOILAGE OF SEED TUBERS STORED FOR 75 DAYS AT AMBIENT CONDITIONS

Days after harvest	Treatment	Spoiled tubers (%)	Sprouted tubers (%)	Sprout yield (g/100 tubers)
	Control	25.1 ± 3.5	61.4 ± 4.0	20.7 ± 3.2
1	$HgCl_2$+Bg	19.5^{ns} ± 9.8	44.0* ± 10.7	21.8^{ns} ± 3.2
	Control	26.5 ± 5.0	61.2 ± 4.8	17.1 ± 3.7
3	$HgCl_2$+Bg	13.3* ± 0.4	21.7* ± 4.3	10.8* ± 1.0
	Control	29.9 ± 0.3	63.5 ± 7.0	14.2 ± 1.7
5	$HgCl_2$+Bg	9.8* ± 2.8	27.4* ± 9.2	5.9* ± 0.9
	Control	24.5 ± 2.0	75.7 ± 5.9	18.9 ± 7.6
10	$HgCl_2$+Bg	19.5^{ns} ± 6.4	43.1* ± 6.2	10.1* ± 2.4
	Control	29.7 ± 9.5	86.3 ± 8.2	17.4 ± 5.9
20	$HgCl_2$+Bg	14.6* ± 2.9	82.7^{ns} ± 7.6	9.5* ± 1.8

$HgCl_2$+Bg—Mercuric chloride (1%)+Brilliant green (0.05%).
*Denotes significant at $P \leq 0.001$.
ns—not significant.
Mean ± S.D.

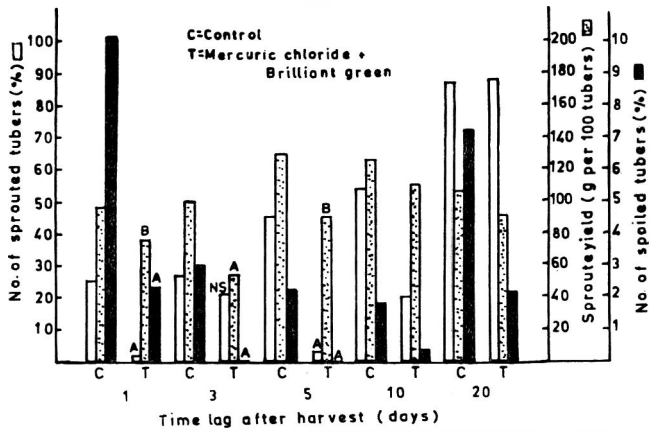

Fig. 4. Effect of time lag from harvest to treatment on the efficacy of mercuric chloride (1%) in combination with Brilliant green (0.05%) in the control of sprouting and spoilage of seed potato tubers during cold storage for 75 days. A & B denote significance at $p \leq 0.001$ and $p \leq 0.005$ respectively; NS—not significant.

harvest was ideal (Table 4). Less sprouting was recorded in the treated tubers than the untreated control lots.

Tubers given treatment with mercuric chloride and brilliant green after 3rd or 5th day of harvest showed reduced spoilage and sprouting (Fig. 4), when the treated tubers were stored at low temperature (2±1°C). These results indicated that treating the tubers on the 3rd or 5th day after harvest ensured effective control of spoilage and sprouting.

The results of the pot experiments revealed that the treated tubers differed in no way from the untreated lot (control) in all the four parameters of plant growth, namely (*i*) length of plants (*ii*) fresh weight of shoot system, (*iii*) root system and (*iv*) tuber yield which was confirmed further under field conditions (data not shown).

Residue analysis for mercury in the tubers obtained from the plants of treated tubers indicated that the mercurial residues were well within the permitted levels 0.025 p.p.m. (0.025 μg/kg fresh weight)[14].

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Tamaroggtt — A New Product from Date and *Oggtt*

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***Tamaroggtt* a combination of *tamar* (date) and *oggtt* was developed by mixing different ratios of pitted, minced date with fermented milk (butter milk) prepared from skim milk powder. The mixture was shaped and dehydrated at room temperature under continuous aeration. The new product is not only rich in protein, sugar and minerals but also acceptable. It can be kept at room temperature without noticeable changes.**

Tamar (date) is the ripe dried fruit of date palm (*Phoenix dactylifera* L.). Without any treatment, it can be kept at room temperature without microbial spoilage. *Oggtt*, *Mathier*, *Jameed* or *Buggl* are different names given to a dried fermented milk product which is made by bedouins from milks of cows, camels, sheep, etc.

Oggtt is made primitively by different methods in different areas. The two most common types are cooked *oggtt* and salted *oggtt*. Cooked *oggtt* is known throughout the Arabian peninsula. It is made by allowing the milk to ferment naturally in a warm place for one or two days. The fermented milk is churned and butter is removed. The butter milk is left to boil on fire with continuous stirring until it thickens. The thick product is left to cool down to a paste. The warm paste is shaped in the form of small patties by hand. The patties are then pressed on tent cloth and left to dry in the sun for several days. The colour of the final product is yellowish white. Salted *oggtt* is common in the northern region of the Arabian peninsula and in Lebanon, Syria and Jordan. This product is named *Jameed*. It is made by fermenting and churning the milk as mentioned earlier. No heat is applied; instead salt is added. Then, the salted fermented product is placed in a sac of cheese cloth and left to drain the whey for one day. The paste is then shaped by fingers in larger and more regular patties and then left to dry in the sun. The final product is white. Both types of *oggtt* can be consumed either dry "as is" or after reconstitution. The salted *oggtt* should be rinsed with water before reconstitution. The reconstituted product is called merase for either type of *oggtt*.

Tamar (date) and *oggtt* have been known in the Arabian peninsula for a long time and have a great sentimental value to the nomads, as they were particularly useful during long trips in the desert.

The objective of this work was the development of a new sanitary product that combines both *tamar* (date) and *oggtt*, namely *Tamaroggtt*.

Materials and Methods

Skim milk powder, *tamar* (date, Rezaiz), salt, cocoa and *oggtt* samples were bought from the local market in Hofuf, Saudi Arabia.

Preparation of Tamaroggtt: *Tamaroggtt* was prepared from reconstituted skim milk powder. Yoghurt was used as starter for carrying out fermentation at 42°C for 5 hr. Samples were drawn periodically to determine the acidity; when it reached 0.6 per cent, the fermented milk was heated with continuous stirring until most of the moisture evaporated. Heating was stopped when the fermented milk became pasty. At this stage, pitted chopped date as well as other additives were added and mixed well. The mixture was shaped by biscuit shaping equipment. The product was dried overnight by a draft of air using a fan. *Tamaroggtt* containing the highest ratio of date was further dried in an oven at 50°C for 8 hr. The final product was packed in separate units in small polyethylene bags and sealed. The products were: (1) *tamaroggtt*-1, 1:1 of date and SMP; (2) *tamaroggtt*-2, 1:2 followed by 0.1 per cent anise and 0.2 per cent sesame seeds; (3) *tamaroggtt*-3, 1 per cent cocoa added to *tamaroggtt*-1.

Chemical analysis: Moisture was determined by using a vacuum oven at 70°C. Nitrogen was estimated by the Kjeldahl procedure and multiplied by 6.38 to obtain crude protein[1]. Fat, ash and total and reducing sugars were determined as described by Pearson[2].

Physical and sensory properties: Texture was determined by Penetrometer (Precision Scientific Co., Chicago Illinois) by using standard needle 73524 with 150 g weight as a driving force. The penetration of the needle into the product during 3 sec was determined. The

average penetration distance per second was calculated.

Sensory properties were determined by a panel of ten Saudi members. The panel members were selected, familiarized with the new product *tamaroggtt* as well as with the classical (market) *oggtt*. Then they were asked to evaluate the *oggtt* (as a comparison) and the different *tamaroggtt* samples on a hedonic scale according to Stone and Siedel[3] designed in five descriptive terms namely: very desirable, mildly desirable, neutral (neither desirable nor undesirable), mildly undesirable, and very undesirable. Furthermore, the panel members were asked to grade three main characteristics (colour, 40 points; texture, 20 points; and flavour 40 points).

Statistical analysis: The data were analyzed for least significant difference (LSD) at 5 per cent probability according to Snedecor and Cochran[4].

Results and Discussion

Chemical composition: Table 1 shows the chemical composition of date, *oggtt* and different *tamaroggtt* samples. 'Rezaiz' tamar is the most abundant cultivar in Al-Hassa. Sixty per cent of dates produced in Al-Hassa is 'Rezaiz'[5,6]. The chemical composition of date used in this study differs slightly from that reported by other investigators[7-10]. With the exception of protein content, oggtt was similar in its analysis to that reported by El-Erian[11]. *Tamaroggtt* products are rich in protein, carbohydrates and minerals.

Physical and sensory properties: *Tamaroggtt* was shaped regularly in different biscuit shaping equipments. The average weight of each *tamaroggtt* unit is about 40 g for formula 1:1 and 35 g for formula 1:2. The colour of *tamaroggtt* depends on the percentage of date and other materials such as cocoa present. But the final colour is homogenous and stable. The colour of *oggtt* is usually creamy with darker ends.

The distribution of the panel members on the five previously mentioned descriptive terms is indicated in Table 2. *Tamaroggtt* products (except the one with cocoa) were superior to the market *oggtt*, inspite of the traditional and sentimental value of the latter. No significant differences were observed between *Tamaroggtt*-1 and *Tamaroggtt*-2. The addition of sesame seeds and anise to *Tamaroggtt*-2 seem to make up the difference that resulted from reducing the amount of date of *Tamaroggtt*-1. *Tamaroggtt*-3 (with cocoa) was

TABLE 1. COMPOSITION OF DATE ('REZAIZ' TAMAR), OGGTT AND TAMAROGGTT

Sample	Moisture (%)	Fat (%)	Protein N×6.25 (%)	Reducing sugars (%)	Total sugars (%)	Ash (%)
Date ('Rezaiz' *tamar*)	8.70	0.46	2.77*	77.50	82.40	1.30
Oggtt	4.90	22.76	37.42	13.87	23.50	11.87
Tamaroggtt-1	10.25	—	25.71	56.05	59.10	2.79
Tamaroggtt-2	9.75	—	34.35	44.30	48.95	2.48
Tamaroggtt-3	10.10	—	33.50	46.25	49.90	3.25
Least significant diff.	0.60	—	1.50	2.50	3.50	0.50

TABLE 2. PENETROMETER VALUES, FREQUENCY DISTRIBUTION OF PANELISTS RATINGS AND COMPOSITE SCORES FOR PRODUCTS

Sample	Penetration (mm/sec)	Frequency %					Composite score			
		E	D	C	B	A	Colour	Texture	Flavour	Total
Oggtt	0	50	50	0	0	0	32	16	35	83
Tamaroggtt-1	0.50	80	10	10	0	0	36	17	39	92
Tumaroggtt-2	0.17	80	20	0	0	0	34	18	38	90
Tamaroggtt-3	0.25	10	20	50	20	0	31	16	20	68
Least sig. diff.	0.05						2.5	1.4	5.0	8.3

Values are means of 3 determinations.
E, very desirable; D, mildly desirable; C, neutral; B, undesirable; A, very undesirable.

much less desirable. Since quality attributes such as colour, texture and flavour interact among each other[12], the composite score was also determined by the panel.

The yellowish colour of market *oggtt* was considered to be desirable by most of the panel members. The addition of date increased the desirable colour of *tamaroggtt*. Texture as determined by penetrometer varied considerably. There was no penetration at all in market *oggtt*, Yet it was very acceptable. *Tamaroggtt* has much improved texture but scored slighly above market *oggtt*. *Tamaroggtt*-1 (with 50 per cent date) was the softest. Flavour of *tamaroggtt* (without cocoa) was superior to market *oggtt*. But *tamaroggtt* with cocoa was inferior. From these results, it is concluded that *Tamaroggtt*-1 and *tamaroggtt*-2 are desirable but *tamaroggtt*-3 is not acceptable.

The cost of preparing either *tamaroggtt*-1 or *tamaroggtt*-2 does not exceed 20 per cent of retail value of market *oggtt*.

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Application of Reverse Osmosis in the Manufacture of Khoa. Process Optimization and Product Quality

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A process has been developed for the manufacture of *khoa* using reverse osmosis (RO) to preconcentrate the milk. Flux in spiral-wound cellulose acetate membranes was pressure-dependent up to 27 kg/cm² and then became independent of pressure. No permeation was observed until a pressure of 6 kg/cm² was applied due to the osmotic pressure of milk. Flow rate affected flux only in the pressure-independent region. The average flux when concentrating cow's milk from 12.5% total solids to 31% total solids was 8–10 liters per square meter per hour at 30°C. *Khoa* manufactured from pre-concentrated (31% total solids) whole milk was typical in flavour and texture compared to *khoa* made in the traditional open-pan boiler. The mineral and ash contents of RO-*khoa* were slightly lower due to permeation of these compounds through the RO membrane. The overall economics and feasibility of a continuous *khoa* manufacturing operation based on reverse osmosis is attractive primarily because of the large savings in energy compared to traditional open-pan boilers.

The use of synthetic membranes in the chemical, medical, food and bio-processing industries is rapidly increasing around the world[1]. Membrane processes such as reverse osmosis (RO) and ultrafiltration (UF) can play a significant role in food processing, especially the dairy industry. To date, the major application on a world-wide basis has been the use of ultrafiltration for fractionating cheese whey or for pre-concentrating milk prior to cheese making. In India. however, owing to the nature and demand of indigenous dairy products, reverse osmosis, which is essentially a dewatering operation, should have wider application.

Khoa is an important indigenous milk product. It has been estimated that as much as 7 per cent of India's total milk production is converted into *khoa*[2]. It is presently manufactured on a small scale by continuous boiling of milk until the desired concentration (65-70 per cent total solids) is reached. Several attempts have been made to develop new methods for commercial production of *khoa*, including the use of scraped-surface kettles or heat exchangers, evaporation for partial moisture removal[3] and use of dehydrated milk[4]. The general conclusions were that using pre-concentrated milk above 31 per cent total solids resulted in poor quality *khoa*. The superior energy efficiency of reverse osmosis during initial stages of milk concentration could be used to advantage in developing an economical low-energy continuous *khoa*-manufacturing process. The aim of this study was to develop such a process. The specific objectives were to optimize the processing of milk by reverse osmosis and to manufacture *khoa* from RO-concentrated milk and evaluate product quality.

Materials and Methods

The processes used in this study are shown schematically in Fig. 1. Cow's milk was obtained from the University of Illinois dairy herd. In all the 4 processes shown, reverse osmosis was used to pre-concentrate the milk to either approximately half its initial volume (2X) or to 40 per cent of its initial volume (2.5X). The concentrated milk was then converted into *khoa* in an open stainless steel kettle using a Teflon-coated stirrer. *Khoa* made by the traditional open-pan method served as the control. The following variations in the basic process were studied:

Process A: Raw milk, with no further treatment, was processed in the reverse osmosis module prior to open-pan boiling.

Process B: Milk was pasteurized at 63°C for 30 min before reverse osmosis. The process was otherwise identical to Process A.

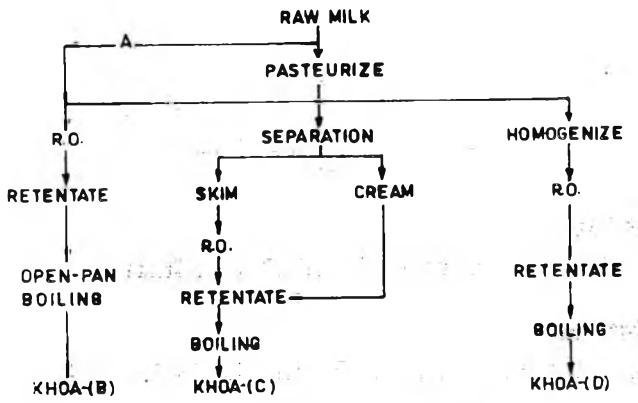

Fig. 1. Schematic of reverse osmosis process for manufacturing *khoa*

Process C: Milk was pasteurized and separated in a cream separator. The skim milk was concentrated by RO. The RO retentate was then combined back with the cream before converting it to *khoa*.

Process D: Milk was pasteurized and homogenized in a Manton-Gaulin homogenizer at a total pressure of 2000 psig (135 kg/cm^2). The homogenized milk was concentrated by reverse osmosis.

A fifth process, referred to as the "Two Stage Membrane Process for *Khoa*", was also evaluated (Fig. 2). After pasteurization, the milk was ultrafiltered to a concentration factor of 4.5X. The permeate from the UF operation was then concentrated by reverse osmosis

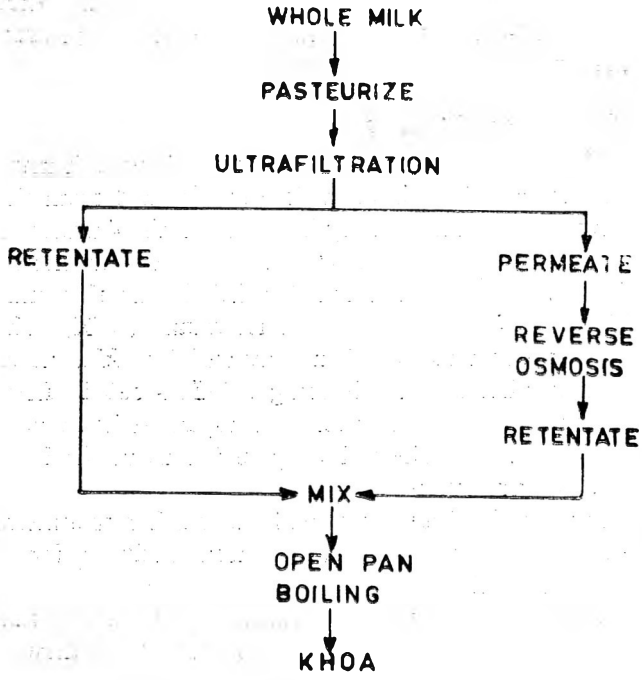

Fig. 2. Schematic of two-stage membrane process for manufacturing *khoa*.

to a 4.4X concentration factor. The UF retentate and the RO retentate were then combined before manufacturing *khoa*.

Membrane systems: Reverse osmosis was done on a spiral-wound pilot-scale module manufactured by Osmonics Inc, Minnetonka, Minnesota, USA. The membrane was type SEPA-97(CA) in a Model 192 housing. The membrane is made of cellulose acetate rated at 97 per cent rejection of sodium chloride, with a nominal pore size of 0.5 nm and maximum recommended operating temperature of 30°C. The module was installed in a process loop consisting of a feed tank, a high-pressure reciprocating pump fitted with a variable speed motor, a pulsation dampener, a shell-and-tube heat exchanger to control the temperature, the appropriate valves, pressure gauges, flowmeters and thermocouples to control and measure temperature, inlet and outlet pressures, flow rate through the module (recycling rate, Q) and permeate flux (J). Unless otherwise mentioned, RO was done at 30°C and pH 6.8.

The ultrafiltration system was a pilot-scale Romicon hollow fiber unit. The module was the HF15-43-PM50; it had a polysulfone membrane of 1.4 m^2 area with a nominal molecular weight cut-off of 50,000. Ultrafiltration was done at 50°C and pH 6.8. Further details on the operation of hollow fibers with milk are available elsewhere[5,6].

The membrane systems were operated in the batch mode[7]. Both retentate and permeate were recycled to the feed tank during the fouling and optimization studies. During concentration, the permeate was discarded and the feed allowed to concentrate. The concentration factor (X) or Weight Concentration Ratio (WCR) is expressed as:

$$\text{WCR or (X)} = \frac{\text{Initial weight of feed}}{\text{Weight of retentate}} \quad (1)$$

The performance of a membrane unit is measured in terms of flux and rejection[7]. Flux, the rate of removal of permeate, is expressed as the permeate volume per unit membrane area per unit time (i.e., l/m^2/hr or LMH). Rejection is a measure of the membrane's ability to retain or separate a particular component in the feed. It is expressed as:

$$R = (1 - Cp/Cr) \times 100 \quad (2)$$

where Cp is concentration in the permeate and Cr is concentration of that component in the retentate.

The driving force for permeation is the transmembrane pressure. It is calculated as:

$$\Delta P_T = \frac{Pi + Po}{2} - Pp \quad (3)$$

where *Pi* is the inlet pressure and *Po* the outlet pressure of the RO module. Pp is the permeate back pressure

measured with a pressure gauge fitted as close as possible to the permeate outlet of the module.

Analytical methods: Total solids (gravimetric), fat (Mojonnier), ash (ignition at 550°C) and nitrogen (Kjeldahl) were measured by standard methods[8]. Lactose was measured by the phenol-sulphuric acid method of Dubois *et al.*[9] Protein is expressed as Kjeldahl nitrogen × 6.38. Colour was measured by the Hunter colorimeter. The Hunter "L" value varies from 100 for perfect white to zero for black. The "a" value measures redness (when positive) or green (when negative), and "b" values are indication of the intensity of yellow (when positive) or blue (when negative). All analyses were performed in duplicate.

Sensory evaluation of *khoa* samples was done by a panel of 7-10 judges who were mostly of Indian and Pakistani origin, using the methods suggested by Pal and Gupta[10].

Results and Discussion

Optimization of reverse osmosis processes: The relationship between flux (J) and applied pressure is expressed as:

$$J = A\,(\triangle P_T - \triangle \pi) \qquad (4)$$

where $\triangle \pi$ is the transmembrane osmotic pressure and A is the membrane permeability coefficient. Since deionized distilled water will exert no osmotic pressure, a linear relationship between flux and pressure was obtained (Fig. 3). The average pure water permeability coefficient (Aw) for this membrane is 1.45 l/m²/hr/atm.

