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CONTENTS

Research Papers

Research Notes

Changes in Protease Inhibitory Activity in Plant Seeds on Heat Processing

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Effects of heat processing on the antitryptic and antichymotryptic activities in thirteen seeds were evaluated. Antitryptic activity decreased by 77% in red gram, 83% in lentil seed, 93% in Bengal gram, 93% in green pea, 98% in lima bean and 99% in green gram on cooking at 100°C for 30 min. Effects were similar on pressure-cooking for 15 min. On preparation of gruels, antitryptic activity decreased by 56% in ragi, 70% in bajra and 62% in Italian millet. Inhibitory activity in sorghum, however, did not decrease. Preparation of unleavened bread had similar effects on the four millets. On roasting, antitryptic activity decreased by 83%, 87% and 100% respectively in tamarind, groundnut and green pea. In cashew nut, the activity did not decrease on roasting (10 min). Antichymotryptic activity was absent in tamarind, Italian millet, bajra and green gram. The activities which were relatively low in other seeds, behaved in a similar fashion like antitryptic activity on heat processing. In all cases except cashewnut, concentration of 0.15 M NaCl extractable protein decreased to varying degrees on heat processing.

Trypsin inhibitors in plant seeds adversely affect the nutritional quality and safety of foods[1-3]. Heat treatment is known to improve the nutritional quality of plant proteins which also causes partial inactivation of protease inhibitors[2]. Considerable amount of work has been done on the effect of food processing on trypsin inhibitor levels in soybean, and its products as also in other plant foods[4-7]. Even though the presence of protease inhibitors in millets, legumes and other seeds commonly used in the Indian diet is established[8,9], the effect of conventional food processing on these systems has not been studied in detail. In a recent communication from this laboratory[10], it was shown that the loss in antitryptic activity in seed extracts on heat treatment does not parallel the loss in activity when the seeds were cooked for the same time-duration. In view of this, it was considered worth-while to evaluate protease inhibitor levels in common edible plant seeds used in this country on food processing. The data obtained with thirteen seeds on different types of heat processing are reported in this communication.

Materials and Methods

Seeds of Italian millet (*Setaria italica* 'Co-5'), sorghum (*Sorghum bicolor*, 'Co-24'), bajra (*Pennisetum typhoideum*, 'Co-6' and ragi (*Eleucine coracana*, 'Co-10') were procured from Tamil Nadu Agricultural University, Coimbatore, India. Lima bean (*Phaseolus lunatus*), lentil beans (*Lens esculenta*), green pea (*Pisum sativum*), groundnut (*Arachis hypogea*), cashewnut (*Anacardium occidentale*), Bengal gram (*Cicer arietinum*), red gram (*Cajanus cajan*), green gram (*Phaseolus aureus*) and tamarind seeds (*Tamarindus indica*) were procured locally. Bovine trypsin (twice crystallized) and bovine α-chymotrypsin (thrice crystallized) were from Worthington Biochemical Corporation, Freehold, NJ, U.S.A.

Active site titrations of trypsin and chymotrypsin were performed according to the method of Kezdy and Kaiser[11]. Caseinolytic activities of the two enzymes and their inhibition by plant extracts were assayed as described before[12]. Under the assay conditions (10 min, 37°C. pH 7.6) 6 μg of active trypsin and 6.2 μg of active chymotrypsin gave an absorbance of 0.6 (λ 540) in the caseinolytic assay. Magnitude of inhibitory action was expressed in terms of μg of enzyme inhibited based on inhibition in the range 20-50 per cent.

Processing of seeds: For the control studies, 1g of the seed powder was extracted with 10 ml of 0.15 M NaCl, stirred for 30 min and centrifuged at 10,000 × **g** for 20 min at 4°C. The precipitate was reextracted at least twice with 10 ml of 0.15 M NaCl and processed as above. The combined supernatants containing more than 99 per cent activity in most cases were assayed for inhibitory action. The final residue was dispersed in 10 ml of 0.15 M NaCl and was also assayed. The seed extracts did not contain measurable amount of caseinolytic activity under the assay conditions.

To study the effect of cooking on lima bean, Bengal gram, lentil, red gram, green pea and green gram, the seeds were cooked in 10 ml of 0.15 M NaCl at 100°C for 30 min and extraction was done as described above. For ragi, sorghum, bajra and Italian millet, one gram of seed powder was boiled with 10 ml of 0.15 M NaCl for 15 min to yield a semi-solid mass. This was

extracted further with 0.15 M NaCl as described above to get a gruel. To study the effect of pressure-cooking, the legume seeds (1 g) were autoclaved for 15 min at 115°C at 10^5 Pa in presence of 10 ml of 0.15 M NaCl. The samples were processed as described above. With the millets, *roti* (unleavened bread) was prepared as follows. The seed powder was made into a dough with hot 0.15 M NaCl and baked. The preparation was extracted thrice with 0.15 M NaCl to get inhibitor extracts. With groundnut, green pea, cashewnut and tamarind seeds, roasting was done without oil for about 8-10 minutes in a hot pan and the roasted seeds were extracted with 0.15 M NaCl as detailed above.