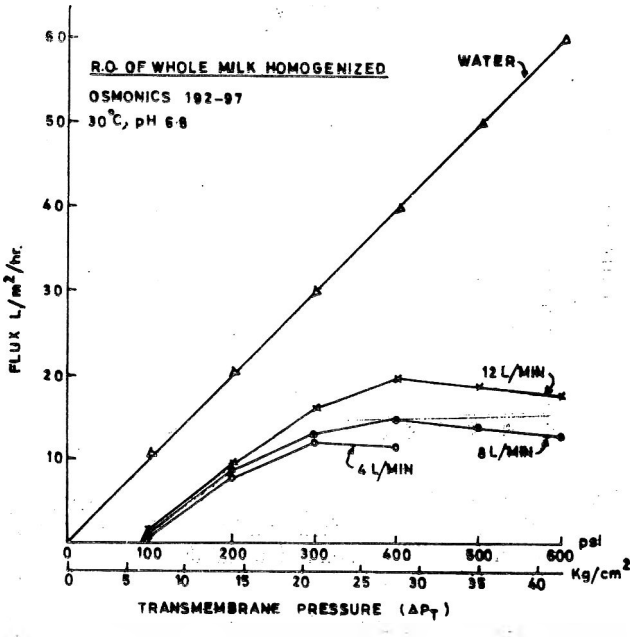

Fig. 3. Effect of transmembrane pressure and flow rate on flux of homogenized whole milk (3.5% fat, 12.6% total solids). Flux for pure water is also shown for comparison (1 psi = 0.068 kg/cm²).

Milk, however, shows a significant departure from linearity and its flux is much lower than that of water. There are two possible reasons for this:

(*i*) According to equation 4, no permeation will occur until the applied pressure exceeds the osmotic pressure of the retained solids. Due to the presence of the solids in milk, especially the dissolved salts and lactose, the osmotic pressure of milk is quite significant. Experimental data and calculations using the van't Hoff equation indicate osmotic pressure of milk to be about 100 psi (6.8 atm)[7]. This explains why there is essentially no permeation until the transmembrane pressure is about 100 psig (Fig. 3) and why flux of milk will always be less than that of water.

(*ii*) In addition to osmotic pressure, another resistance must be overcome due to concentration polarization and the associated boundary layer. Depending on the pressures in the module and the porosity of the membrane, a certain portion of the feed is removed as it flows down the membrane channel. This results in a "polarization" of the solute molecules on the membrane surface. This polarized layer decreases flux either by increasing the hydrodynamic resistance of the membrane and/or by further increasing the effective osmotic pressure. Increasing the applied pressure merely brings additional solute to the membrane, negating the beneficial effects of higher pressure and resulting in the asymptotic flux behaviour shown in Fig. 3. In extreme cases, the polarized layer may "gel" or precipitate out on the membrane leading to severe membrane fouling. Thus, in the polarized region, improvements in flux can only be obtained by reducing solute concentration at the membrane surface. This can be done using high shear at the surface or by increasing the turbulence in the module which will enhance the rate of back-transport of polarized solute into the bulk of the solution.

This explains why higher flow rates improved the flux with milk (Fig. 3). The fat and protein contribute little to the osmotic pressure compared to the salts and lactose, but will be the major reason for the increased hydrodynamic resistance. Greater polarization will occur at higher pressures and thus the effect of flow rate will be more noticeable at higher pressures.

Similar results were obtained with non-homogenized milk (Process B) and with skim milk (Process C)[11]. Flux of skim milk was usually higher at equivalent pressures and flow rates than whole milk. Homogenization improved the flux at the lower flow rates, but was not beneficial at the higher flow rates. It appears that the optimum pressure is 400 psig (27.3 kg/cm²) and flow rate with this module should be at least 12 l/min, which is the highest flow rate recommended by the manufacturer.

Fig. 4 shows the effect of solids concentration on flux

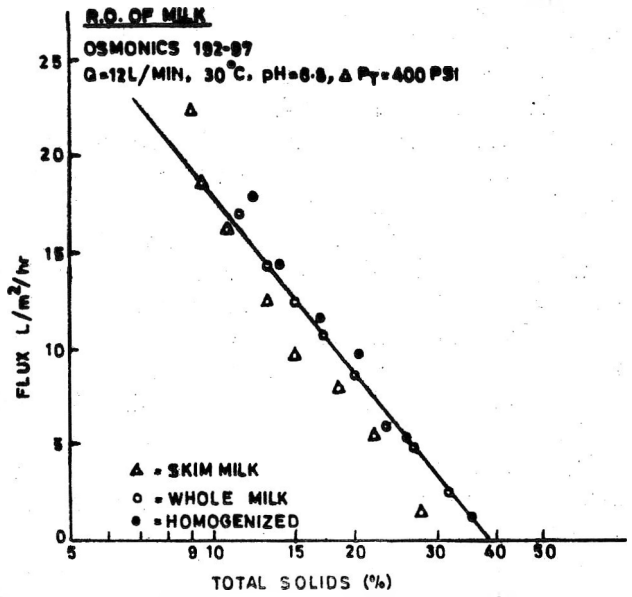

Fig. 4. Relationship between total solids and flux during reverse osmosis of milk. (△=skim milk; O=whole milk; ●= homogenized whole milk). The transmembrane pressure was 400 psig and the flow rate (Q) was 12 L/min.

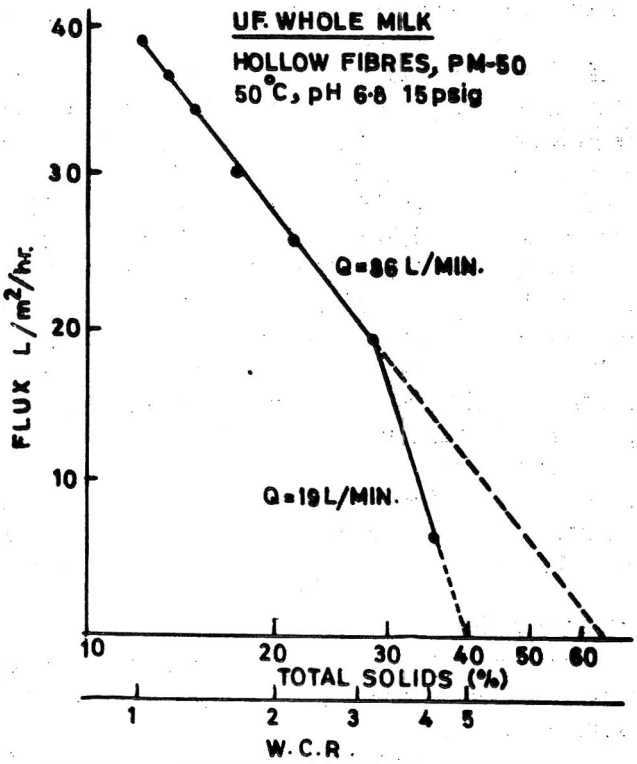

Fig. 5. Ultrafiltration of whole milk: effect of retentate solids concentration of flux. The flow rate (Q) through the module was 86 L/min upto 3X, then it was lowered to 19 L/min. Transmembrane pressure was 15 psig.

using these optimum operating parameters. Since the system is operating in the mass transfer controlled region, the relationship between flux and solids concentration appears to follow the film theory[7]. The differences between the three milks are due to slight differences in their initial composition and rates of fouling of the membrane. By extrapolation, it appears that the maximum concentration of milk that can be obtained under these conditions is 36-38 per cent total solids.

Two-stage membrane process: Fig. 5 shows the relationship between flux and solids during the hollow fiber ultrafiltration of whole milk (the two-stage process shown in Fig. 2). Semi-logarithmic relationship was observed as long as the flow rate could be maintained constant. However, due to increases in viscosity at higher solids, large pressure drops were required to maintain the flow rate. This exceeded the pressure rating of the hollow fiber module. Above 30 per cent solids, the flow rate was reduced to 19 l/min. In practice, hollow fibers are limited to about 36-40 per cent solids with whole milk.

Fig. 6 shows the relationship between flux and total solids during RO of the UF permeate. Since the UF permeate was free of macromolecules and fat, concentration polarization was insignificant compared to milk. There was no asymptotic flux behaviour and little effect of flow rate on flux[11]. Thus, since osmotic pressure of the feed is the major resistance, the optimum process conditions for reverse osmosis of UF permeate would be highest possible pressure and low flow rates in contrast

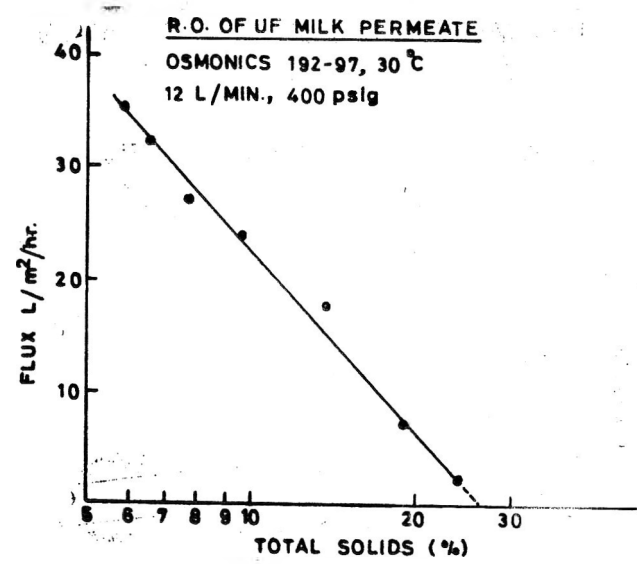

Fig. 6. Concentration by reverse osmosis of permeate from ultrafiltration of whole milk: relationship between retentate solids concentration and flux.

to the optimum conditions for reverse osmosis of milk (highest flow rate and intermediate pressure).

Since the osmotic pressure of UF permeate would be close to that of milk and the applied pressure is 400 psi,

the maximum concentration possible (assuming a linear relationship between UF permeate solids and osmotic pressure) would be 4.4X, according to equation 4. This is approximately what we obtained before the flux became too low to be measured. The initial solids content of the UF permeate was 5.81 per cent and the maximum concentration possible (obtained by extrapolating the data in Fig. 6) is about 27 per cent solids. Higher applied pressures would no doubt have permitted a higher final concentration.

However, there is a practical limit to the degree of concentration which is dictated by the loss of solids. Permeation of the solids is a function of the difference in concentration on either side of the membrane. The higher the solids in the feed/retentate, the greater the passage of the solids into the permeate. This is shown in Fig. 7, where the concentration factor in terms of WCR is plotted against the permeate and retentate composition during the experiment shown in Fig. 6. Above WCR 3, the "leakage" of ash and lactose increased significantly. However, even at the highest retentate concentration, the overall rejection of the solids was more than 95 per cent. If losses of the solids are to be minimized with this particular cellulose acetate membrane, the concentration factor should be kept below 3X.

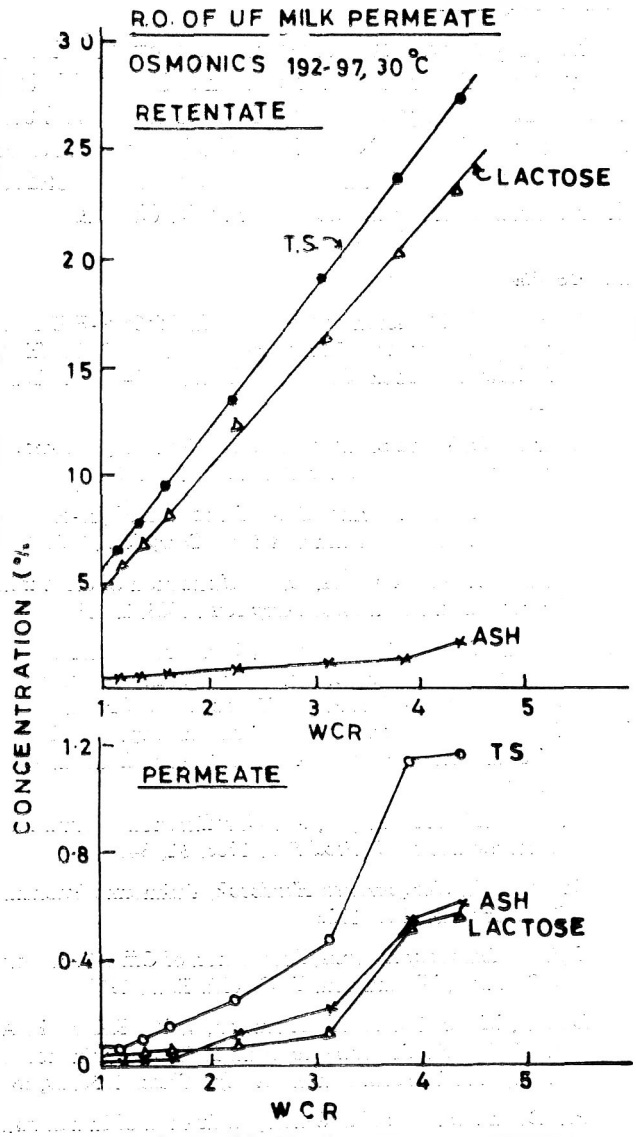

Fig. 7. Rejection of total solids, ash and lactose during reverse osmosis of UF-milk permeate. Top: Concentration in retentate. Bottom: concentration in permeate.

Product composition: Based on previous research[2-4] and our own optimization studies, it was decided to limit the pre-concentration of milk by RO to less than 3X. This should keep RO losses low and the flux high and the sensory properties of the *khoa* made from the concentrates should be satisfactory. With each process shown in Fig. 1, two concentrates were prepared; one where the milk was concentrated to 2X and the other to 2.5X. The composition of the *khoa* made from milk and its concentrates is shown in Table 1. Preparation of *khoa* from RO retentates took less than half the time of the control *khoa* made by the traditional method. In general, the *khoa* from RO concentrates showed higher moisture contents than the controls. The fat content of all *khoa*, including the control, was usually less than 20 per cent, since the raw material was cow's milk. However, fat content can be adjusted during the process, either before or after RO.

Throughout all the experiments[11], a significant difference was observed in the fat and ash contents between control and RO-*khoa*. The ash was always lower in the RO-*khoa*. This could be due to the passage of some

TABLE 1. COMPOSITION OF KHOA (AS IS BASIS)

Process	Concn. factor	Total solids (%)	Fat (%)	Protein (%)	Ash (%)	Lactose (%)
A	Control	69.9	17.0	19.9	4.4	26.5
	2X	63.2	16.1	18.1	3.7	25.3
	2.5X	60.5	15.7	17.4	3.4	24.1
B	Control	67.7	19.6	19.3	4.0	24.9
	2X	61.4	18.6	17.7	3.2	22.0
	2.5X	60.3	18.3	17.5	3.0	21.5
C	Control	66.7	19.3	18.8	4.3	23.8
	2X	66.4	20.6	18.8	3.6	23.3
	2.5X	65.9	20.5	18.7	3.5	23.2
D	Control	62.5	17.8	17.4	3.9	23.5
	2X	61.3	18.1	17.1	3.3	22.8
	2.5X	60.6	17.9	17.0	3.2	22.5

ionic species through the membrane during concentration. At WCR of 2-2.5X, rejection of ash was typically about 78 per cent (Fig. 7) and thus the lower ash content of the RO-*khoa* is to be expected. Lactose loss was much less since the rejection of lactose was in excess of 97 per cent. Some non-protein nitrogen may pass through the membrane, but Table 1 indicates nitrogen rejection was also very high since protein contents of RO-*khoa* were comparable to the control. Fat rejection is essentially 100 per cent with RO membranes. Since there was a loss of other components, the fat content of RO-*khoa* is expected to be slightly higher.

Sensory properties: Table 2 shows sensory properties of RO-*khoa*. There were essentially no differences in the colour values (L,a,b) among any of the RO-*khoa* samples i.e., neither the process used (A, B, C or D) nor the degree of pre-concentration (2X or 2.5X) had any effect on the colour values. The RO-*khoa* showed less of a greenish tinge than control *khoa*, but the differences were not significant. All *khoa* samples showed a noticeable yellowish-orange colour, due to the presence of carotene in the cow's milk.

There were no differences in other sensory properties between 2X and 2.5X RO-*khoa* samples. The RO-*khoa* samples did not show any large differences in flavour and body from their respective control, except for Process A samples. Since Process A used raw milk, the RO-*khoa* was distinctly rancid. This is due to the hydrolysis of fat by lipase which was not inactivated before concentration. RO-*khoa* tended to lack graininess, but this may not be an undesirable quality. In fact, in preparation of products such as *burfi*, this will help in producing a homogeneous and smooth product. It is also possible to control the graininess during the final finishing/heating stages. Except when homogenized, the RO-*khoa* showed slightly higher free fat which was evident from deposition on the sides of the container.

It is concluded that the application of reverse osmosis for the manufacture of *khoa* appears to have great potential in India. The process is quite simple, requiring only the pumping of milk through a membrane module. The *khoa* prepared from RO-concentrated milk has a slightly higher moisture content, but the flavour and body do not appear to be affected. This should result in higher yields of the product. Since there were no major differences between the products (except for the *khoa* from Process A), Process B is preferred due to its simplicity: no separation is needed as with Process C, and no homogenization as with Process D.

TABLE 2. SENSORY PROPERTIES OF RO-KHOA

Colour by Hunter colorimeter (average of all trials)

	L	*a*	*b*
Control khoa	69.3	—4.1	19.5
RO-khoa (2X)	67.0	—1.5	16.7
RO-Khoa (2.5X)	67.8	—3.1	15.7

Flavour and Texture (average ratings for all samples)

Control khoa*	: Normal, granular, typical flavour
RO-Khoa (Process A)	: Rancid, free fat, lacks grains
RO-Khoa (Process B)	: Normal, free fat, no grains
RO-Khoa (Process C)	: Cooked, slight free fat, no grains
RO-Khoa (Process D)	: Normal, no free fat, no grains

*Except for Process A control, which had a rancid flavour.

Acknowledgement

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Study of Paneer Quality in Relation to Pressing Conditions

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The study was conducted on a laboratory model paneer press for correlating final paneer quality to pressing conditions. Three quality factors, namely, moisture content, shear strength and porosity were considered and the effect of pressure and time of pressing on these quality factors was studied. Mathematical models for predicting final moisture content and shear strength as a function of pressure and time of pressing were developed. Effect of pressing conditions on porosity of paneer product was not significant even at 10% level of significance. From the point of view of uniformity of product quality, pressing at lower pressures around 1 kg/cm^2 is recommended.

Paneer (Indian cheese) is a common milk product of high nutritive value. In India, paneer production is mostly limited to domestic level because of lack of technical details of production. The process of paneer making consists of coagulation of milk with the help of a suitable coagulant and separation of curd from whey. The curd is subsequently pressed for increasing the cohesion and removal of excess moisture.

Quality of paneer is judged on the basis of its cohesive strength, moisture content, sponginess and nutritive value. All these quality factors, except the nutritive value, are dependent on the pressing parameters which include the pressure and the time of pressing. Product pressed under a high pressure or for a long time, would probably be very compact lacking sponginess. On the other hand, pressing with very low pressure, or for a short time, may result in poor handling and storage properties owing to low cohesive strength and high moisture content. Hence, optimization of pressing condition is needed to produce paneer of desirable quality.

Kundu and De[1] and De[2] employed the method of hanging for removing excess water from the curd by dripping. No pressure was applied. The dripping of water stopped in 30-60 min. De *et al*[3] employed a pressure of 2 kg/cm^2 and a pressing time of 25 min while studying paneer production from acidic milk. The study was conducted only at a single pressing condition and the effect of pressing conditions was not investigated. A general description of paneer making process has been discussed by Warner[4]. He did not specify the pressure but mentioned a pressing time of 15-20 min. Kulshreshtha[5] presented a mathematical analysis of the process under low pressure short time pressing, based on rheological properties of paneer. No experimental data were presented and the developed model was not verified experimentally.

Considering the limited extent of work on the effect of pressing conditions on the quality of paneer, the present study was undertaken. Cohesive strength, moisture content and porosity were chosen as quality factors and were studied in relation to pressure and time of pressing.

Materials and Methods

Experiments were conducted at 1, 2 and 3 kg/cm^2 pressures, and pressing time intervals of 5, 10, 20, 60 and 120 min. Moisture content, cohesive strength and porosity of final product were the dependent parameters.

Paneer press: A vertical paneer press (Fig. 1) was developed for conducting the experiments. It consisted of a vertical perforated cylinder (5.5 cm internal diameter, 12 cm height), made of a G.I. pipe and threaded

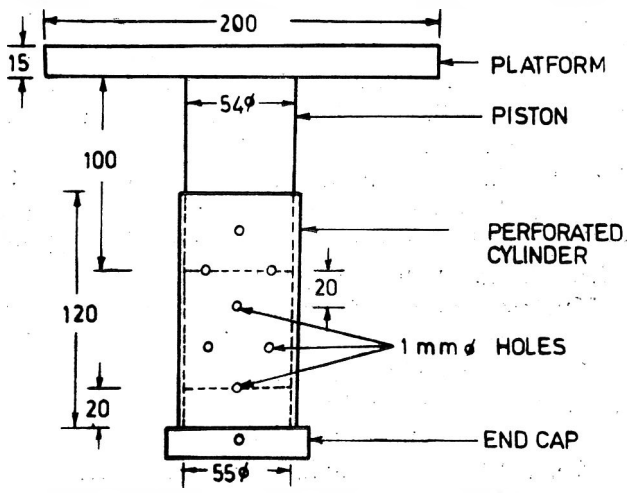

Fig. 1. The paneer press (all dimensions are in mm)

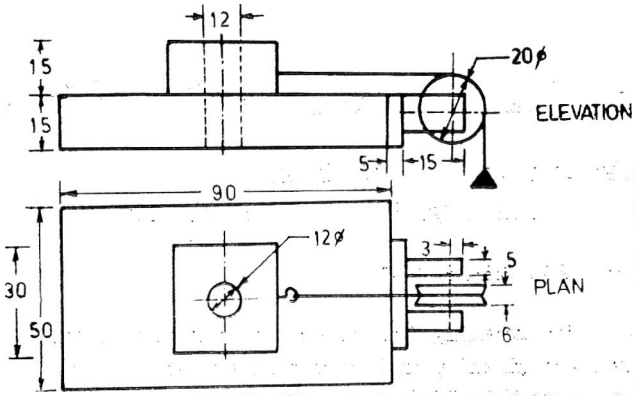

Fig. 2. The shear test apparatus (all dimensions are in mm)

internally upto a height of 2 cm from the bottom for securing an end cap, provided to facilitate the removal of pressed sample. The cylinder had five horizontal rows of 1 mm diameter perforations at a vertical spacing of 2 cm, each row containing four perforations. Pressing was done through a wooden piston of 5.4 cm diameter, with a clearance of 0.05 cm between the piston and the walls of the cylinder. A 1.5 cm thick wooden platform of 20×20 cm^2 was fixed on top of the piston for loading.

Shear test apparatus: A sliding block shear test apparatus was developed to determine shear strength of paneer samples. The apparatus (Fig. 2) was made of wood and consisted of a stationary and a sliding block, both having a hole of 1.2 cm diameter for placing the sample. The sliding block was connected to a weight pan through a string passing over a pulley mounted on the stationary block. The force required to shear the sample was determined by increasing the weights on the pan gradually without any impact.

Experimental technique: After estimating its fat content and taking a lactometer reading, the milk was boiled and 1 per cent citric acid solution was added to it slowly till clear whey was separated. The curd was, then, filtered through a muslin cloth. The whey was drained out by hanging the cloth till dripping of water practically stopped. A sample (about 5 g) of this curd was taken out for determination of initial moisture content. A 40g curd sample was pressed under preselected conditions in the press. After the pressing was over, the sample was taken out and tested for quality.

Moisture content: Moisture content was determined by oven drying a 5 g sample at 90°C for a period of 24 hr.

Cohesive strength: Shear strength of a sample is given by the following Coulomb's equation[6]

$$\tau = \sigma \tan \phi + c$$

where, τ=shear strength, σ=normal stress,
ϕ=angle of internal friction and c=cohesion

For $\sigma = 0$, $\tau = c$, hence shear strength of a sample, without any normal loading is equivalent to cohesion. A sample, 1 cm diameter plug, of paneer was taken out from the central location with the help of a sampler and its shear strength was determined.

Porosity: Porosity was calculated using the following formula:

$$\epsilon = 1 - \frac{W_d}{V.\rho_p}$$

where, ϵ=porosity, W_d=dry weight of samples, V=volume of sample, and ρ_p=true density of milk particles

The volume of sample used for moisture determination was recorded. True density of milk particles for use in above formula was calculated on the basis of its composition[7].

Results and Discussion

Product obtained in each experiment was tested for moisture, shear strength and porosity and they were correlated with the pressing conditions.

Effect on moisture content: Since initial moisture content of different samples was observed to differ significantly from each other, the ratio of moisture

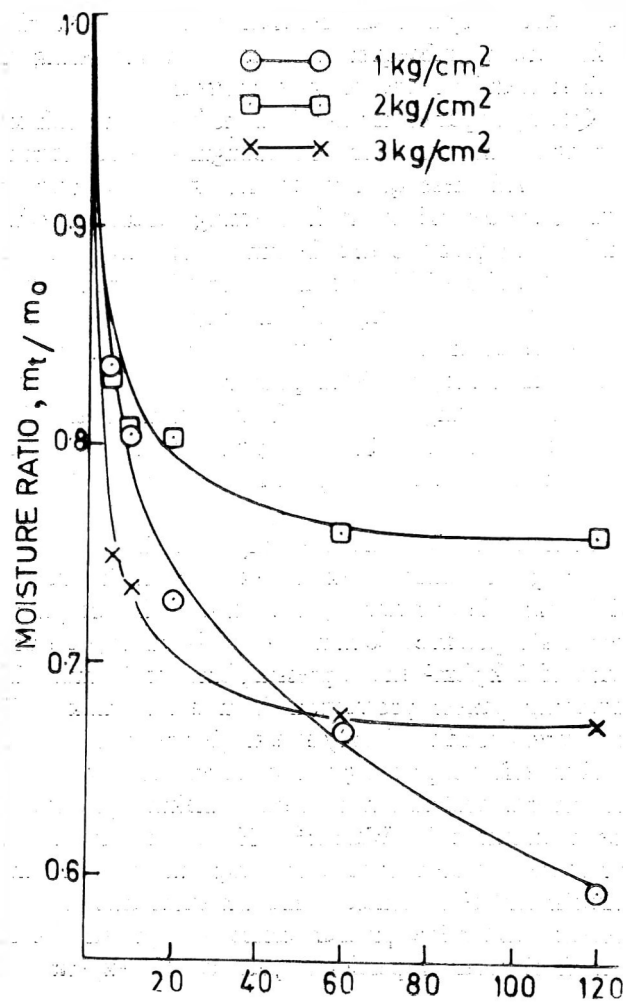

Fig. 3. Effect of time of pressing on moisture ratio

content at time t (m_t) and initial moisture content m_o, i.e. $\frac{m_t}{m_o}$ was used to arrive at uniformity of data.

Fig. 3 shows the effect of pressing time on $\frac{m_t}{m_o}$ at different pressures. The trend of moisture variation is nearly exponential. In case of higher pressures, initial rate of moisture ratio reduction is greater while ultimate reduction is lesser. This results in intersection of curves. This behaviour indicated the following mechanism of moisture expulsion:

As pressure is applied, a hydraulic gradient is established in the sample and water starts migrating, radially, towards the perforated walls of the press. Along with water, finer particles may also be transported and may get deposited in the outer pores. Consequently, porosity in the outer zone is reduced and further expulsion of water is restricted although excessive moisture is present in the central zone. At higher pressures, the velocity of water and hence that of the particles, is more. Therefore, the permeability of peripheral zone may be reduced to the extent of formation of an impervious layer. In this case further expulsion of water shall be restricted though excessive moisture and sufficient hydraulic pressure are present in sample. Consequently, the ultimate moisture ratio reduction, in case of higher pressures, is lesser. The moisture ratio can be correlated to pressing time by a relationship of the form:

$$MR = \exp(-K_1 t^{K_2})$$

where, K_1 and K_2 are pressure dependent constants. Constants of the equation were determined by least square regression[8] of experimental data after log transformation at different pressures. The variation of K_1 and K_2 with pressure is represented in Fig. 4. K_1 and K_2 were related to pressure according to the following equations:

$$K_1 = 0.068 \exp(0.412\ p)$$
$$K_2 = 0.516 \exp(-0.553\ p)$$

Hence, the unified model for moisture content may be represented by the following equation:

$$MR = \exp\left[\{-.068 \exp(.412p)\} t^{\{.516 \exp(-.553p)\}}\right]$$

Effect on cohesive strength: Cohesive strength of paneer increased with time of pressing (Fig. 5). For pressing times greater than 10 min, cohesive strength decreased as pressure was increased.

These observations may again be explained on the basis of particle transport. Cohesive strength is basically due to the interlocking of particles. Since interlocking itself is a function of compaction of sample, the increase in cohesive strength with time may be said to be due to the compaction of the sample. As pressure is increased,

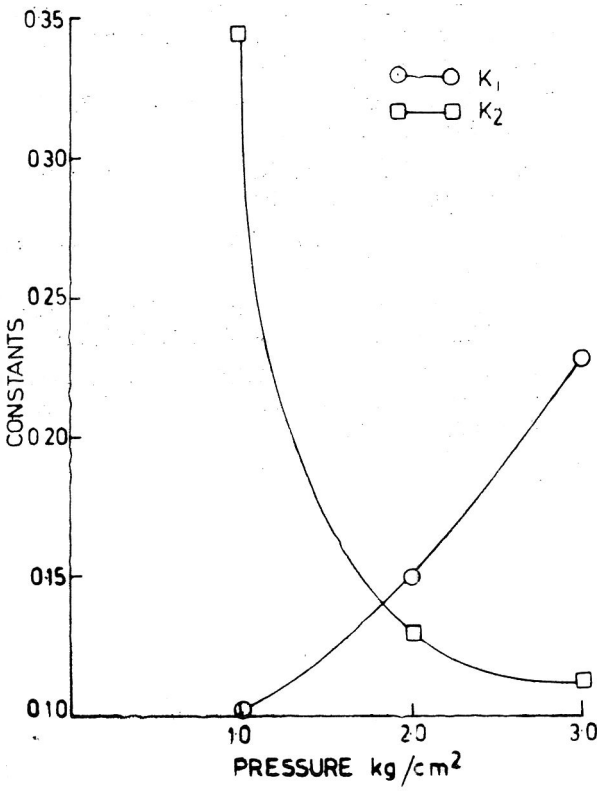

Fig. 4. Effect of pressure on constants K_1 and K_2

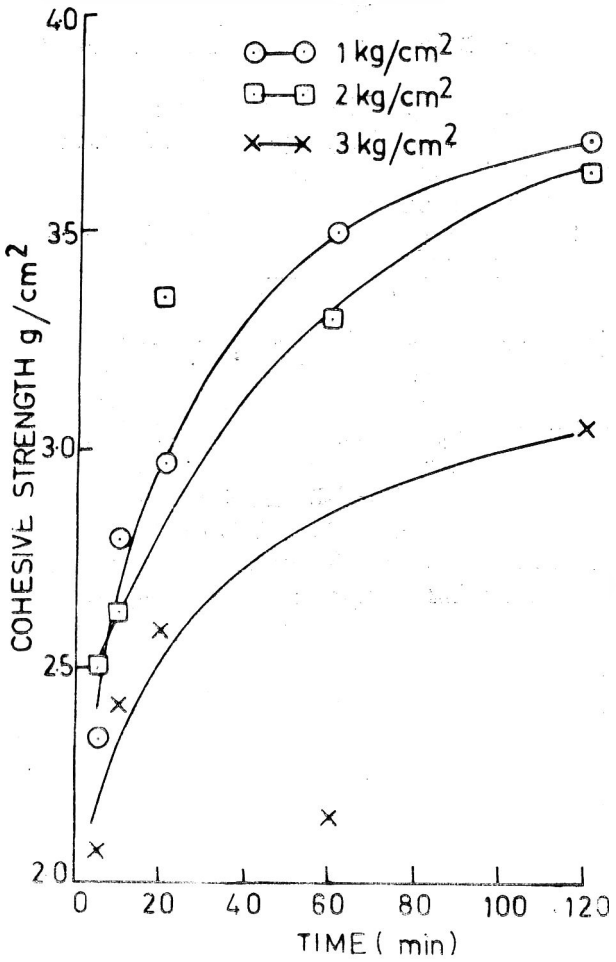

Fig. 5. Effect of time of pressing on cohesive strength

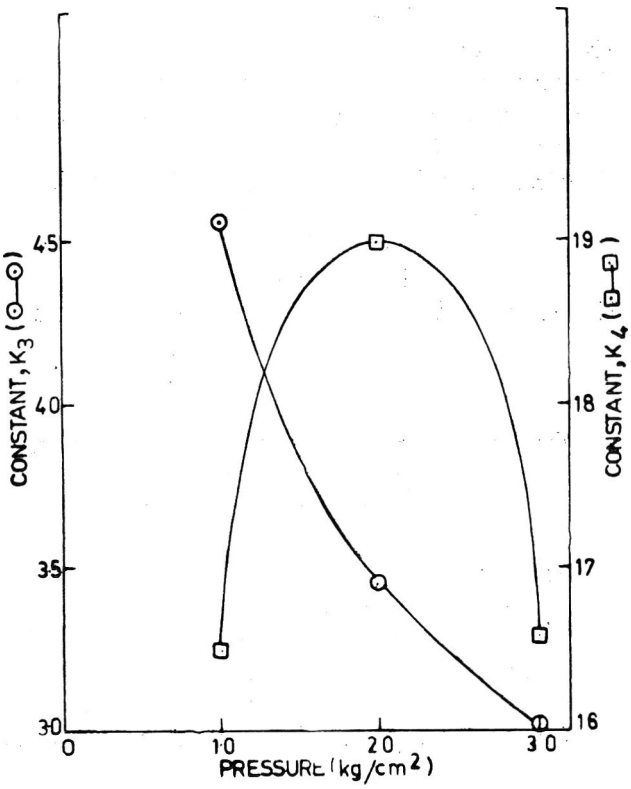

Fig. 6. Effect of pressure on constants K_3 and K_4

more and more particles move away from the central zone and the interlocking of particles in the central zone weakens. Consequently, cohesive strength of the central zone decreases with increase in pressure.

Cohesive strength was correlated to time of pressing by a relationship of the form

$$C = K_3 \ln t + K_4$$

where, K_3 and K_4 were pressure dependent constants. Constants of equation at different pressures were evaluated by least square regression of data. The variation of K_3 and K_4 is plotted against pressure in Fig. 6. The relationship can be expressed as:

$$K_3 = 5.47 \ (\exp^{-0.2p})$$

$$K_4 = 18.99 - 2.48 \ (p\text{-}2)^2$$

A unified model for cohesive strength in a sample of paneer may thus be represented by the equation

$$C = 18.99 - 2.48 \ (p-2)^2 + \{ 5.47 \exp (-0.2 \ p) \}. \ln t$$

It may be noted that this equation is valid for cohesive strength of central zone only. For peripheral zones, the strength is expected to be more and may follow a different pattern.

Effect on porosity: The porosity of final paneer ranged from 0.46 to 0.68. The variation of porosity did not show a definite relation with the pressing conditions. Analysis of variance of porosity data indicated the calculated 'F' values for pressure and time of pressing to be 0.359 and 1.263 respectively, against the table values (at 10 per cent significance level) of 3.11 and 2.81 respectively. Hence, neither pressure nor time of pressing had any significant effect on porosity.

It is concluded that: (1) variation of moisture content and shear strength with pressing conditions can be described by empirical mathematical relationships., (2) porosity of paneer is not influenced by pressing conditions, and (3) lower pressure with higher press time is more suitable for obtaining paneer of uniform quality in terms of moisture content and shear strength.

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Effect of Freezing and Sodium Citrate Treatment on the Association-Dissociation of Proteins from Shrimp (*Parapenaeopsis stylifera*)

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The effect of freezing at –20°C and treatment with sodium citrate on proteins of shrimp (*Parapenaeopsis stylifera*) has been investigated by analytical ultracentrifugation. The results indicate that the proteins dissociate during storage at –20°C. The rate of dissociation of proteins of shrimp by sodium citrate treatment was slower; and they had less drip loss and the overall quality of the shrimp was better. These are considered to be due to partial dehydration during storage and the effect of inorganic salts, mainly on the non-covalent interactions.

The shelf-life of fish depends upon the physical and chemical treatments given to it both on-shore and on-board fishing vessels. Generally, temperatures in the range of –20 to –40°C are used to extend the shelf-life of fish. Chemical treatments with hydrophilic additives antioxidants, neutral lipids and inorganic salts also extend their shelf-life to a considerable extent[1–5]. Frozen shrimp is an important export item and accounts for about 90 per cent of the total fishery export amounting to nearly 55000 MT[6].

However, apart from studies on cod meat by Mathews *et al.*[7] and Laird *et al.*[8], no systematic investigation is available on the deteriorative changes in proteins of shrimp upon storage. These treatments, both physical and chemical, can lead to dissociation-association and denaturation-aggregation of the proteins, particularly in lean fish and thus bringing about deterioration of the texture of the product. In the present study, such changes induced by storage at –20°C and treatment with sodium citrate are monitored mainly by analytical ultracentrifugation and measurement of drip loss and proteolytic activity.

Materials and Methods

Raw material: Shrimp belonging to the commercial species *Parapenaeopsis stylifera* was used in these studies. They were obtained immediately after landing at Mangalore (West Coast of India). After washing with municipal water, they were beheaded, peeled and deveined. One portion of the fresh meat was then subjected to quick freezing, filled in waxed cartons (400 g capacity) and stored at –20°C. Another portion was given a dip treatment in sodium citrate (8 per cent solution) for 5 min, drained over inclined stainless steel mesh for 5 min and then subjected to quick freezing and stored at –20°C like the control sample. The samples were stored for five months. At predetermined intervals, the blocks were taken out, thawed at 4°C and the meat and drip were taken for analysis.

Sodium carbonate, sodium chloride and sodium citrate were of reagent grade from BDH Chemicals, India; denatured haemoglobin was from Worthington Biochemicals USA. Distilled water was used in all the experiments. All other chemicals used were of reagent grade. Sodium citrate was estimated by a standard procedure[9,10].

Extraction of protein: Twenty grams of shrimp meat was homogenised for 2 min in a Waring blender with 140 ml of buffer solution, consisting of 0.02M sodium carbonate, pH 7.8, containing 5 per cent sodium chloride. The homogenate was centrifuged at 4000 rpm for 45 min at 4°C and the supernatant was used for analysis.

Protein concentration: The concentration of protein in the supernatant was estimated by micro-Kjeldhal procedure[11]. For routine determination of protein concentration, a calibration curve relating the protein content in the sample to its absorbance at 280 nm was established using a Shimadzu spectrophotometer ($E^{1\%}_{1cm,\ 280nm} = 15.52$).

Analytical ultracentrifugation: Sedimentation velocity experiments were carried out at 25°C in a Spinco Model E analytical ultracentrifuge equipped with a phase plate schlieren optics and a rotor temperature indicator and control (RTIC) unit. For a typical run, a standard 12 mm

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Kel F centerpiece cell was used. Protein sample of 1.6 per cent was routinely used and centrifuged at 59,780 rpm unless otherwise stated. Photographs were taken at frequent intervals and plates were read on a Gaertner microcomparator and $S_{20,w}$ value calculated by the standard procedure[12]. The per cent composition of the various peaks were calculated from the sedimentation velocity patterns after enlarging them on a Gaertner microcomparator and accurately weighing the tracing paper equivalent in area to the various peaks.

Proteolytic activity: The proteolytic activity of the total soluble proteins in the shrimp was determined by using denatured haemoglobin as substrate[13]. The reaction mixture contained 1 ml of supernatant from muscle homogenate (muscle: buffer 1:6) and 2.5 ml of 2 per cent denatured haemoglobin in 0.1M acetate buffer, pH 4.0. The reaction was carried out at 37°C for 30 min in a constant temperature shaking water bath and was terminated by addition of 3 ml of 10 per cent trichloroacetic acid (TCA). Blanks were prepared by the addition of TCA solution to the denatured haemoglobin substrate followed by immediate addition of the tissue extract. These extracts were filtered and the absorbance monitored at 280 nm with respective blanks in a Shimadzu spectrophotometer and the enzyme activity calculated as described by Wojtowicz and Odense[13].

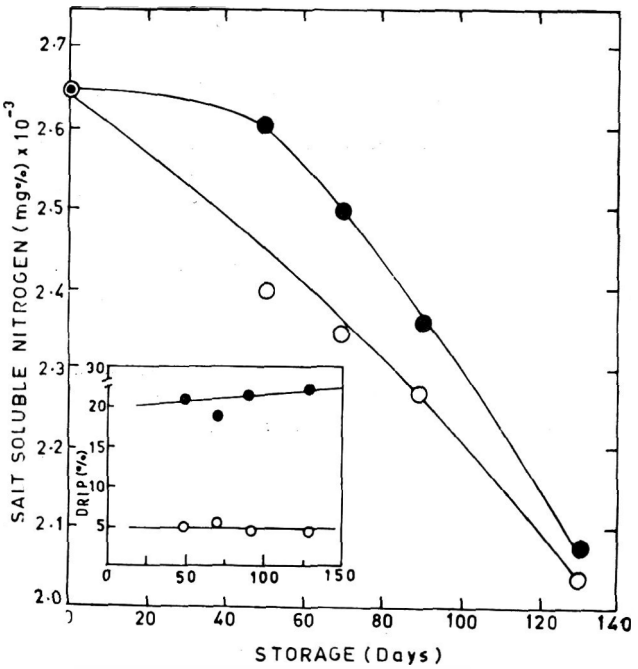

Fig. 1. Salt soluble nitrogen versus number of days of storage of shrimp: treated (O), untreated (●), (Inset) percentage drip loss *versus* number of days storage.

Results and Discussion

Fig. 1 shows the drip loss and the salt soluble nitrogen as a function of storage. The sample treated with sodium citrate has a better water holding capacity than the untreated sample. The residual sodium citrate in the meat was 1 per cent. This has a profound effect on the shelf-life as well as the colour, texture and flavour of shrimp[9]. However, there appears to be a gradual change in the salt soluble nitrogen upto 40 days followed by a sharp decrease in the treated and untreated samples. These results indicate that sodium citrate has a profound effect on the water holding capacity of shrimp proteins and hence the changes with respect to the association-dissociation profiles of the proteins were monitored.

The sedimentation pattern for total protein extracted from fresh shrimp indicates four distinct components of

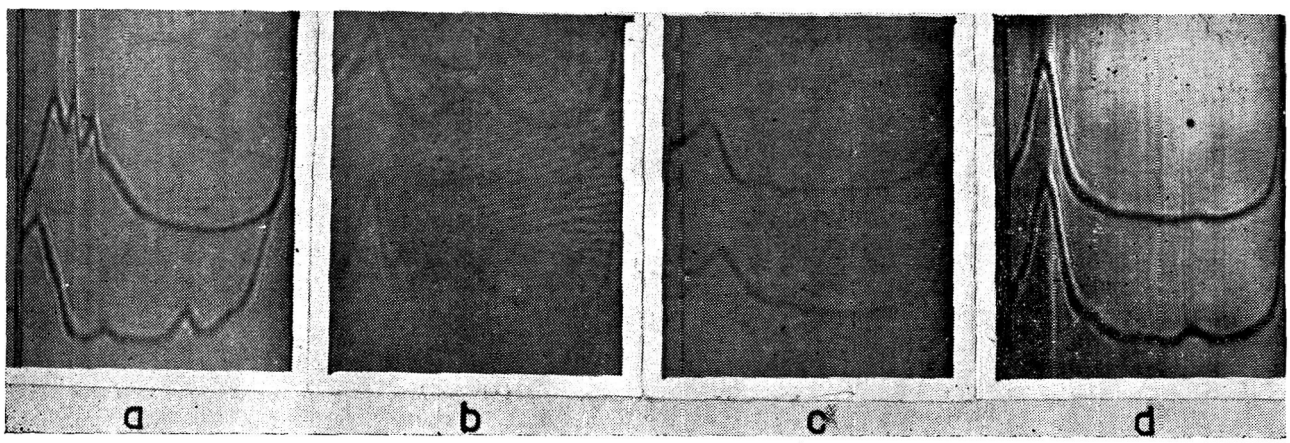

Fig. 2. Sedimentation velocity pattern of (a) *upper*: shrimp proteins 0-day storage, *lower*: shrimp proteins 0-day storage + *in vitro* addition of sodium citrate (b) *upper*: 49 days storage at -20°C and *lower*: treated with 8% sodium citrate and 49 days storage at -20°C (c) *upper*: 128 days storage at -20°C and *lower*: treated with 8% sodium citrate and 128 days storage at -20°C (d) *upper*: drip of 30 days stored at -20°C untreated sample and *lower*: drip of 97 days stored at -20°C and treated with 8% sodium citrate. All the runs were performed at 59,780 rpm and at 27°C.

sedimentation coefficients of 3,5,6 and 7S the percentage of each component being 43,22,23 and 12 respectively (Fig. 2-a upper). As the time of storage increases the proteins dissociate to the 3S component with a concomitant decrease in the percentage of 5,6 and 7S components. Fig. 2-b and c show representative sedimentation profiles of the total protein as a function of time of storage. In order to monitor the changes in the treated and untreated samples, the components are grouped as low molecular weight (LMW) proteins (consisting of only 3S component) and high molecular weight (HMW) proteins (consisting of 5,6 and 7S components).

Fig. 3 and 4 show the changes of LMW and HMW components as a function of storage. Results with the untreated sample indicate that the increase in LMW component follows a sigmoidal pattern with a concomitant decrease in the per cent fraction of the HMW component; nearly 50 per cent of each is present at 50 days of storage (Fig. 3). On the other hand, in the treated sample, the pattern does not change significantly upto nearly 50 days of storage period, after which there appears to be a rapid increase in the LMW components (Fig. 4). The rate of protein dissociation is much less in the initial 50 days of storage in the treated samples than the untreated samples. These results along with the drip loss data (Fig. 1) indicate that sodium citrate has a profound effect on the association-dissociation of shrimp proteins probably mediated through the water holding capacity.

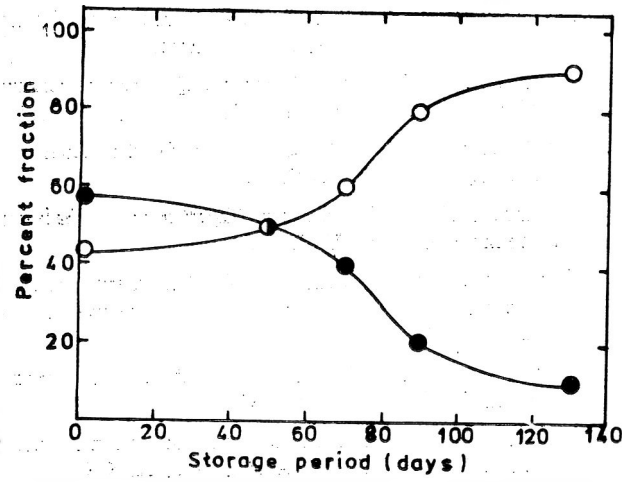

Fig. 3. Per cent fraction *versus* period (days) of storage of untreated sample, frozen stored at -20°C (O—O LMW component, ●—● HMW component).

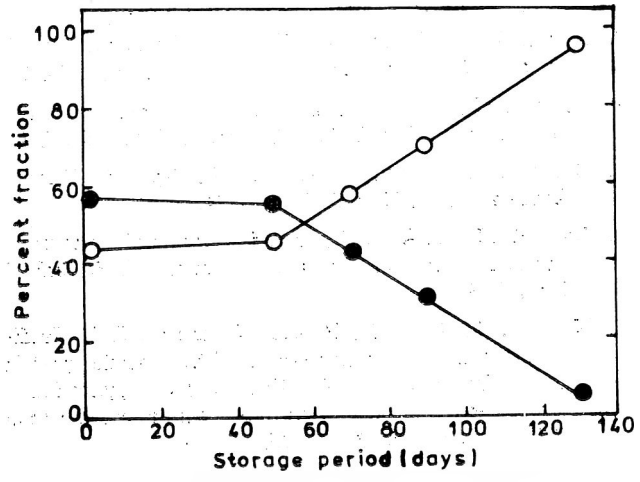

Fig. 4. Per cent fraction versus period of storage (days) of treated sample (5 min dip) with 8% sodium citrate and then frozen stored at -20°C. O—O LMW, component ●—●, HMW, component.

Fig. 2-d shows the sedimentation pattern of the drips collected at 30 and 97 days storage period from which it is clear that both in the treated and untreated samples, the 2S fraction predominates in the drip fraction.

In order to find out whether these association-dissociation profiles are not mediated by proteolytic enzymes, the proteolytic activity of the shrimp extract was monitored as a function of storage. Over a period of 120 days, there is only a 0.75 per cent increase in the proteolytic activity of the system. However, at 150 days, nearly 4.5 per cent of proteolytic activity was observed. These results indicate that probably proteolytic activity is not the only causative agent for the degradation of the protein in the shrimp. However, in some fish proteins, proteolytic enzymes are attributed to be the solely deteriorative cause.

The deterioration of fish proteins during frozen storage is reflected mainly by alterations that occur in the myosin-actomyosin system. Accelerated denaturation of myosin has been reported in fish on the basis of ultracentrifugal analysis and electron photomicrographic studies[14]. However, the properties of cod actin did not change significantly during prolonged storage at -14°C[15]. The results observed here indicate that untreated shrimp proteins dissociate during storage at -20°C. These results are similar to those obtained by King[16] where a rapid dissociation of F-actomyosin into G-actomyosin has been observed before the protein finally precipitates out. Similar results are obtained here with sodium citrate treated samples, but the rate of dissociation is slower. In Fig. 2-a (lower) is demonstrated the *in vitro* effect of sodium citrate on the dissociation of shrimp proteins when added directly after extraction.

These results can be explained by (*a*) partial dehydration during storage and (*b*) effect of inorganic salts. It is well known that the orientation of the water molecules around the protein is different from the rest of the water. During freezing, the surrounding water and the water-mediated hydrophobic/hydrophillic bonds which keep conformation of the protein intact are disrupted[17,18].

Hence, in these protein fractions which are predominantly held by noncovalent interactions, freezing of water has a profound dissociative and/or denaturing effect. This inference is supported by the fact that a fully denatured and hydrated protein in a state of random coil is characterized by a high entropy value. The entropy of the surrounding water, however, is lower because of the ordering of its molecules induced by the hydration of all polar groups. On the other hand, inorganic salts affect proteins in frozen fish most probably by depressing the freezing point of tissue fluids, dehydration, by altering interfacial tension and by ionic interaction with charged groups of the side chains[1]. The characteristic capacity of ions to hydrate and interfere with water structures, makes the various salts at different concentrations exert specific influences on the conformation of proteins. Snow[19] has shown that sodium chloride at low concentration has a protective effect against freezing-induced 'denaturation' of myosin. Other ions like calcium and magnesium can form ionic cross-linkage between polypeptide chains and hence involved in changes in protein conformations. Further, sodium chloride and calcium chloride also influence the hydrolysis of lipids[20] which in turn affects protein solubility. Based on these reports, it may be that sodium citrate can exert indirect action on freezing changes in proteins of shrimp. However, at the concentration used they are not capable of efficiently decreasing the amount of ice formed in the tissues at low temperatures[21].

The sodium citrate treated shrimps were much better in their colour, flavour and texture than untreated samples in terms of glossyness, juiciness, taste and retention of their original shape[9]. These results are in agreement with *in vitro* screening tests, especially of sodium aspartate, malonate, maleate, malate, glycolate and sodium citrate which effectively prolonged the frozen storage life of horse mackerel mince[22]. However, more information is needed on the extent of conformational changes of individual specific proteins upon freezing and storage and the effect of individual inorganic ions on the water structure and the relation of these parameters to the changes in texture and functional properties of shrimp meat, so that rational methods can be developed for better processing of shrimp/fish at low temperatures.

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Study of Pectolytic Factors and Processing in Relation to Rheological Characteristics of Tomato Juice

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The effect of processing conditions on the pectolytic factors and rheological properties of juice of two commercially, grown cultivars of tomatoes, 'Pb. Chhuhara' and 'Pb. Kesri', was studied. The high viscosity of hot break tomato juice was related to its high pectin content and the nature of pectin. Water soluble pectin was the predominant fraction in juice. Gel filtration studies of soluble pectin showed that more of the higher molecular weight fraction was retained in the hot break juice than the control. The methoxyl content of serum pectin in hot break juice was higher than in cold break. Differences in electrophoretic pattern of cold *vs* hot break juice soluble pectin were also observed. Effect of long term storage on the elution profile and electrophoretic mobility of pectin fractions is described.

The pectin components and the associated enzymes in tomato are highly important factors which determine processing and possible innovations to be introduced for obtaining juice of consistent commercial quality. Inactivation of pectic enzymes by the hot break process yielding a high viscosity juice has been reported by several workers[1-4]. Xu *et al.*[5] observed a relationship between microstructure and rheological properties of tomato juice. Differences in molecular weight and changes of pectic substances of two cultivars of tomato have been reported by Stein and Brown[6].

The purpose of this investigation was to study the integrated effects of pectolytic changes in relation to rheological characteristics of cold *vs* hot break tomato juice of two extensively grown cultivars, 'Pb. Chhuhara' and 'Pb. Kesari' as a corollary to the extraction of viable seed of the new cultivars and utilizing the juice as a by-product.

Materials and Methods

Ripe tomatoes of 'Pb. Chhuhara' and 'Pb. Kesri' cultivars obtained from a local farm were graded according to size, ripening, freedom from damage and blemishes.

Processing: To enable recovery of viable seed, the juice was extracted by mechanically pressing 30 to 40 kg lots of tomatoes in a pulper (Raylons Metal Works, Bombay) twice, using 1mm sieve. The extract was brought to near boiling in a steam jacketed kettle for 5 min filled hot in glass bottles (700 ml capacity), crown corked and processed in boiling water for about 55 min. Another portion of the juice was homogenized at 65°C/1000 psi, followed by boiling and bottling as above.

For hot pressing, tomatoes were crushed mechanically, immediately transferred to a steam jacketed kettle and brought to near boiling for 5 min before passing through the pulper twice, boiled and filled hot. A second portion of the juice was homogenized before bottling and processed as above. Bottles of juice were kept under ambient summer and winter conditions.

Composition: Proximate composition of tomato juice and reducing sugars were determined using the AOAC methods[7]. Tannins were estimated by the colorimetric method[8] using Folin-Denis reagent. Lycopene was determined according to Adsule and Dan[9]. Colour was also determined using the Universal Lovibond Tintometer (Associated Instrument, Mfg., India) and expressed in Yellow (Y), Red (R) and Blue (B) Tintometer Units (TU).

Pectinesterase assay: The enzyme extract was prepared according to Al-Delaimy and Ali[10] and the assay carried out according to Lineweaver and Jansen[11] using pure pectin substrate (Loba-Chemie Indoaustranal Co. Bombay) and Hinton's indicator[12]. The activity was expressed as the amount of enzyme which produced one milliequivalent of ester hydrolysed/min/g of sample (PE, μ/g).

Pectic fractions: Four pectic fractions present in tomato juice were determined by adopting sequential extraction in distilled water, 0.2 per cent ammonium

oxalate, 0.05 N HCl and 0.05N NaOH[13]. The pectin content in each fraction expressed on juice weight basis was quantified by using the carbazole method of McComb and McCready[14].

Equivalent weight and methoxyl content of soluble pectin were determined according to methods of Owens *et al.*[15] and Stier *et al.*[16], respectively.

Gel filtration: An accurately measured aliquot (0.3 ml) containing about 800 μg of demethoxylated soluble pectin with pH adjusted to 5.5, was eluted with distilled water using a Sephadex G-200 column (0.9×30 cm) and a flow rate of 5 ml/hr according to Stein and Brown[6]. Pectin in the fractions (1 ml) was quantified by using the carbazole method[14]. Elution profiles were established by plotting the absorbance at 520 nm against elution volume (Ve).

Electrophoretic studies: Electrophoresis was carried out in 7.5 per cent polyacrylamide gels prepared essentially as prescribed by Davis[17] and set in 75 mm long glass tubes with 4 mm inner diameter. The electropherograms, were developed according to Do *et al.*[18] and stained by using periodic-fuschin-sulfite method[19].

Rheological characteristics: Rheotest 2 viscosimeter (VEB MLW Prufgerate-Werk, Medingen, Sitz, Freital) with S/S_1 coaxial cylinder system and an ascending and descending range of shear rates (3-1312s^{-1}), was used to characterize the flow behaviour of tomato juice.

Apparent viscosity (ηa) was calculated by dividing shear stress values (τ, dynes cm^{-2}) by shear rate (Ds^{-1}) and expressed in centipoise (cp). The relationship between log ηa and log Ds^{-1} was developed. Effect of processing on the rheological parameters of Power Law[20], Casson's[21] and Michaels and Bolger[22] models, was evaluated.

Results and Discussion

The yield of the juice was higher for both the varieties of tomatoes when pressed hot, the recovery being 86.6 to 86.8 per cent as compared to 78.6 to 84.3 per cent of juice by the cold extraction method. While evaluating 12 varieties of tomatoes including 'Pb. Kesri', Singh *et al.*[23] reported a juice yield of 46.5 to 56.0 per cent which seemed very low. Much higher yields of tomato juice were obtainable by using appropriate equipment[24-26].

Effect of variety and processing on composition of juice: On hot extraction, the juice had 15 per cent higher total soluble solids (TSS) content. Homogenization of cold extracted juice also increased the TSS by 12 per cent. The juice of 'Pb. Kesri' was more acidic than that of 'Pb. Chhuhara'. The pulp content of cold pressed juice was considerably less than that of hot pressed juice (Table 1). The cold pressed juice of 'Pb. Kesri' tomatoes, however, contained 15 per cent more pulp than that of 'Pb. Chhuhara' juice.

The TSS of a commercial juice included for comparison was considerably more than that of the pure tomato juice of both the varieties and was found to be due to added sugar. The amount of reducing sugars in the commercial juice was nearly twice that of the natural

TABLE 1. EFFECT OF VARIETY AND PROCESSING ON THE COMPOSITION OF TOMATO JUICE

Processing methods	Total soluble solids (°Brix)	Pulp (%)	Acidity (as CA %)	Red. sugars as dextrose (%)	Tannins* (mg/100g)	Lycopene (μg/g)	Colour (Tintometer units)		
							Red	Yellow	Blue
Pb. Chhuhara									
Cold break	4.0	0.54	0.42	2.52	32.0	5.30	21	10	0.3
Cold break-H	4.5	0.58	0.54	3.10	27.2	5.07	23	10	0.4
Hot break	5.0	0.77	0.49	2.91	32.0	5.19	23	10	0.7
Hot break-H	5.0	0.78	0.51	3.20	31.2	5.19	25	10	0.4
Pb. Kesri									
Cold break	4.0	0.62	0.67	2.27	27.2	2.88	19	10	1.0
Cold break-H	4.5	0.62	0.63	2.70	22.4	2.76	20	10	1.0
Hot break	5.0	0.76	0.67	2.47	31.4	2.59	19	10	1.0
Hot break-H	5.0	0.70	0.63	3.06	25.6	2.54	20	10	1.0
Commercial	9.5	0.80	0.67	6.10	41.6	1.30	18	12	—
Least sig. diff. (0.01)	—	—	—	—	—	1.25	12	—	—

H: Homogenized, CA: Citric acid, * As tannic acid.

juice of the tomato varieties. Lycopene content and Red Tintometer units of the commercial juice were distinctly less than those of the juice of 'Pb. Chhuhara' and 'Pb. Kesri' tomatoes; the juice of the former variety was deep bright red in colour and is much sought for culinary use and for pastes and ketchups. Its lycopene content was significantly higher ($P<0.01$) than that of 'Pb. Kesri' juice.

Effect of storage on the composition of tomato juice: The composition of 'Pb. Chhuhara' cold extracted juice prepared in 1978 and preserved in glass bottles (700 ml capacity) was compared with juice similarly processed during 1984–85 (Table 2). The ready-to-use 1984 and 1978 tomato juice samples contained added salt (0.8 per cent), sugar and citric acid to obtain a lower pH of 3.71 to 3.75 as compared to freshly processed juice without any additions. The 1978 samples of juice turned dark with suppressed typical red colour. Extent of browning was more as seen from the appearance of darker serum. It did contain lycopene, though notably reduced over the years as compared to the 1985 juice. Tintometer red colour units of the three juices were nearly the same i.e. 20 to 21. However, the yellow units of 1978 juice increased by about 40 per cent as compared to the 1984 and 1985 samples. Increased blue (TU) value shown by the 1978 juice as compared to 1984 and 1985 samples was indicative of its darker and dull appearance but it had acceptable flavour of tomato juice.

Processing in relation to pectin methylesterase (PME) activity: PME-activity (milli equivalents of ester hydrolysed/min/g) of 'Pb. Chhuhara' residue was 2.65 as compared to 1.64 of its unprocessed juice. A similar picture emerged regarding the PME-activity distribution in the residue and juice of 'Pb. Kesri' tomato. The occurrence of pectic enzymes in highest concentration is near the inner surface of the fruit as reported by McColloch *et al.*[27] and is therefore susceptible to heat inactivation.

Effect of variety and processing on pectic fractions in tomato juice: Water soluble pectin content was higher in the juice of 'Pb. Chhuhara' tomato than that of 'Pb. Kesri' (Table 3). Homogenization of cold extracted juice of 'Pb. Chhuhara' and 'Pb. Kesri' increased the water-soluble pectin contents to the extent of 37 and 32 per cent, respectively. The hot extracted juice of the varieties was richer in pectin content than the cold extracted juices, the increase being 75 per cent for 'Pb. Chhuhara' juice as compared to 120 per cent for 'Pb. Kesri'. The commercial sample contained less water-soluble pectin than the hot extracted juice of both the varieties.

The differences in the other pectic fractions were less conspicuous quantitatively than water soluble pectin fractions. Luh and Daoud[1] observed that the total pectin content of tomato pulp and serum increased as the temperature and holding times were increased. The higher consistency of firm ripe tomatoes was attributed to the higher protopectin content by Luh *et al.*[28]

Methoxyl content and equivalent weight of soluble pectin: The methoxyl content of soluble pectin of 1985

TABLE 2. EFFECT OF STORAGE ON THE COMPOSITION OF 'Pb. CHHUHARA' TOMATO JUICE UNDER AMBIENT CONDITIONS*

Composition	1985	1984	1978
TSS (°Brix)	4.00	7.00	9.00
Acidity (as CA %)	0.42	0.56	0.67
Tannins (mg/100g)	32.00	32.00	16.00
Lycopene (ug/g)	5.30	2.71	1.30
Salt (%)	0.00	0.80	0.79
Pulp, db (%)	0.54	0.54	0.44
Colour, (Tintometer units)			
Red	21.0	20.0	20.0
Yellow	10.0	10.0	14.0
Blue	0.3	0.8	2.0

Range of summer and winter temperatures: 34 to 37°C, 15 to 25°C

TABLE 3. EFFECT OF VARIETY AND PROCESSING ON THE PECTIN FRACTIONS IN TOMATO JUICE

Processing method	Pectin in juice as an hydrogalacturonic acid (%), soluble in			
	Water	Ammonium oxalate	0.05N HCl	0.05N NaOH
	Pb. Chhuhara			
Cold break	0.220	0.017	0.007	0.023
Cold break-H	0.301	0.035	0.007	0.017
Hot break	0.385	0.074	0.038	0.028
Hot break-H	0.325	0.034	0.026	0.016
	Pb. Kesri			
Cold break	0.167	0.053	0.004	0.020
Cold break-H	0.221	0.048	0.001	0.018
Hot break	0.367	0.020	0.019	0.025
Hot break-H	0.376	0.023	0.016	0.028
Commercial	0.311	0.046	0.010	0.012

H : Homogenized; AGA : Anhydrogalacturonic acid.

crop cold extracted juice of 'Pb. Kesri' and 'Pb. Chhuhara' was 6.2 and 10.4 per cent as compared to 11.0 and 10.0 per cent of the respective hot extracted juice. Soluble pectin in the 7-year old cold extracted juice of 'Pb. Chhuhara' tomato showed a high methoxyl content of 14.1 per cent as compared to 10.2 and 10.4 per cent of 1984 and 1985 juices, respectively. The equivalent weight of the pectin of hot extracted 'Pb. Kesri' juice was 728 as compared to 1041 of 'Pb. Chhuhara'. The equivalent weight of the pectins in cold extracted juice of both the varieties was considerably more (ranging 1238 to 1439) than that of the hot extracted juice pectins which was attributed to the presence of higher molecular weight pectin fractions resistant to degradation.

Effect of storage on changes in pectin fractions: Water soluble pectin in the juice decreased considerably during storage. The pectin content of 1 year old juice was 0.174 per cent as compared to 0.220 and 0.101 per cent of the fresh and 7 years old juice, respectively. The ammonium oxalate soluble fraction in the long stored juice was 0.037 per cent which was double that of 1984 and 1985 samples. There was also a decrease from 0.028 to 0.009 per cent in the alkali soluble pectin fraction with storage.

Gel filtration profiles of pectin: There were two major fractions of pectin in the serum of juice prepared by cold and hot extraction methods (Fig. 1). The hot extracted juice of 'Pb. Chhuhara' contained 24.1 per cent of higher molecular weight pectin fraction as compared to 16.6 and 15.5 per cent in the cold extracted juice prepared during 1978 and 1985, respectively. Similarly, hot extracted juice of 'Pb. Kesri' contained 29.2 per cent of higher molecular weight fraction as compared to 16.5 per cent for the cold extracted juice. Lower molecular weight pectin fraction was predominant in the cold extracted juice of both the varieties and this would partly account for higher viscosity of hot extracted tomato juice.

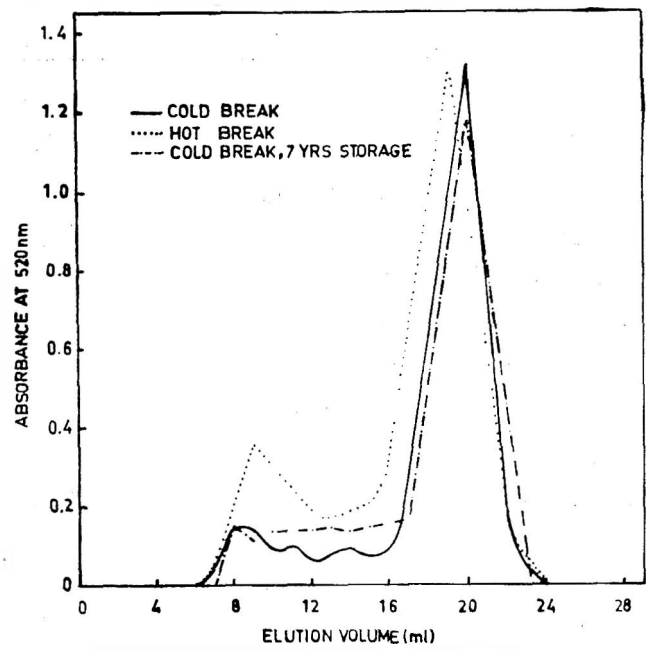

Fig. 1. Effect of cold *vs* hot break on the pectin elution profile of Pb. Chhuhara tomato juice

Electrophoretic pattern of soluble pectin: A major band of pectin common to all juices was located in proximity to the site of loading the samples (Fig. 2). This was also observed by Stein and Brown[6] in their studies on the electrophoresis of water soluble pectin fraction in alcohol insoluble solids (AIS) of tomatoes. Another common major band of pectin which migrated to a distance of 7 to 12 mm from the origin was observed. One or two minor bands were observed in between these two major bands depending on the variety or period of storage of tomato juice.

The cold extracted juice of 'Pb. Chhuhara' (1984 and 1985) showed the presence of three distinct pectin bands. From the pectin of 1978 juice, the minor band was missing. The distance travelled by the second pectin band of the hot extracted juice of 'Pb. Chhuhara' varied from 1.2 to 2.0 mm as compared to 9.5 mm of the 'Pb. Kesri'. There was no third pectin band in the hot extracted juice of 'Pb. Kesri' unlike the ubiquitous presence of this band in the cold extracted juice of both the varieties and the hot extracted juice of 'Pb. Chhuhara'. There were only two major pectin bands in the 1978 juice of 'Pb. Chhuhara' tomato.

Apparent viscosity: The log-log relationship of apparent viscosity *vs* shear rate over the range of 16 to 730s^{-1} was linear. Shear thinning of the tomato juice occurred with increasing shear rate. On homogenization, apparent viscosities (100s^{-1}) of cold break juice of 'Pb. Chhuhara' and 'Pb. Kesri' tomatoes showed an increase from 39.5 to 52.0 cp and 32.0 to 58.0 cp, and of hot break juice from 74.0 to 84.0 cp and 66.0 to 82.0 cp, respectively. Settling of cold break juice was effectively reduced by homogenization.

Rheological characteristics: The values for the flow behaviour index 'n' computed from the slope of log-log plots of shear stress *vs* shear rate according to power law[20] were <1 which was indicative of the pseudoplastic nature[29] of tomato juice (Table 4). Neither the variety of tomato nor the method of juice extraction had any significant effect on values for 'n'. The values for consistency coefficient 'K' were affected notably by the method of juice extraction. However, the effect of tomato variety on this parameter was minimal. Homogenization of cold extracted juice of 'Pb. Chhuhara' and 'Pb. Kesri' tomatoes increased the 'K' values by 15.1 and 46.1 per cent, respectively. There was a notable increase in the 'K' values of hot extracted juice of the

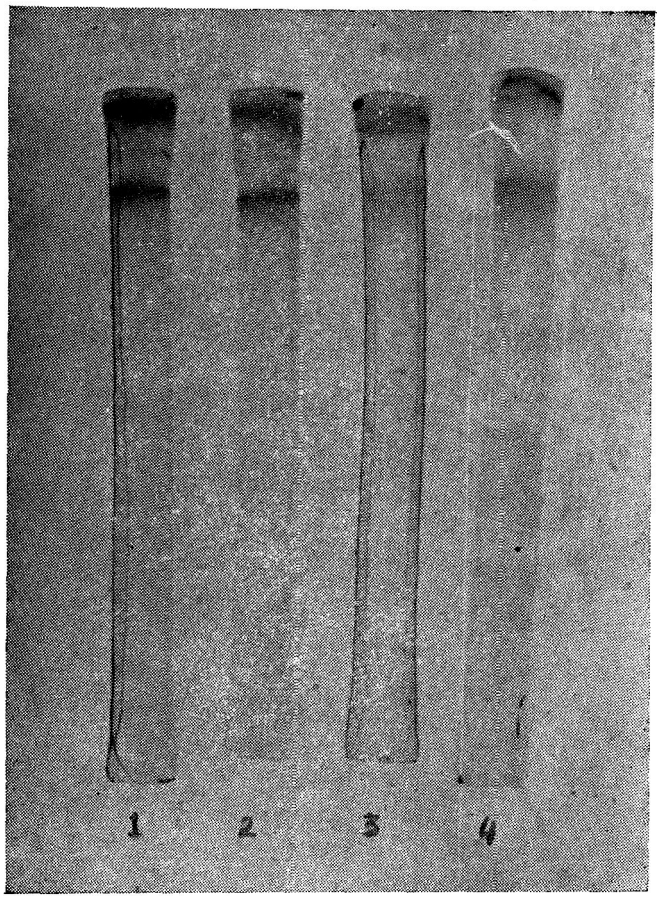

Fig. 2a. Polyacrylamide gel electropherograms of soluble pectin in 'Pb. Chhuhara' 1985 juice

1. Cold break, 2. Hot break, 3. Cold break + Urea, 4. Hot break + Urea.

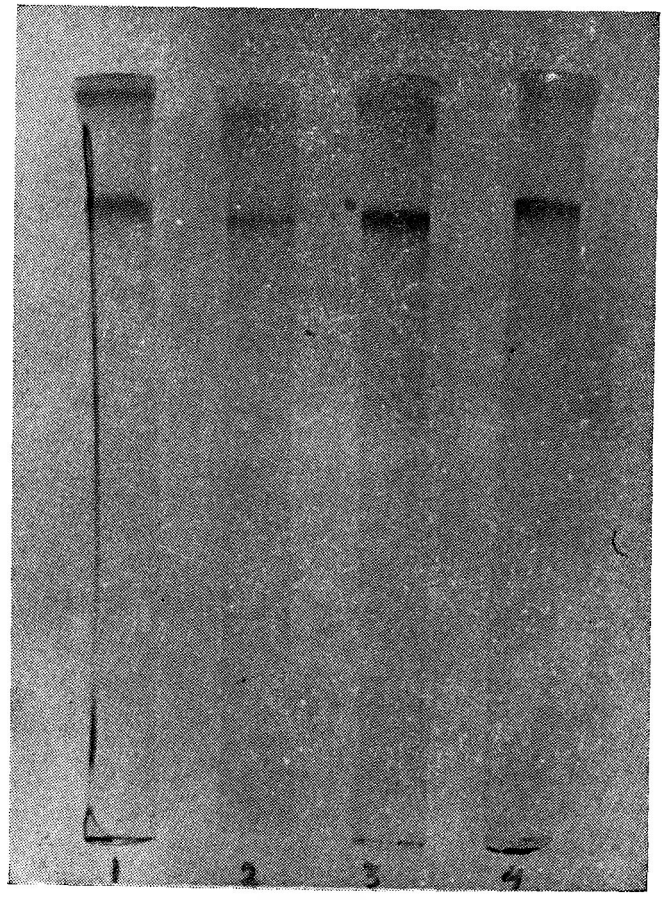

Fig. 2b. Phlyacrylamide gel electropherograms of soluble pectin in 'Pb. Chhuhara' cold break stored juice

1. 1984, 2. 1978, 3. 1984 + Urea, 4. 1978 + Urea.

varieties as compared to that of cold extracted juice. On homogenization, the 'K' values further increased by 31.3 and 18.5 per cent, respectively.

Casson's yield value: The effect of processing on the Casson's[22] yield (τoc) values of tomato juice was perceptible. The values for hot extracted juice of 'Pb. Chhuhara' and 'Pb. Kesri' tomatoes increased by 97.3 and 78.3 per cent, as compared to the respective values of 22.1 and 23.0 dynes cm^{-2} for the cold extracted juices. Homogenization also increased the yield values of juice but the effect of hot break method of juice extraction was more pronounced (Table 4).

Michaels and Bolger model parameters: The values for shear stress (τcr) necessary for breaking of aggregates of particles in the hot break juice of 'Pb. Chhuhara' increased to the extent of 90.9 per cent as compared to cold break juice. A further increase of 30.9 per cent was obtained by homogenization as compared to 19.1 per cent for a similar juice of 'Pb. Kesri'. For viscous flow, the magnitude of shear stress (τv) was nominal as compared to the values for the destruction of net work (τn) and the breaking of aggregates (τcr) in the juice system, respectively (Table 4).

Besides tomato cultivar, the break temperature and mechanical factors of juice extraction considerably affected the viscosity[30] of tomato juice. With prolonged higher break temperatures, reduction in juice consistency has been attributed to pectin degradation[31]. Pectolytic factors and the particulate nature of suspended pulp determine largely the body of tomato juice.

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TABLE 4. EFFECT OF VARIETY AND PROCESSING ON THE RHEOLOGICAL PARAMETERS OF TOMATO JUICE

Processing method	Power law		Casson's model	Michaels and Bolger model		
	n	K	τ_{oc}	τ_n	τ_{cr}	τ_v
			Pb. Chhuhara			
Cold break	0.162	27.8	22.1	—	22.0	0.023
Cold break-H	0.168	32.0	26.0	24.0	24.0	0.029
Hot break	0.175	45.0	43.6	40.0	42.0	0.017
Hot break-H	0.176	59.1	50.4	50.0	55.0	0.029
			Pb. Kesri			
Cold break	0.191	26.0	23.0	—	20.0	0.010
Cold break-H	0.171	38.0	34.8	28.0	36.0	0.017
Hot break	0.205	42.6	41.0	42.0	42.0	0.017
Hot break-H	0.167	50.5	45.0	48.0	50.0	0.053
Commercial juice	0.242	31.5	29.2	—	38.5	0.023

H : Homogenized
n : Flow behaviour index
K : Consistency coefficient, dynes sec^n cm^{-2}
τ_{oc} : Yield value, dynes cm^{-2}
τ_n : Shear stress for destruction of net work, dynes cm^{-2}
τ_{cr} : Shear stress for breaking of aggregates, dynes cm^{-2}
τ_v : Shear stress for viscous flow, dynes sec^n cm^{-2}

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Inhibitory Effect on Lithogenesis by Ingestion of a *Curcuma* Mixture (*Temoe lawak Singer*[R])

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Rabbits were fed high-cholesterol diets without or with 0.1 or 1.0% (w/w) of a *Curcuma* mixture (*Temoe Lawak* Singer[R]), which consisted of an extract of the roots of *Curcuma aromatica rhizoma* and whole roots of *Curcuma amara rhizoma* and *Rhamni purshianae cortex*. After 28 days, the *Curcuma* mixture had not influenced the concentrations of cholesterol in serum and plasma. The *Curcuma* mixture partly counteracted the increase in lithogenic index of bile seen after cholesterol feeding. It is suggested that the *Curcuma* mixture under study could be of value in the prevention of recurrence of cholesterol gallstone formation.

Mixtures and extracts of the roots of *Curcuma* species are widely used as cholerectic and cholagoguic drugs. Experiments have been performed in order to identify the components responsible for the increase in bile flow caused by *Curcuma* preparations. It became clear that both the so-called etherical oil components and the curcuminoids cause an increase in bile flow and changes in bile composition[1–6]. It could be suggested that *Curcuma* preparations affect lithogenicity of bile. The objective of the present study was to find out whether a *Curcuma* mixture consisting of the extracts of roots of *Curcuma aromatica rhizoma*, *Curcuma amara rhizoma*

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and *Rhamni purshianae cortex* would counteract the increase in lithogenicity of the bile fluid of rabbits seen after cholesterol feeding. In the Netherlands, this *Curcuma* preparation is marketed as a cholerectic drug.

Materials and Methods

Random-bred, male rabbits of the New Zealand White strain were obtained from the Broekman Institute, Helmond, The Netherlands. The animals were fed a commercial rabbit diet (Hope Farms, Woerden, The Netherlands). All rabbits were kept individually in cages with mesh bases constructed of galvanized steel in a room with controlled lighting (14 hr/day), constant temperature (16-20°C) and relative humidity (55-65 per cent).

At day O of the experiment, when they were aged about 4 months, the rabbits were divided into 4 groups, consisting of 8 animals each. The groups were formed so that the distributions of serum cholesterol concentration and body weight were similar. The rabbits were transferred to semipurified diets for a period of 4 weeks. The composition of the cholesterol-free, control diet was as follows (per cent, w/w): casein, 21 corn starch, 17; dextrose, 21; molasses, 5; corn oil, 1; coconut fat, 9; sawdust, 18; dicalciumphosphate, 2.9; sodium chloride, 0.6; magnesium carbonate, 0.3; magnesium oxide, 0.2; potassium bicarbonate, 1.8; vitamin premix, 1.2; mineral premix, 1.0. The composition of the vitamin and mineral premixes has been described[7]. The three cholesterol-enriched diets contained 0.1 per cent (w/w) of cholesterol, which was added at the expense of the dextrose component. Two cholesterol-rich diets also contained *Temoe Lawak* Singer[R], RVG 08637 (The Singer Import b.v., Leusden, The Netherlands), either at a level of 0.1 or 1.0 per cent (w/w). *Temoe Lawak* Singer[R], which was added at the expense of the dextrose component, consisted of 75 per cent of extract from the roots of *Curcuma aromatica rhizoma*, 12 per cent of whole roots of *Curcuma amara rhizoma*, 4 per cent of whole roots of *Rhamni purshianae cortex* and 9 per cent of sucrose. The analysed content of curcumin and etherical lipids of this mixture was 0.74 and 2.34 per cent, respectively All diets were in pelleted form. Food was provided each day at 09.00 hr on a restricted basis, the animals receiving 70 g/day. Most rabbits consumed all their food within 4 hr. Tap water was available *ad libitum*.

Body weight of the animals was recorded at the beginning, day 14 and at the end of the experiment. Samples of blood were taken between 08.00 and 10.00 hr after the removal of any remaining food at 16.00 hr the previous day. Blood was taken from a marginal ear vein into tubes without anticoagulant; serum was prepared by low speed centrifugation. Cholesterol in serum was measured enzymatically using the kit (Monotest) supplied by Boehringer-Mannheim GmbH, FRG. At the end of the experiment, the rabbits were killed by cervical dislocation. The livers were rapidly removed, the gall bladder was excised and the bile was collected. Liver wet weight was determined as liver without gall-bladder and bile. Liver cholesterol was extracted and analysed according to Abell *et al*[8]. Biliary lipids were analysed as described earlier[9] and the lithogenic index calculated using the formula of Thomas and Hofmann[10] and data of Hegardt and Dam[11] and Holzbach *et al*[12].

Results and Discussion

Table 1 documents that the experimental diet had no differential effect on body weight of the rabbits. The feeding of cholesterol at a level of 0.1 per cent (w/w) of diet caused a significant increase in serum total cholesterol, the increase being about 3-fold after 28 days. *Temoe Lawak* Singer[R] at either 0.1 or 1.0 per cent of diet did not influence the cholesterol-induced rise in serum cholesterol (Table 1). This contradicts the results obtained in a study with rats[13]. In this study, 1.0 per cent of *Temoe Lawak* Singer[R] in the diet completely counteracted the increase in serum cholesterol seen after cholesterol loading. Subba Rao *et al.*[14] reported that curcumin, the active principle of turmeric, also nullified the dietary-cholesterol-induced increase in serum cholesterol concentrations in rats. It should be noted that in this study[14] using rats the concentration of curcumin in the diet was about 100-fold higher than that in the present study with rabbits. Nevertheless, there might be a difference between rats and rabbits with respect to their response of serum cholesterol to curcumin or a *Curcuma* mixture.

Cholesterol feeding did not affect liver wet weight. The *Curcuma* mixture tended to cause an increase in liver weight, an effect that reached statistical significance at the lowest dose (0.1 per cent). As would be anticipated[9] the consumption of cholesterol produced a significant increase in liver cholesterol (Table 1). The *Curcuma* mixture did not influence the cholesterol-induced increase in liver cholesterol concentration. Again, there appears to be a discrepancy between rabbits and rats in their response of liver cholesterol concentration to curcumin. Both Subba Rao[14] and ourselves[13] have reported that curcumin and a *Curcuma* mixture, respectively, partially counteracted the increment in liver cholesterol in rats seen after cholesterol loading.

Dietary cholesterol caused a significant increase in biliary cholesterol concentration, whereas the concentrations of phospholipids and bile acids in the bile were not affected (Table 1), in conformity with our earlier work[9].

The addition of the *Curcuma* mixture to the cholesterol-enriched semi-purified diet did not significantly

TABLE 1. PLASMA LIPIDS, LIVER CHOLESTEROL AND BILIARY LIPIDS IN RABBITS FED HIGH-CHOLESTROL DIETS WITH OR WITHOUT A CURCUMA MIXTURE

	Dietary cholesterol/Dietary *Curcuma* mixture (%/%)			
	0/0	0.1/0	0.1/0.1	0.1/1.0
Body weight (kg)				
Day 0	2.66±0.19	2.67±0.10	2.66±0.16	2.65±0.16
Day 28	2.82±0.10	2.83±0.10	2.88±0.20	2.84±0.10
Serum cholesterol (mmol/l)				
Day 7	1.21±0.22	1.23±0.22	1.18±0.20	1.19±0.23
Day 14	1.90±1.09	3.01±0.83[a]	3.17±1.06	3.65±1.66
Day 28	2.03±1.22	6.40±4.08[a]	6.45±2.71	6.99±3.33
Liver wet wt (g)	98 ±12	91 ± 12	107±8[b]	103±18
Liver cholesterol (μmol/g)	6.4±0.7	11.6±2.2[a]	10.9±2.8	11.8±2 6
Biliary lipids (mmol/l)				
cholesterol	4.5±0.4	5.9±0.9[a]	5.1±0.7	5.5±0.9
phospholipids	12.0±4.2	11.0±3.8	12.7±4.3	15.8±6.3
bile acids	175± 9	186±11	181±19	168±32
Lithogenic index of bile	0.67±0.10	0.86±0.13[a]	0.74±0.07[b]	0.79±0.20

Results are means ± SD for 8 animals per group. Values for liver wt, liver cholesterol and biliary lipids refer to the end (Day 28) of the experiment. [a]$p<0.05$, *versus* cholesterol-free diet; [b]$p<0.05$, *versus* high-cholesterol diet without *Curcuma* mixture. All rabbits were fed a commercial diet until day 0 of the experiment, and were then transferred to the semi-purified, experimental diets.

alter the biliary concentrations of lipids. However, the *Curcuma* mixture tended to reduce the concentration of biliary cholesterol. Such an effect was also observed when bile was collected from rats which had been fed a diet containing curcumin for 4 weeks[6]. In the same experimental design, however, curcumin caused a pronounced increase in the concentration of bile acids in bile[6].

Cholesterol feeding markedly elevated the lithogenic index of bile. An increase in the lithogenic index reflects an enhanced risk for the precipitation of cholesterol stones in the bile. *Temoe Lawak* Singer[R] partly counteracted the increase in lithogenic index, the effect being statistically significant at the lowest dose (0.1 per cent). At the level of 1.0 per cent of diet, the *Curcuma* mixture also caused a decrease in the lithogenic index, but this effect did not reach statistical significance because of the relatively large inter-individual variation, as indicated by the high standard deviation (Table 1). In the lowest dose group, the rabbits ingested only about 25 mg of *Temoe Lawak* Singer[R]/kg body weight/day. Assuming that rabbits and humans are equally responsive, this would be equivalent to about 1.75 g/day for humans. In practice, the use of such a dose is feasible.

In conclusion, it is suggested that *Temoe Lawak* Singer[R], which is widely used because of its cholerectic action, may be of value in the prevention of recurrence of gallstone formation.

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A METHOD TO DETERMINE SHRINKAGE OF MUSHROOMS DURING PROCESSING

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A modified liquid displacement method was developed to measure volume of porous biological materials of low density and that absorb water. The method consisted of immersing the sample in a glass container with an overflow spout. Water treated with a surfactant was used as the displacement liquid in the glass container. This technique was found to be reliable for determining shrinkage of mushrooms during processing.

Shrinkage during processing is a major problem in the mushroom processing industry[1]. In order to maintain the desired drained weight in the final product, the common industry practice is to induce major portion of shrinkage during the blanching operation and thus minimize the shrinkage during thermal processing of canned mushrooms.

In order to improve existing processes and to control shrinkage, it is desirable to be able to monitor the change in volume along with change in weights in mushrooms during different processing steps. The research reported in the literature dealt with loss of weight during processing[2-4], but not with the volume change during processing.

Methods presently available to measure volumes of biological materials use either air comparison pycnometer or specific gravity bottle[5]. For porous materials such as fresh mushrooms, the air comparison pycnometer method requires coating of the sample with wax by immersing in a molten wax bath. Since mushrooms shrink when subjected to high temperatures, this method cannot be used. Use of the specific gravity bottle is also not feasible since the mushrooms absorb water and moreover, the diameter of the specific gravity bottle restricts the size of the mushroom that can be used in the test.

The objective of this study was to develop a liquid displacement method to measure volume of porous biological materials, such as mushrooms that are of low density.

A glass container with an overflow spout and a funnel with a long stem was fabricated (Fig. 1). The shape and size of the funnel was chosen such that the mushroom sample can be enclosed within the funnel in order to immerse the fresh mushroom tissue completely in the glass container; the fresh mushroom sample will otherwise float on top of the water. The angle between the wall of the glass container and the protruding tube was kept as small as possible for free flow of the displaced liquid. Water was used as the displacement liquid. Tween-80 (Fisher Scientific, Pittsburgh, PA) was added to this water to reduce its surface tension and to promote its free flow through the overflow spout.

The glass container was initially filled with water and the excess water was allowed to flow through the overflow spout. Then, the funnel was immersed in it and the water displaced by the funnel was collected in the beaker

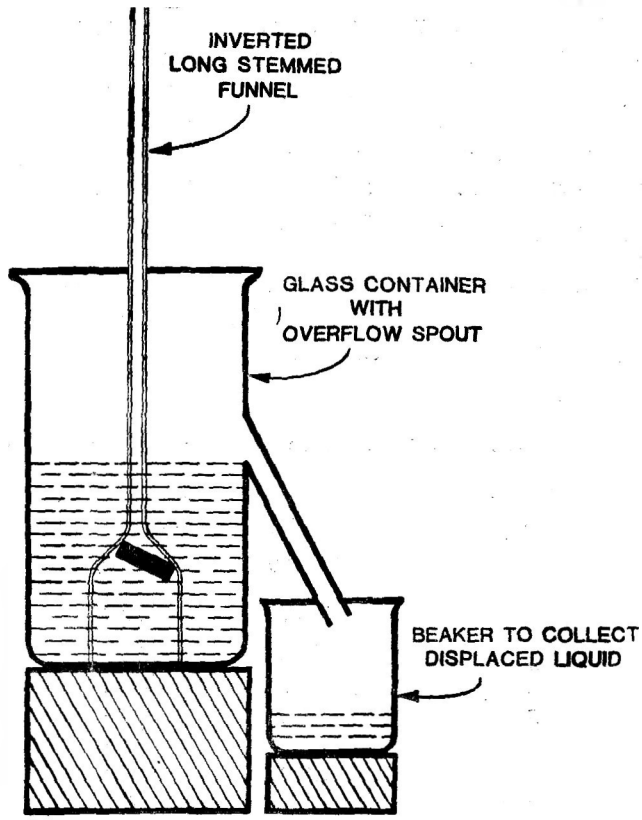

Fig. 1. Experimental set up.

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and weighed. This was converted into the volume of the material of the funnel.

Disc shaped samples that were cut from mushroom cap were used in the experimental studies. The weight, diameter and thickness of the samples were measured and were kept in a humidified environment to prevent moisture loss. The volume of the sample was calculated using the mensuration formula for a cylinder. The sample was then coated with a thin layer of water proofing compound (consisting of a mixture of wax and tetrolatum) to prevent absorption of water. The glass container was filled with water and once the overflow of water stopped, the mushroom sample was gently placed in the glass container. The sample was completely immersed by means of the funnel. The water that was displaced by the sample and the funnel was collected and weighed. This was converted into the volume displaced by the sample and the material of the funnel. By subtracting the volume displaced by the funnel alone, as measured initially, the volume of the sample as measured by the displacement technique was obtained.

Samples were then blanched in boiling water for 5 min (as done during commercial processing). The waterproofing compound had a melting point well below the blanching temperature and hence did not interfere with the shrinkage of mushrooms. The blanched samples were kept in the humidified environment after the diameter and thickness were measured. Volumes of blanched samples were measured by displacement technique with two modifications: (1) the blanched sample was not coated since it did not absorb any liquid, and (2) no funnel was required since the blanched sample did not float. Volumes of the blanched samples were also calculated by mensuration formula. This process was repeated with ten different samples.

The shrinkage ratio was calculated using the equation:

$$\text{Shrinkage ratio} = \frac{\left(\begin{matrix}\text{Initial vol of}\\ \text{sample}\end{matrix}\right) - \left(\begin{matrix}\text{Vol of sample after}\\ \text{blanching}\end{matrix}\right)}{\text{Initial vol of sample}}$$

The volumes of 10 samples and the shrinkage ratios evaluated by the displacement technique and calculated by the mensuration formula are presented in Table 1. There was no significant difference ($\alpha = 0.05$) between the volumes and shrinkage ratios as determined by the two different methods. Errors arising from the volume of waterproofing compound were found to be less than 1.5 per cent. For blanched samples, the differences between the calculated and measured volumes were generally greater than for fresh tissue. This may be due to errors in the calculated values because of distortion of the shape of the sample due to blanching.

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TABLE 1. CALCULATED AND MEASURED VOLUMES OF DISC SHAPED MUSHROOM TISSUES, BEFORE AND AFTER BLANCHING

Sample No.	Before blanching		After blanching		Shrinkage ratio	
	Calculated vol. (cc)	Measured vol. (cc)	Calculated vol. (cc)	Measured vol. (cc)	Using calculated vol.	Using measured vol.
1	2.252	2.205	0.882	0.852	0.608	0.614
2	2.214	2.183	0.868	0.859	0.608	0.607
3	2.282	2.286	0.701	0.691	0.692	0.698
4	2.218	2.219	0.772	0.781	0.652	0.648
5	1.865	1.906	0.763	0.758	0.591	0.602
6	2.442	2.479	0.909	0.891	0.628	0.641
7	2.178	2.177	0.893	0.907	0.590	0.583
8	2.334	2.364	0.887	0.793	0.620	0.665
9	2.009	1.963	0.781	0.822	0.611	0.581
10	2.493	2.500	1.139	1.069	0.543	0.572
Mean	2.229	2.228	0.859	0.842	0.614	0.621
S. D.	± 0.187	± 0.194	± 0.121	± 0.104	± 0.040	± 0.047

PREPARATION OF FULL FAT SOY FLOUR AND ITS USE IN FORTIFICATION OF WHEAT FLOUR

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Whole soybeans were sand roasted, boiled for 5 min and steamed for 5, 10, 15 and 20 min at 15 psi. The treated beans were sun-dried to 7–8% moisture content. The minimum trypsin inhibitor (T.I.) activity was observed in steam samples. Nitrogen solubility indices indicated that the steaming of beans beyond 5 min at 15 psi was not desirable. Therefore, full fat soy flour was prepared from beans steamed at 15 psi for 5 min. Different blends of full fat soy flour and wheat flour were prepared and chapaties were made using traditional method. T.I. activity in chapaties was found to be 0.73 to 2.18% indicating almost complete elimination of trypsin inhibitor in the processes of flour preparation and chapati making. The results of sensory evaluation indicated that chapaties containing 25% full fat soy flour were found neither acceptable nor disliked, but those with 10% full fat soy flour were quite acceptable.

Soybean with 40 per cent good quality protein and 20 per cent oil holds great promise for combating the problem of malnutrition in developing countries. The lower cost of soy protein when compared with milk, meat and fish is the most favourable point in utilizing soybean in human food preparations. Though foods from whole soybean (including seed coat) have been developed[1,2], soy flour, which is comparatively easy and inexpensive to process is one of the most promising forms in which it can be used in the Indian diet, provided the problem concerning flavour, anti-nutritional factors and stability of the product are minimised if not completely eliminated. The existing solvent extraction plants in the country are not in a position to produce food grade soy flour due to inadequate preparatory equipment[3]. Preparation of full fat soy flour by extrusion cooking has been discussed earlier[4].

Chapaties from wheat constitute the major portion of Indian diet. Wheat flour used for chapati making, if fortified with soy flour would be a good source to counteract protein malnutrition. In view of this, the present investigation was undertaken to prepare full fat soy flour free from anti-nutritional factors and use it with wheat flour to prepare chapati.

Soybeans (var. 'Bragg') and wheat (var. 'RR 21') obtained from Crop Research Centre, G. B. Pant University of Agriculture & Technology, Pantnagar, Nainital, U.P. were cleaned and graded manually. Four different lots of graded bean were autoclaved separately at 15 psi for 5, 10, 15 and 20 min and then sun-dried. The sun-dried lots of beans were analysed for proximate composition and nitrogen solubility index (NSI) by the AACC[5] and AOCS[6] procedures, respectively. Trypsin inhibitor activity was determined in the bean after boiling in water for 5 min, autoclaving at 15 psi for 5 min and after sand roasting by the method of Kakade *et al.*[7] The flours of autoclaved and sun-dried beans and wheat were prepared by milling in a hammer mill. Chapaties were prepared with different ratios of soy flour and wheat flour by the traditional method using fire wood. Chapaties thus prepared were analysed for trypsin inhibitor activity and subjected to sensory evaluation on a nine-point Hedonic scale using a trained panel of five judges. All the determinations were done in triplicate and the average values have been reported. The data were analysed by two way ANOVA[8].

The percentage composition of soybean was: moisture, 7.5, protein, 42.2, fat, 21.5, ash, 5.85, and carbohydrate 22.9. The NSI of raw soybean was about 79 per cent which showed a decreasing trend due to steaming (Table 1). The minimum value of 8.1 per cent NSI was observed in beans steamed at 15 psi for 15 min. Mustakas[9] observed NSI value of 16 per cent in beans steamed for 30 min. at atmospheric pressure. Since the NSI value of less than 23 per cent is not desirable, the steaming of beans at 15 psi for more than 5 min should be avoided.

It is evident from Table 2 that steaming of beans for 5 min at 15 psi showed about 45 per cent inactivation of trypsin inhibitor activity and this was the maximum

TABLE 1. EFFECT OF STEAMING SOYBEANS AT 15 PSI ON NITROGEN SOLUBILITY INDEX (NSI)

Steaming period (min)	Water soluble N (%)	Total N (%)	NSI
0	5.05	6.39	78.9
5	1.68	7.23	23.2
10	1.40	7.23	19.3
15	0.56	6.91	8.1
20	0.56	6.49	8.6

TABLE 2. EFFECT OF HEAT TREATMENT OF SOYBEANS ON ITS TRYPSIN INHIBITOR ACTIVITY (TIA)

Treatment	Trypsin units		% Inhibited	% TIA Inactivation
	Present	Inhibited		
Control (no heat treatment)	10.50	23.75	69.3	—
Boiling 5 min in plain water	16.30	17.45	52.4	26.1
Steaming 5 min at 15 psi	19.65	14.60	42.6	38.5
Sand roasting on an electric heater	18.40	15.85	45.7	33.2

inactivation that could be achieved. These results are in conformity with the findings of Wu *et al.*[10] and Dumka[11] who reported that trypsin inhibitor activity could not be completely eliminated even by autoclaving at 121°C for 15 min.

Beans steamed for 5 min at 15 psi with minimum trypsin inhibitor activity and maximum NSI value were, therefore, selected for full fat soy flour preparation. As indicated in Table 3, trypsin inhibition ranged from 0.73 to 2.18 per cent in chapaties prepared from blends of wheat and full fat soy flour. The results of the present investigation thus indicated almost complete inactivation (99.27 per cent) of trypsin inhibitor activity in chapaties made from blend of 10 per cent soy and 90 per cent wheat flour. Results of sensory evaluation of chapaties prepared from different blends presented in Table 3 indicate that chapaties made from 25:75 and 20:80 soy-wheat flour blends scored lowest. Statistical analysis of overall sensory score showed that the differences in the overall sensory score of the chapaties prepared from mixtures containing 15, 20 and 25 per cent soy flour were significant when compared with control (100 per cent wheat flour) whereas those prepared from blends containing 5 and 10 per cent soy flour were non-significant.

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TABLE 3. TRYPSIN INHIBITOR ACTIVITY AND SENSORY QUALITY OF CHAPATIES PREPARED FROM THE BLENDS OF SOY FLOUR AND WHEAT FLOUR

Soyflour (%)	Wheat flour (%)	% Inhibition of trypsin units	Tenderness	Chewiness	Colour	Flavour	Overall acceptability
25	75	2.04	5.75	4.50	5.00	4.75	5.00
20	80	2.18	5.50	5.00	5.00	4.50	5.00
15	85	1.46	6.00	6.00	6.25	4.75	5.70
10	90	0.73	6.25	6.50	6.25	5.50	6.10
5	95	1.90	6.00	6.50	6.00	6.00	6.10
0	100	—	7.00	7.25	7.00	7.00	7.60

Chapaties were evaluated by a trained panel of five members.
Critical difference, 1.506.
Hedonic scale: Dislike extremely 1, Dislike very much 2, Dislike moderately 3, Dislike slightly 4, Neither like nor dislike 5, Like slightly 6, Like moderately 7, Like very much 8, Like extremely 9.

SURFACE MICROFLORA OF SEED POTATOES (*SOLANUM TUBEROSUM* L., CV. KUFRI JYOTI): ISOLATION AND IDENTIFICATION OF ORGANISMS RESPONSIBLE FOR SPOILAGE OF POTATOES GROWN AT DEVANAHALLI

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Seed potatoes suffer from microbial spoilage to the extent of 8 to 15% during the post-cold storage transport, handling and holding at tropical ambient conditions. Bacterial soft rot and fungal dry rot were noticed as the principal types of spoilage in Kufri Jyoti variety grown largely in Karnataka. Surface mycoflora consisted of genera like *Fusarium*, *Penicillium*, *Aspergillus* and *Rhizopus*. Gram positive rods and Gram negative coccobacilli constituted the bacterial surface flora. It has been established that in 'Kufri Jyoti', the dry rot is caused by *Fusarium oxysporum* while the soft rot is by *Erwinia carotovora* var *carotovora* (Jones) Dye. Isolated organisms produced characteristic disease symptoms when inoculated to healthy tubers under laboratory conditions.

The popular potato cultivar grown in Karnataka is 'Kufri Jyoti'. The seed tubers are generally produced in the northern states of Himachal Pradesh, Bihar and Punjab, put into cold storages for about 2-3 months, thereafter removed and transported at ambient conditions to southern states for use as seed for the ware crop. This process involves a time lag of 2-4 weeks from the time of removal from cold storage to their planting in the southern states. During this post-harvest handling and transport of seed potatoes, the tubers are prone to microbial spoilage. In terms of the total production of 85,000 tonnes of potato in Karnataka, the spoilage inflicts considerable economic loss both to the farmer and to the nation[1]. Dry rot and soft rot are the principal forms of microbial spoilage. Although *Fusarium* and *Pseudomonas* or *Erwinia* are implicated as causative agents of dry rot and soft rot respectively, so for no report on the isolation and characterisation of the spoilage microorganisms has appeared from 'Kufri Jyoti' cultivar grown at Devanahalli—a principal potato growing region in Karnataka.

Although storage of potatoes at ambient conditions (21-35°C, RH 40-75 per cent) appears to be an economical alternative method to refrigerated storage, it is beset with serious problems like microbial spoilage. If the causative organisms are known, microbial spoilage of potatoes, especially seed tubers, could be avoided adopting the seed plot technique[2-6], and/or by appropriate post-harvest control measures. However, no systematic studies on the microbial spoilage of potatoes of cultivar 'Kufri Jyoti' were carried out and hence attempted in the present study. Fungi and bacteria were isolated from the surface of seed potatoes not only to identify the spoilage organisms relevant to the variety and to the agrometeorological conditions of the region, but also to use them as test materials for evolving control measures.

Tubers weighing 50 g were thoroughly shaken for 10 min in 450 ml sterile distilled water in a sterilised screw type bottle and plated on various growth media at appropriate dilutions. For the estimation of total bacterial and fungal counts, nutrient agar and potato dextrose agar were used respectively[7]. Gram negative bacteria were enumerated on McConkey agar of pH 7.1[7]. Stewart's medium was used to isolate the total pectolytic bacteria and also to distinguish *Pseudomonas* from *Erwinia*[8]. For the estimation of yeasts and moulds, malt extract agar was used[7]. All media were autoclaved at 121°C for 20 min.

The colonies developed were examined and scored for bacteria and yeasts after 24 hr and for moulds after 72 hr. Five replicates were maintained. Study was carried out selecting tubers carrying visible infections and those which appeared to be free from infection (designated as healthy). The fungal isolates were identified based on the colony morphology, pigmentation and spore characteristics[9].

The bacterial isolates were identified[10] on the basis of both morphological features and biochemical properties detailed below. In addition, the arrangement of flagella was examined by scanning electron microscopy.

The oxidase and catalase tests were carried out following the method of Kovacs[11]. Production of gas from carbohydrates, gelatin liquefaction, nitrate reduction, indole production, litmus milk reaction, production of H_2S, hydrolysis of starch, citrate and ethanol utilisation, methyl red and Voges-Proskauer tests were carried out according to standard procedures[12]. *Pseudomonas* culture was used as a positive control during biochemical studies.

Confirmatory tests by inducing the soft rot production in healthy tuber slices were done by inoculating about 10^8 cells in the form of thick paste on to the surface of healthy tuber slices. The inoculated samples were incubated for 24-48 hr at 37±2°C, in a petri dish having 5 ml of sterile water, kept in such a way that the tuber

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TABLE 1. SURFACE MICROFLORA* OF DISEASED AND HEALTHY POTATO TUBERS

Sample	Total counts				Pectolytic bacteria counts ($\times 10^2$)
	Fungi ($\times 10^2$)	Fusarium ($\times 10^2$)	Bacteria ($\times 10^3$)	Gram–ve ($\times 10^2$)	
Diseased	15.8	12.5	39.0	30.0	30.0
Healthy[+]	15.2	3.3	4.6	0.5	0.5

[+] The tubers without apparent infection are designated as healthy.

* Each value is the mean of five independent estimations.

TABLE 2. IDENTIFICATION OF GRAM NEGATIVE COCCOBACILLI ISOLATES BASED ON VARIOUS MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS

	Pseudomonas	*Erwinia*
Generic level		
Flagella arrangement	Polar	Peritrichous produce, gas and acid under aerobic and anaerobic conditions
Acid/gas production from sugar	Produce acid; but no gas under aerobic conditions only	
Oxidase test	+	—

	1. *Amylovora*	2. *Herdicola*	3. *Carotovora*
Specific level			
Gelatin liquefaction	—	—	+
Soft rot in slices	—	—	+
Nitrate reduction	—	±	+
Litmus milk reaction	Alkaline	acidic	acidic
Acid from lactose	—	+	+
Yellow, water insoluble pigment	—	+	—
Growth factor requirement	+	—	—

	Carotovora	Atroseptica	Rhampontia	Chryosynthemi	Cypripedii
Varietal level					
Growth at 37°C	+	—	+	+	+
Sucrose reduction	—	+	±	±	—
Indole reaction	—	—	—	+	—
Pink, water soluble pigment	—	—	+	—	—
Pectate liquefaction	+	+	—	+	—
Confirmatory tests					
V..P. test	+	—	§	§	§
Ethanol utilisation	+	—	§	§	§
Acid from maltose	—	+	§	§	§
Acid from ∝-methyl glucoside	—	+	§	§	§

Reaction is : + positive; — negative; ± variable; § not tested.

The isolates were identified as belonging to *Erwinia carotovora*, var. *carotovora* (Jones) Dye and were confirmed by Koch[12] in healthy tubers with the isolates.

slices did not come in direct contact with the water. Softening of the slices, accompanied by production of characteristic odour was taken as positive reaction. In addition, sucrose inversion, growth factor requirement and pigment production tests were carried out as indicated by Dye[13,14] and Perombelon[15], From the experimentally induced soft rot, bacteria were isolated and compared with the original isolate of *Erwinia carotovora*.

The results indicate that surface microbial load on the apparently healthy tuber was markedly less than the diseased tubers (Table 1). The isolates from both the samples showed the presence of fungal genera, *Fusarium*, *Penicillium*, *Aspergillus* and *Rhizopus*. Even though the total fungal count did not deviate considerably in healthy and diseased tubers, *Fusarium oxysporum* dominated the latter and accounted for 80 per cent of the total mycoflora, indicating *Fusarium oxysporum* as the causative agent for dry rot of 'Kufri Jyoti' potatoes. This fungus has the following properties: parasitic on potatoes; mycelium extensive and cottony in culture, often with some tinge of pink in the mycelium or medium; conidial layer cushion-shaped, conidiophores grouped into sporodochia; macroconidia typically canoe shaped, 3-7.5μ in diameter, usually with five septa; microconidia with 1-3 cells; yeasts were absent.

The bacterial isolates included Gram positive rods and Gram negative coccobacilli. Since Gram positive bacteria are not considered to be primary pathogens[16], further identification was restricted to only Gram negative coccobacilli. Biochemical properties studied are shown in Table 2 and the isolate had the following distinctive characters.

Growth on beef extract agar slants greyish white, Gram negative, short rods measuring 0.7 to 2μ, motile with peritrichous flagella; produce acid and gas from glucose, sucrose and lactose; use ethanol as carbon source; liquefy pectate and gelatin; coagulate litmus milk in 4 days; do not produce indole and hydrogen sulphide; methyl red test positive and produce acetylmethyl carbinol; nitrite is formed from nitrates, grow at 37°C and produce rapidly soft rot of potato. Based on these properties, the isolate is identified as *Erwinia*. Further tests were also carried out to characterise the strain at species and varietal level. Results in Table 2 indicate that the isolated bacteria is *Erwinia carotovora* var *carotovora* (Jones) Dye.

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VARIATION IN QUALITY TRAITS OF LITCHI (*LITCHI CHINENSIS* SONN.) CULTIVARS*

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Physico-chemical composition in four promising cultivars of freshly harvested litchi fruits was investigated. The ranges of values were: fruit weight 6.8–21.2 g, edible protein 61.7–84.4%, seed 3.3–26.1%, peel 12.2–19.5%, total soluble solids (TSS) 21.0–21.5%, total acidity 0.51–0.54%, moisture 80.0–82.0%, protein 1.12–1.20%, fat 0.21–0.35%, carbohydrates 16.06–17.9%, total minerals 0.52–0.55%, reducing sugars 5.60–6.44%, total sugars 15.51–16.22%, protopectin 0.11–0.14%, pectinic acid 0.41–0.57%, total pectins 0.55–0.71%, ascorbic acid 35.76–44.36 mg/100 g, tryptophan 1.17–1.34 g/16 gN, methionine 0.18–0.25 g/16 gN, anthocyanin (in skin) 0.30–0.38 O.D./10 discs and total phenols 116–151 mg/100 g fresh tissue. 'Muzaffarpur' was found to be superior to other cultivars.

Litchi, called 'summer sweet' is a single seeded nut and varies in size and shape according to variety[1]. The edible portion of litchi called aril, is a good source of minerals particularly calcium and phosphorus and has got medicinal importance. The fruit is a good appetizer and useful in dyspepsia and small pox[1]. Information regarding the quality constituents of the fruit is of interest and of great importance to breeders, agronomists, nutritionists and others. The quality composition of litchi cultivars was determined and the information is recorded here.

Fully ripe fruits of 'Early Large Red', 'Calcuttia', 'Muzaffarpur' and 'Bedana' were procured from the trees growing in the Government Garden, Faizabad during the last week of May, 1986. After harvesting, the samples were kept in polythene bags and brought immediately to the laboratory in ice-box. Four replicate samples were selected from each cultivar and all the analyses were carried out in duplicate.

After weighing the fruits, pulp and seeds were separated and weighed after removing the peel. Moisture content was calculated by subtracting dry weight from fresh weight and expressed as per cent of fresh weight. Total soluble solids (TSS) of fresh extracted juice was determined with the help of a hand refractometer. The methods as described in A.O.A.C.[3] were used to analyse acidity, ascorbic acid and fat contents. Total carbohydrates were determined by difference[4]. Reducing, non-reducing and total sugars were estimated by Shaffer-Somogyi micromethod[3]. Determination of protein content was done by the method of Lowry *et al.* using Folin-Ciocalteau reagent[5]. Tryptophan and methionine contents were analysed according to Spies and Chambers[6] and Horn *et al.*[7] Pectin was estimated according to Ranganna[8] and total mineral content according to Hart and Fisher[9]. The method of Swain and Hillis[10] was followed for the quantitation of total phenols using gallic acid as standard. Anthocyanin was determined by the colorimetric method as described by Kliewer[11] and expressed as OD/10 discs.

Fruit weight, edible portion, seed percentage and peel percentage exhibited remarkable variation among the cultivars (Table 1). 'Early Large Red' showed significantly highest fruit weight, edible portion and lowest seed percentage while 'Bedana' gave highest value for peel percentage.

As shown in Table 2, there were considerable differences in the level of all the constituents except TSS, moisture and protopectin content. Total acidity was significantly high in 'Calcuttia'. The highest and lowest values for carbohydrates were observed in cultivars 'Early Large Red' and 'Calcuttia' respectively, while 'Muzaffarpur' showed highest fat content (0.35 per cent).

TABLE 1. PHYSICAL MEASUREMENTS OF LITCHI CULTIVARS

Trait	Early Large Red	Calcuttia	Muzaffarpur	Bedana	Mean	C.D. at 5%
Fruit wt. (g)	21.2	13.8	17.9	6.8	14.90	2.7
Edible part (%)	84.4	61.7	68.2	71.7	71.50	5.2
Seed (%)	3.3	26.1	18.1	8.8	14.07	2.5
Peel (%)	12.3	12.2	13.7	19.5	14.04	1.5

*The paper was accepted for presentation in the 22nd International Horticultural Congress, held at Davis, California, 11th–20th. August, 1986.

TABLE 2. COMPOSITION OF LITCHI CULTIVARS

	Early large Red	Calcuttia	Muzaffarpur	Bedana	Mean	C.D. at 5%
TSS (%)	21.5	21.0	21.5	21.0	21.2	NS
Acidity (%)	0.51	0.54	0.51	0.53	0.52	0.01
Moisture (%)	80.2	82.0	80.3	81.2	80.9	NS
Carbohydrates (%)	17.9	16.06	17.60	16.93	17.12	0.34
Fat (%)	0.21	0.27	0.35	0.23	0.26	0.02
Reducing sugars (%)	10.17	9.88	9.78	9.91	9.93	0.12
Non-reducing sugars (%)	5.86	5.76	6.44	5.60	5.91	0.15
Total sugars (%)	16.03	15.64	16.22	15.51	15.85	0.32
Proteins (%)	1.15	1.13	1.20	1.12	1.15	0.01
Tryptophan (g/16gN)	1.25	1.24	1.34	1.17	1.25	0.01
Methionine (g/16gN)	0.18	0.21	0.22	0.25	0.21	0.01
Total minerals (%)	0.54	0.54	0.55	0.52	0.53	0.01
Protopectin (%)	0.14	0.12	0.14	0.11	0.13	NS
Pectinic acid (%)	0.41	0.53	0.57	0.56	0.52	0.03
Total pectins (%)	0.55	0.65	0.71	0.67	0.64	0.01
Ascorbic acid (mg/100g)	38.43	35.76	44.36	37.88	39.11	0.70
Total phenols (mg/100g)	127	116	143	151	134	0.30
Anthocyanin (OD/10 discs)	0.35	0.33	0.38	0.30	0.34	0.01
Energy (Kcal)	78.09	71.19	78.35	74.27	75.47	0.85

The highest amounts of non-reducing and total sugars were observed in 'Muzaffarpur' whereas 'Early Large Red' gave highest value for reducing sugars.

Protein content of the cultivars varied from 1.12 to 1.20 per cent. The essential amino acids viz. tryptophan and methionine were also analysed, keeping in view the importance of amino acids during ripening[12]. Tryptophan and methionine were highest in 'Muzaffarpur' and 'Bedana' cultivars. The highest total mineral and ascorbic acid contents were observed in 'Muzaffarpur' cultivar. Pectinic acid and total pectins ranged from 0.41 to 0.57 per cent and from 0.55 to 0.71 per cent respectively. West and Todd[13] suggested that 0.30-0.70 per cent protein is required for gel formation and because of its capacity to form gel, it may reduce irritation at the intestinal wall[14]. The highest content of total phenols was observed in 'Bedana' and the lowest in 'Calcuttia' while anthocyanin which is responsible for the bright red colour of litchi, was found to be highest in 'Muzaffarpur'. The highest energy value would be attributable to 'Muzaffarpur' and the energy value for all the cultivars were higher than those reported earlier[15]. Cultivar 'Muzaffarpur' was observed to be nutritionally excelling other three cultivars on the basis of the constituents analysed.

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NUTRIENT STATUS DURING *SHRIKHAND* MAKING

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Nutrient contents were determined at various stages during *shrikhand* making, using the conventional LF–40 and a new yoghurt culture YH as starters. At the stage of *dahi* making, there was an increase in the contents of vitamin B_2 (121–131%), folic acid (165–331%) and niacin (160–201%) Protein and methionine contents were unaltered. During *chakka* making, there were substantial losses of all three vitamins in whey. The product made with YH culture was superior in nutrient contents to that made from LF–40.

Shrikhand is the indigenous fermented milk based dessert, most popular in many parts of the country. Recent studies reported from our laboratory showed better nutritional quality of proteins in *shrikhand*[1,2]. In view of these observations and the varying ability of lactic cultures to synthesize B-group vitamins, changes in certain nutrients during *shrikhand* making with two different cultures YH (*Streptococcus thermophillus* and *Lactobacillus bulgaricus* 1:1) and LF-40 (*Lactic fermentii* consisting of different strains of *S. lactis*) were studied. Data are presented in this report.

Shrikhand was prepared using fresh buffalo milk (standardised to 3.0 per cent fat) as described earlier[1]. At appropriate stages, *dahi*, *chakka* and whey portions were saved for nutrient analysis. Protein[3], methionine[4] and cholesterol[5] were determined by chemical methods. Vitamin B_2, folic acid and niacin were determined by microbiological procedures using *L. casei* (ATCC 7469), *S. faecalis* (ATCC 8043) and *L. plantarum* (ATCC 8014), respectively as test organisms[6]. Nutrients at various stages were compared by taking the amount of a particular nutrient in 100 g milk as the basis and its concentration at various stages of *chakka* making was expressed as per cent of this value.

The changes in various nutrient contents are given in Fig. 1. During conversion of milk to *shrikhand*, the recovery of *chakka* was about 37 and the whey drained off amounted to 59 per cent with both the cultures. The protein content did not change on conversion to *dahi* (Fig. 1-a). Khambatta and Dastur[7] also did not observe any change in the total nitrogen as a result of progressive fermentation of milk. The retention of methionine in *dahi* made with both the cultures was almost 98 per cent of the methionine in the untreated milk, suggesting no loss during fermentation. The methionine losses in whey ranged between 13 and 15 per cent while more than 80 per cent methionine was retained in *chakka* and subsequently in *shrikhand*. Although methionine is the first limiting amino acid in milk proteins, a small decrease in its content as observed during *chakka* making may not alter the biological value of milk proteins as reported earlier[1]. As regards cholesterol content, *dahi* preparation showed a small decrease of 8-15 per cent in comparison with milk. The cholesterol content in *chakka* was about 78 per cent of milk cholesterol. A similar decrease in the cholesterol content during yoghurt preparation was earlier reported by Antila and Antila[8].

The use of YH culture for *dahi* making produced more of vitamin B_2, folic acid and niacin in comparison with LF-40. The retention of vitamin B_2 and folic acid in *chakka* was also found to be distinctly higher when YH culture was used.

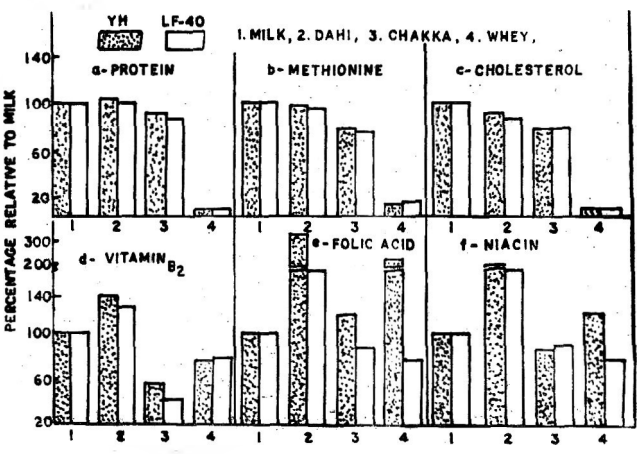

Fig. 1. Distribution of nutrients during *Shrikhand* making

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The starter cultures used in this study were mixed ones. Culture LF-40 was *Lactic fermentii* containing different strains of *S. lactis* while YH comprised *S. thermophilus* and *L. bulgaricus*. The increase in vitamin contents observed in this study was in keeping with the earlier observations, that levels of certain vitamins of B-group markedly increase during fermentation of milk using mixed cultures[9-13].

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PHYSICO-CHEMICAL AND FUNCTIONAL PROPERTIES OF SPENT HEN COMPONENTS

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Physico-chemical and functional characteristics of deboned meat components (breast, leg and wing+back meats) and low value components (skin, gizzard and heart) were studied in 'White Leghorn' spent hens. Breast meat was found to be superior with higher protein, extractable proteins, emulsifying capacity and lower fat and collagen contents for use in further processed or comminuted meat products. Skin contained higher fat and lower protein with maximum collagen. Gizzard was slightly preferable to heart and skin was least preferable for use in products due to poor functional properties. Determination of optimum level of incorporation of low value components into products needs greater consideration.

In India, spent hens contribute about 83 thousand tonnes of poultry meat comprising about 46 per cent of the total poultry meat. One of the greatest needs of the poultry industry is increasing the utilization of spent laying hens considered to be a by-product of the egg industry[1]. Ground or emulsion type products were attempted for efficient utilization of spent hen components. Information on the various physico-chemical and functional properties of spent hen components is essential for development of such further processed products as these properties determine the quality of the finished products. Reports are available on some of the compositional[2-4] and functional properties[2,5,6] of spent hen components. In the present study, an attempt was made to evaluate the deboned meat components and low value components from spent hens for the physico-chemical and functional properties.

Spent hens of 'White Leghorn' breed (above 500 days and maintained on deep litter) available from a research farm were utilised in the study. Birds selected at random were fasted for 15 hr, slaughtered and hand dressed after scalding for 1 min at 60°C. After evisceration, birds were hot deboned within 3 hr of slaughter. Deboned meat components namely, breast meat, leg meat and wing+back meat; and low value components like skin, gizzard and heart were packed separately in polythene bags and frozen for 7 days at -10°C and thawed at 5°C for 15 hr, fine minced and analysed. Components from more than one bird (2 to 20) were grouped in order to

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TABLE 1. PHYSICO-CHEMICAL AND FUNCTIONAL CHARACTERISTICS OF DEBONED MEAT COMPONENTS AND LOW VALUE COMPONENTS FROM SPENT HENS

Quality characteristics	Deboned meat components			Low value components		
	Breast	Leg	Wing + back	Skin	Gizzard	Heart
Moisture (%)	72.09±0.59^a	69.87±1.12^a	62.96±0.21^a	52.22±1.36^b	72.97±0.58^a	70.60±0.17^a
Protein (%)	23.07±0.56^b	19.52±0.65^a	17.71±0.09^a	15.17±0.31^a	20.28±0.36^b	17.55±0.33^c
Fat (%)	3.42±0.72^a	8.79±1.44^b	17.79±0.44^c	31.91±2.36^b	6.43±1.32^a	10.94±0.32^a
Ash (%)	1.21±0.05^a	0.98±0.01^b	0.73±0.02^c	0.44±0.06^b	0.94±0.03^a	0.82±0.05^a
pH	5.94±0.07^a	6.08±0.05^a	6.07±0.04^a	5.98±0.24^b	6.67±0.09^a	6.50±0.06^a
Water holding capacity (ml/100g)	12.81±3.67^a	7.19±1.87^a	2.75±1.89^a	23.12±4.89^{ab}	31.38±0.20^b	13.81±1.84^a
Emulsifying capacity (ml oil/2.5g)	131±5.64^a	122±2.73^b	102±2.69^c	47±4.41^b	89±2.08^a	94±2.33^a
Cooking loss (%)	21.72±1.42^a	22.89±1.10^a	21.73±0.63^a	23.04±2.24^a	27.42±1.00^a	39.65±0.68^b
Water soluble proteins (%)	7.87±0.30^b	4.90±0.57^a	4.72±0.23^a	1.45±0.03^b	6.73±0.23^a	5.50±0.35^a
Salt soluble proteins (%)	12.14±0.94^b	7.75±0.25^a	6.41±0.15^a	2.04±0.04^b	5.27±0.14^a	5.02±0.27^a
Extractable proteins (%)	81.94±2.98^b	60.24±4.54^a	63.01±1.38^a	23.44±1.00^b	59.79±0.44^a	60.07±3.82^a
Collagen (%)	0.68±0.11^a	1.03±0.16^a	1.16±0.25^a	5.16±0.44^a	2.79±0.02^b	1.17±0.07^c

Mean with the same superscript in the same row do not differ significantly ($P<0.05$) when compared separately under deboned meat components and low value components.

obtain sufficient quantities of sample for analysis and a minimum of 3 such pooled samples were analysed for the various physico-chemical and functional properties as per the procedures reported earlier[7]. Analysis of variance was conducted and means were compared using Duncan's multiple range test[8], separately under deboned meat components and low value components.

Quality characteristics of different spent hen components are presented in Table 1 and the analysis of variance in Table 2. A highly significant difference was

TABLE 2. ANALYSIS OF VARIANCE OF QUALITY CHARACTERISTCS

Quality characteristics	Deboned meat component			Low value components		
	error d.f	Error m.s	Components' m.s	Error d.f	Error m.s	Components' m.s
Moisture (%)	15	0.018	0.036	12	3.95	660**
Protein (%)	17	2.46	45.77**	9	0.49	29.25**
Fat (%)	17	8.64	276.14**	9	9.81	1366**
Ash (%)	11	0.006	0.256**	7	0.009	0.26**
pH	15	0.018	0.037	6	0.069	0.388*
Water holding capacity	9	27.36	101.67	11	36.35	309.18*
Emulsifying capacity	23	102.86	1256**	6	29.24	2045**
Cooking loss (%)	13	7.77	2.55	9	8.65	296**
Water soluble proteins (%)	9	0.62	12.51**	6	0.106	22.90**
Salt soluble proteins (%)	9	1.29	35.93**	6	0.095	9.69**
Extractable proteins (%)	9	41.78	558**	6	15.76	1331**
Collagen (%)	6	0.105	0.18	6	0.20	12**

1 : components d.f=2 *significant $P<0.05$; **significant $P<0.01$.

observed between the deboned meat components for the parameters of protein, fat, ash content, emulsifying capacity (EC), water soluble proteins (WSP), salt soluble proteins (SSP) and extractable proteins (EP) while under low value components, all the parameters showed significant differences (Table 2).

Deboned meat components: Breast meat contained relatively higher moisture and significantly higher protein than the other components (Table 1). Fat was significantly higher in wing+back meat followed by leg meat and breast meat. Ash was significantly lower in leg and wing+back meat due to higher contents of fat in these components.

pH was significantly different between the components but was relatively lower in breast meat. Even though no significant difference was observed in the water holding capacity (WHC) of different components, the mean differences clearly indicate that breast meat had superior WHC followed by leg meat and wing+back meat (Table 1). Emulsifying capacity differed significantly between the components and was maximum in breast meat followed by leg and wing+back meat. Cooking loss did not differ significantly between the components. WSP, SSP, and EP were significantly higher in breast meat than the other components due to the relatively higher protein content and lower fat content in breast meat. Collagen content was maximum in wing+back meat (1.16 per cent) indicating a higher proportion of connective tissue followed by leg meat (1.03 per cent) and breast meat (0.68 per cent).

The trend of differences between breast and leg meat for the parameters of pH, moisture, protein, fat and SSP was similar to that of McCready and Cunningham[2] for light and dark meat of fowls. Higher SSP per cent of breast meat was in agreement with the findings of other workers[2,3]. Significantly higher EC observed in breast meat than in leg meat did not confirm the findings of other workers[2,9,10]. However, in support of the present study Maurer and Baker[6] and Parkes and May[10] reported the superiority of breast meat over other parts for emulsification. Breast meat with better compositional and functional properties was found to be more desirable for use in the preparation of products with better yields and quality compared to leg and wing+back meats. Wing+back meat showed relatively poor functional properties due to higher fat and lower protein contents.

Low value components: In general, lower values were observed for all the parameters in skin than gizzard and heart except for fat per cent, WHC and collagen per cent (Table 1). Moisture and protein were significantly lower in skin due to higher fat content. The composition of gizzard and heart were nearly comparable with the reported values[3] but in case of skin much variation was observed due to the subcutaneous fat which is dependent on the condition (fatty or non fatty) of the birds.

pH of the skin (5.98) has been found to be similar to the normal pH of poultry meat (6.0) and significantly lower than gizzard and heart. Water holding capacity was significantly lower in heart than gizzard showing that cardiac muscle has very poor WHC. Lyon and Thomson[11] also reported that hearts showed less WHC than gizzards and livers. Emulsifying capacity values were comparable with the values reported by Maurer and Baker[6]. Skin emulsified significantly less oil as could be expected because of the high content of fat and low content of total protein. Cooking loss was significantly higher in heart since it has very poor WHC. Water soluble proteins, SSP and total extractable proteins were significantly lower in skin than other components due to the lower protein percent and maximum collagen content.

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BACTERIOLOGICAL QUALITY OF FRESH PORK COLLECTED FROM DIFFERENT ABATTOIRS AND RETAIL SHOPS

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Samples of fresh pork collected from 3 types of slaughter houses (i.e. modern, semi-modern and rural) and two retail shops at 15 days interval were analysed for total bacterial count, (SPC) most probable number (MPN) of coliform, MPN of *E. coli* and salmonella incidences. The values of mean SPC, MPN of coliform and *E. coli* in fresh pork samples ranged from 5.77×log 10 to 9.14×log 10, 4.3×log 10 to 7.81×log 10 and 3.5×log 10 to 7.07×log 10 per gram, respectively. SPC, coliform and *E. coli* count of the pork samples collected from retail shops were significantly higher than those of the samples collected from slaughter houses. Some of the samples were positive for salmonella and serotype isolates were *S. bareilly* and *S. stanley*.

The bacteriological quality of meat is dependent on several factors and the hygienic condition of abattoirs is one of them. Total bacterial count of pork sample increased when it moved from butcheries to home[1]. Among the beef, pork and poultry samples, contamination with salmonella was maximum in pork[2]. Several workers have studied the bacteriological quality of fresh meat[3-6]. Though information is available on the bacteriological quality of fresh pork in general, the present study was undertaken to evaluate the bacteriological quality of pork as influenced by abattoir type and retail shops in Tarai regions of U.P. For comparison, samples of fresh pork were also collected from C.D.F., Aligarh.

Samples of fresh pork were collected from three different types of slaughter houses viz. modern type equipped with fully automatic commercial plant having tap water supply; semi-modern type where slaughtering is done by local butchers on tables and water supply is from well; and rural type where slaughtering is done in the open on ground and water supply is from river and two retail shops under aseptic conditions at 15 days interval. These samples were transported to laboratory in ice boxes and stored in deep freeze at -18°C until analysed (2-10 hr).

Weighed quantity (10 g) of each sample was cut into small pieces and triturated with 90 ml of sterilized normal saline solution in a sterilized blender for 10 min. From this, further dilutions were made for counting. Enumerations of Standard plate count (SPC), most probable number (MPN) of coliform and *E. coli* were carried out by using selective media nutrient agar, Mcconkey's broth and Ejackman lactose, respectively according to the procedure given in Methods of Standard Plate Count of Bactoeria in Food Stuff[7] for standard plate count and that of International Organization for Standardization[8] for MPN of coliform and *E. coli*. Total bacterial colonies in nutrient agar plate were counted with the help of Qubecs colony counter. MPN of coliform and *E. coli* were determined by the production of gas in durham's tubes. The production of gas at 37°C for 48 hr in case of coliform and at 44°C for 48 hr in case of *E. coli* was taken as positive for presumptive test. The MPN/100 ml of dilutions was computed on the basis of positive tubes for presumptive test by using probabilities table[9] and further, it was calculated per gram of sample.

For isolation and identification of salmonella, the method of Roberts *et al.*[10] was used. Minced sample (about 10 g) was inoculated in tetrathionate broth and incubated at 37°C for 18 hr. A loopfull of the inoculated broth cultures was streaked on brilliant green agar plates which were incubated at 37°C for 24 hr. Colonies suspected to be of salmonella were isolated, purified and tested biochemically by using the techniques described by Edwards and Edwing[11]. The biochemically confirmed isolates were sent to Central Research Institute, Kasauli, Himachal Pradesh, for serotyping.

The data of SPC, MPN of coliform and *E. coli* were statistically analysed by two way analysis of variance[12].

Standard Plate Count (SPC): Significantly higher mean SPC were obtained in the samples collected from retail shops as compared to slaughter houses which might be due to successive time lapses between slaughtering and marketing (Table 1). However, among the slaughter houses, the samples collected from modern slaughter houses exhibited higher mean SPC which might be due to the consequences of long chain handling of meat as well as due to the lapse of longer time (about 20 hr) during transportation of samples from modern slaughter house to laboratory as compared to other two local slaughter houses (3-4 hr). Higher mean SPC has been observed by Park[1] in pork samples collected from local meat shop as compared to local slaughter houses. The day of sampling did not significantly influence SPC.

Coliform and E. coli *count:* Variation in MPN of coliform counts due to the source of sampling was significant at 5 per cent level whereas day of sampling did not have any significant effect (Table 1). The samples collected from shop No. 1 exhibited the highest MPN of *E. coli*. The sample from modern slaughter house showed higher MPN of *E. coli* as compared to local slaughter houses. The differences in *E. coli* count of pork

TABLE 1. STANDARD PLATE COUNT (SPC), MOST PROBABLE NUMBER (MPN) OF COLIFORMS AND OF E. COLI (X LOG 10/g) IN FRESH PORK SAMPLES COLLECTED FROM DIFFERENT ABATTOIR AND RETAIL SHOPS AT DIFFERENT TIMES

Sampling interval (days)	Enumeration type	Abattoir			Retail shop		Mean
		Modern	Semi-modern	Rural	No. 1	No. 2	
0	SPC	7.73	5.87	6.22	9.36	6.77	8.68
	Coliform MPN	4.95	3.87	3.47	8.17	4.95	7.55
	E. coli MPN	4.95	2.84	3.36	6.66	4.95	5.97
15	SPC	8.21	5.49	5.77	7.29	8.66	8.07
	Coliform MPN	7.38	3.17	4.17	4.60	7.00	6.85
	E. coli MPN	6.66	2.60	2.95	3.38	6.66	6.25
30	SPC	7.93	5.99	7.50	9.60	7.30	8.91
	Coliform MPN	5.60	3.97	4.55	8.17	4.66	7.47
	E. coli MPN	5.36	3.36	3.32	7.63	4.63	6.93
45	SPC	8.32	6.32	5.30	8.65	8.25	8.20
	Coliform MPN	6.96	3.63	4.60	7.32	6.20	6.79
	E. coli MPN	6.96	3.60	3.95	5.36	6.17	6.34
60	SPC	8.00	5.55	6.25	8.46	6.63	7.95
	Coliform MPN	6.32	4.60	4.95	7.04	5.60	6.43
	E. coli MPN	6.17	3.95	4.36	7.04	4.60	6.39
Mean values							
	SPC	8.08	5.83	5.77	9.14	8.11	
	Coliform MPN	6.85	4.30	4.56	7.81	6.44	
	E. coli MPN	6.49	3.51	3.87	7.07	6.07	

C.D. for variation due to source of samples (at 5%): SPC = 9.0 × log 10, MPN of coliform = 7.67 × log 10, MPN of *E. coli* = 7.06 × log 10.

TABLE 2. INCIDENCE OF SALMONELLA IN FRESH PORK SAMPLES COLLECTED FROM DIFFERENT ABATTOIRS AND RETAIL SHOPS

Source	Samples tested (No.)	Salmonella	
		Samples + ve (No.)	Serotype identified
Abattoir			
Modern	5	1	*S. stanley*
Semi-modern	5	0	—
Rural	5	1	*S. bareilly*
Retail shops			
No. 1	5	1	*S. bareilly*
No. 2	5	0	—

samples collected from shop No. 1, shop No. 2 and modern slaughter house were non-significant. However, *E. coli* count of samples collected from rural slaughter house was significantly lower than that of the samples collected from shop No. 1.

Salmonella: The results of salmonella analysis showed that 12 per cent of samples of fresh pork were positive for salmonella and serotype isolates were *S. bareilly* and *S. stanley* (Table 2). The samples collected from modern and rural slaughter houses and from retail shop No. 2 showed the presence of salmonella. Studies of Oesterem *et al.*[13] and Swaminathan *et al.*[2] showed that 12.9 and 21.5 per cent of samples, respectively were positive for salmonella.

These data underscore the need to observe strict hygienic conditions during production and sale of fresh pork.

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Advances in Biochemical Engineering / Biotechnology, Vol. 32: Agricultural Feedstock and Waste Treatment and Engineering, Edited by A. Fiechter, Springer-Verlag, Berlin, Heidelberg, New York, 1985; pp: 267; Price: DM132.

The first chapter is on Industrial Processing and Products from the Jerusalem Artichoke by N. Kosaric, A. Wieczorek, G. P. Cosentino and Z. Duvnjak. Jerusalem artichoke gives high yields per hectare in terms of biomass. It contains substantial levels of carbohydrates and has been used as food as well as feed. Its production has declined over the years because tubers got preference. Production of ethyl alcohol and high-fructose syrup are described. Preparation of raw material, pretreatment of carbohydrates, optimisation of conditions for fermentation along with energy balance and economics are discussed for ethanol production. In this preparation of syrup, juice extraction, hydrolysis, purification and concentration steps are discussed.

The second chapter is on the Utilisation of Cheese Whey and its Components by N. Kosaric and Y. J. Asher. The author discusses various alcoholic beverages, xanthan gum etc. Hydrolysis of lactose and conversion to high fructose syrup have been described. It has also been shown how lactose could be utilised to produce lactic acid and single cell proteins. Finally, discussion on utilisation of whey proteins is also given. A detailed discussion on functional properties of whey protein concentrates has been presented and also the various uses of whey protein concentrates in food products.

The third chapter is on Bioconversion of Hemicellulosics by R. J. Mages and N. Kosaric. This chapter discusses the structure and availability of hemicellulose carbohydrates which comprise mostly pentose sugars and the biochemistry and process technology involved in their conversion to fuels and chemical feedstocks. In the discussion on hemicellulose utilisation various hydrolysis methods and bioconversion processes to ethanol are described. More discussion on economics of such processes would have been desirable.

The fourth chapter is on Mathematical Modelling, Parameter Identification and Adaptive Control of Single Cell Protein Processes in Tower Loop Bioreactors by R. Luttmann, A. Munack and M. Thoma. Among new types of bioreactors developed for large scale single cell protein (SCP) production, tower and tower loop reactors have been successfully used at pilot plant and production levels. A discussion of optimisation of SCP processes in tower loop reactors is given. Optimisation methods are presented both for dynamically changing cultivation as well as for steady state behaviour with respect to SCP processes. Modelling of cultivation is presented with various parameters such as cell mass, oxygen, substrate, etc. Simulation and parameter identification methods are given and their application is shown with the help of experimental data. Finally, optimisation of continuous pilot plants is given describing the scale up, process parameters and steady state model. Method of evaluating the economy of the production process is also briefly discussed.

The last chapter is on Modelling, Optimisation and Control of semi-Batch Bioreactors by S. J. Parulekar and H. J. Lim. A number of products like antibiotics, organic acids, amino acids, proteins, cellomass and enzymes are produced by fed-batch process. A review of various models, structured and unstructured along with their applications is presented. Discussion on optimisation gives formulation of problem and its solution both for constant and non-constant biomass yield. Various control schemes are described along with the instrumentation and specific examples on control in biomass production and in antibiotic and enzyme production are discussed.

In the fine tradition of Advances of Biochemical Engineering/Biotechnology series, this volume does not come upto the expectation. The title of this volume is justified by first three chapters each of which is a short review of some moderately important areas. 'Cultivation of Jerusalem Artichoke' is so small as it does not have the importance of many other crops which could become potential sources for industrial exploitation. Cheese whey may be considered an important source of agricultural feed stock, a large proportion of which forms a waste at present and can be recovered and converted to useful products. Hemicellulosics have been glorified in the present volume showing only the research possibilities. In reality, the cost constraints are so great, most processes described will be uneconomical under present conditions.

The remaining chapters which form the bulk of the volume, although excellent reviews, do not really belong in this volume. Instead, the editors could have chosen various subjects which could merit mention in this volume. Many different wastes, agricultural and industrial, have promising applications and are being researched. Inclusion of some of these would have enhanced the usefulness of this volume.

J. S. PAI
U.D.C.T., BOMBAY

Evaluation of Certain Food Additives and Contaminants: Twentyninth Report of the Joint FAO/WHO Expert Committee on Food Additives, Technical Report Series 733, World Health Organisation, Geneva, 1986; pp: 59; Price: Sw. fr. 9.

The report of the Joint FAO/WHO Expert Committee on Food Additives which met in Geneva on 3-12 June 1985 deals with topics like principles governing the toxicological evaluation of certain enzyme preparations, organic and inorganic acids and their salts, vegetable gums and also gives its comments on specific food additives based on the data available on toxicological studies. The report deals with the following:

(*a*) While considering enzyme preparations, lot of stress was made on the testing requirements for immobilised enzymes and immobilising agents.

(*b*) ADI values for certain inorganic and organic acids and their salts with cations comprising Al^{+++}, Ca^{++}, Fe^{++}, Mg^{++}, K^{+}, Na^{+} and anions such as, acetate, adipate, fumarate, sorbate, carbamate, silicate, sulphate, etc. were proposed.

(*c*) Specific food additives under the headings: enzyme preparations and enzyme immobilising agents, flour treatment agents, flavouring agents, food acids and their salts, food colour, sweetening agents, thickening agents and miscellaneous food additives have been commented upon based on the available toxicological data.

(*d*) While evaluating food colours, particularly different caramels 2-acetyl-4(5)-tetrahydroxy butyl imidazole (THI) in caramel prepared by ammonia process was considered as lymphocyte depressant and was given an ADI of 0-200 mg/kg b.w. Similarly, 4-methyl imidazole was considered while evaluating caramel prepared by ammonia sulphite process. The report indicates the preparation of a consolidated toxicological monograph on different caramels.

(*e*) Certain details on establishment, revision and withdrawal of certain specifications for antioxidants, emulsifying agents, extraction solvents, sweetening agents, thickening agents were given.

(*f*) The report recommends the future programme on various items including establishment of tolerable intakes of lead for infants and children which is of great relevance to people working on food safety assurance programme.

There are four annexures-Annexure I deals with the report and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food additives, Annexure 2: Acceptable daily intakes, other toxicological recommendations and information on specifications. Annexure 3-Further toxicological studies and information required or desired by the Committee. Annexure 4 deals with matters arising from the reports of Sessions of the Codex Committee on Food Additives.

This report will be useful for persons working on various aspects of food additives and researchers of food safety evaluation.

K. V. NAGARAJA
C.F.T.R.I., MYSORE

Produce Handling, Packaging and Distribution: by Kalman Peleg, AVI Publishing Company Inc., West Post Connecticutt, U. S. A., 1985; pp: 625; Price: $ 135.

The number of books on the subject of fresh produce handling and packaging is rather limited considering the extensive R & D output in this area. It is only in recent years that the number of books on this subject appears to be on the increase. The present volume by K. Peleg is a very welcome addition to the growing list of books in this field.

In 18 chapters, the author has endeavoured to cover various aspects of fresh produce handling and packaging from the field to the distribution centre having the prevelent system in the west as the back drop. Each chapter ends with a comprehensive bibliography and summary. The real feature of the book is the in-depth treatment of the approach to some of the packaging problems which still engage the attention of the researchers. There is a copious use of mathematics obviously treating the subject on a technical plane. However, there are some chapters almost completely descriptive and understandable to those without much mathematical background.

The first three chapters cover preliminary operations from the time of harvest, viz. harvesting techniques, maturity standards, pre-treatments for storage etc. as is in vogue in advanced countries.

The next three chapters deal with sorting operations sizing and sizing machines, grading and the theory and techniques behind these operations. The adoption of optical grading machines X-ray and γ-ray techniques for grading serve to highlight the importance given to the grading, which in India is done only manually and restricted to very few commodities.

Chapter 7 gives a mathematical treatment of packing patterns of the produce in containers, assuming a spheroidal shape for the content. The counts, packing density, have all been worked by using mathematics. The relative merits of jumble packs and pattern packs have also been highlighted.

In chapter 8, computer programmes for stacking pattern forms leading to optimal container selection has been presented. This provides necessary guidance for

tackling problems on better volume utilisation of container space.

The whole sale produce packaging, the FCC produce packaging system and retail produce packaging are discussed in chapters 9, 10 and 11. The chapter on whole sale produce packaging, presents the technological economic and protective features of the different packing systems like the jumble pack, pattern pack and cell packs commonly adopted in Transport packs. The filling machines are also described.

The FCC produce packaging system being the outcome of the author's research over several years is thoroughly discussed in chapter 10. The abbreviation FCC stands for 'Face Centered Cubic 'structure the densest' atomic lattice'. Adoption of this pattern provides for "better volume utilisation of the shipping container, thereby saving on the material storage and transportation costs".

The retail packing or consumer packing and the recent trends therein are presented in Chapter 11. A brief treatment of pre-packaging is also presented.

The subject of shipping containers is dealt in chapters 12 and 15. Corrugated fibreboard containers get a very extensive treatment since they happen to be the most widely used ones in fresh produce trade in all advanced countries.

The highlight of chapter 15 is the detailing of container design procedures using the data on static strength and dynamic strength of containers. The theoretical basis adopted in design procedure is elucidated in each case.

The handling and transportation of unit loads is discussed in chapter 13, detailing the procedures followed in road, rail car, marine and air transports. The pelletisation and containerisation are also dealt in brief.

The next chapter (chapter 14) discusses the transportation environment, introducing the reader to the basics of the theory of vibration and shock.

Chapter 16, on package-produce testing is utilised to describe the testing machines and methods of measurement of shock and vibration and compression. The theoretical basis underlying these tests and their relative merits are given a fairly adequate treatment.

Since the fresh produce are treated as viscoelastic materials, the rheological behaviour of these are sought to be explained by appropriate models. This forms the subject matter of chapter 17.

The concluding chapter (18) discusses mechanical injury to the produce in processing storage and distribution. The needs and different approaches to quantify the damage and indirect methods of damage evaluation are described in brief.

The book ends with 5 useful appendices inclusive of two computer programme print-outs and one on small statistics which is very useful to the research worker whereas the computer programmes will be of interest for problems in pelletising and container loading.

On the whole, the book is sure to delight the researchers in fresh produce packaging. Since here is a single source which fulfills several needs, as an inventory of latest research, an advanced technical text book, and an information guide for developments abroad. Despite limitations like the technical treatment being severely limited to spherical fruits, like the apple, and being biassed heavily towards the situations obtaining in advanced west, the book is one of the best to appear in this field in recent times.

A. R. VIJAYENDRA RAO
C.F.T.R.I., MYSORE

Season to Taste: by Colin Dence, Food Trade Press Ltd., 29, High Street, Green Street Green, Orpington BR6 6LS Kent, England; 1985; pp. 165; Price. £ 19.5 in U.K., & Europe, £ 23, Outside Europe including Air Mail Postage.

The book fulfills the long felt need on the role of seasoning in food by describing the desirability, compatability and the effect of various seasonings on the palatability of food. It has been divided into three basic sections. The first section deals with the various types of seasonings, those which are used for addition of particular aroma to food and those that affect the taste. The importance of interactions of taste and aroma in relation to seasonings have also been discussed.

The second part deals with the history and origin of seasonings, agricultural aspects of each spice and herb and their individual and combined uses, the various recipes of the past, the culinary practice of medieval world and the changes which have led to the present day practice of use of spices. Different seasoning affinities based on the nature of the food viz., sweet, sweet-sour or savory, have been discussed. The past and present cooking practices of Roman Lombard, French and English have been discussed with particular reference to seasonings.

The third part deals with present day seasonings, balancing various spice flavours, compatability of each spice in a recipe and harmony of the flavours in detail. An attempt has been made to answer the question "Why use seasoning at all?"

On the whole, this is a good book throwing some light on the theory and practice of seasoning food. The price of the book is too high.

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

AFST(I) News

Bombay Chapter

The Annual General Body Meeting of the Bombay Chapter was held on Saturday, the 20th June 1987. A lecture on "Emerging Scenario in Chemical Industries" by Prof. M. M. Sharma, FNA, Head, Chemical Engineering Division, B.U.D.C.T., Bombay was also organised on this occasion.

The results of the elections to the Local Executive Committee (LEC) for 1987–88 were declared. The following members and office-bearers were elected.

President	:	Dr. P. J. Dubash
Vice-Presidents	:	Dr. C. L. Nagarsekar
		Mr. S. V. Krishnaswamy
Hon. Secretary	:	Dr. A. S. Gholap
Hon. Jt. Secretary	:	Dr. G. M. Tewari
Hon. Treasurer	:	Dr. S. R. Padwal-Desai
Members	:	Prof. K. M. Agashe
		Dr. H. R. Adhikari
		Dr. S. V. Bhalkar
		Dr. D. R. Bongirwar
		Dr. D. P. Nerkar
		Dr. S. V. Padgaokar
		Mr. N. A. Pandit
		Dr. S. B. K. Warrier
		Mr. L. K. Shah (*Ex-officio*)
Co-opted Members	:	Dr. C. P. S. Menon
		Dr. J. S. Pai
		Mr. J. K. Sanzgiri
		Dr. R. R. Mallya
		Dr. R. Y. Angle
		Dr. (Mrs.) Sucy Eapen

INSTRUCTIONS TO AUTHORS

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

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

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

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

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

6. **Illustrations:** Graphs and other line drawings should be drawn in *Indian ink* on tracing paper or white drawing paper preferably art paper not bigger than 20 cm (oy axis) $\times$16 cm (ox axis). The lettering should be such that they are legible after reduction to column width. Photographs must be on glossy paper and must have good contrast; *three copies* should be sent.

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

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

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

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

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

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

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

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

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

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

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

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Research Papers

Research Notes

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