Protein in the plant extracts was estimated according to the method of Lowry *et al*[13] using bovine serum albumin as standard.

Results and Discussion

In Table 1, the effect of cooking six legume seeds on the anti-tryptic and antichymotryptic activities is shown. Pressure cooking for 15 min or boiling the seeds for 30 min which were adequate to cook them completely, had similar effects on inhibitory activities. The residual antitryptic activities were lowest in green gram (1 per cent) and highest in red gram (22.7 per cent). Green gram alone had no detectable antichymotryptic activity. The antichymotryptic activities in other native and cooked seeds were lower than the antitryptic activities.

Even though sorghum had relatively low trypsin inhibitory activity, it was fully stable on processing (Table 2). While antichymotryptic activity in ragi also was highly stable, 56 and 51 per cent of antitryptic activities were lost during the preparation of the gruel and *roti* respectively. Bajra and Italian millet which had no antichymotryptic activity, lost about 70 and 77 per cent of the antitryptic activities during preparation of *roti*.

In Table 3, the effect of roasting on the protease inhibitory activities in four seeds is shown. Cashewnut which had relatively low antitryptic and antichymotryptic activities was found to have highly heat stable inhibitors. The inhibitory principles in green pea were completely heat labile. Nearly 80 per cent of the antitryptic activity in groundnut was inactivated on roasting. Tamarind seed which had no measurable antichymotryptic activity, but had very high antitryptic activity, lost 83 per cent of it on processing.

In Table 4, the data on the quantity of proteins extractable on processing are presented. The amount of soluble protein was considerably reduced, irrespective of the type of processing, the exception being cashewnut. However, the decrease in protease inhibitory activity could not be attributed to the association of inhibitors with non-extractable proteins. No antitryptic or anti-chymotryptic activity was detected in the insoluble residual portions of Italian millet, sorghum, bajra, groundnut, green pea, red gram, lentil seed, Lima bean, Bengal gram and green gram. In the case of ragi with the native extract the

TABLE 1. DECREASE* IN PROTEASE INHIBITORY ACTIVITIES IN LEGUME SEEDS ON HEAT PROCESSING

Legume	Trypsin (μg/g seed) inhibition			Chymotrypsin (μg/g seed) inhibition		
	Native extract	Boiled extract	Pressure cooked extract	Native extract	Boiled extract	Pressure cooked extract
Lima bean	16000	400	583	5620	48	55
Bengal gram	11300	791	675	1200	60	60
Lentil seed	7250	1240	1200	1030	77	97
Red gram	5600	1270	1220	453	58	56
Green pea	580	40	37	427	25	40
Green gram	2940	29	88	0	0	0

*Values are means of three determinations.

TABLE 2. CHANGES* IN PROTEASE INHIBITORY ACTIVITIES IN MILLETS ON HEAT PROCESSING

Millet	Trypsin (μg/g seed) inhibition			Chymotrypsin (μg/g seed) inhibition		
	Native extract	Gruel	Roti	Native extract	Gruel	Roti
Ragi	654	288	323	121	121	124
Sorghum	40	42	40	53	53	52
Bajra	320	96	100	0	0	0
Italian millet	150	57	35	0	0	0

*Values are means of three determinations.

TABLE 3. CHANGES* IN INHIBITORY ACTIVITIES IN SEEDS ON ROASTING

Seed	Trypsin (μg/g seed) inhibition		Chymotrypsin (μg/g seed) inhibition	
	Native	Roasted	Native	Roasted
Tamarind	9000	1500	0	0
Groundnut	680	87	166	36
Green pea	580	0	427	0
Cashewnut	22	20	20	20

*Values are means of three determinations.

TABLE 4. EXTRACTABLE PROTEIN (mg/g SEED) ON PROCESSING

Seed	Native	Boiled	Pressure cooked	Roti	Roasted
Lima bean	120	23.8	16.7	–	–
Bengal gram	132	46.1	40.0	–	–
Lentil	110	32.0	38.0	–	–
Red gram	67	19.9	25.6	–	–
Green pea	95	36.0	58.0	–	26.0
Green gram	126	62.6	35.8	–	–
Ragi	16	7.2*	–	10.5	–
Sorghum	16	5.6*	–	4.8	–
Bajra	26	8.3*	–	10.5	
Italian millet	12	4.3*	–	5.0	
Tamarind	49	–	–	–	18.6
Cashewnut	38	–	–	–	35.2
Groundnut	69	–	–	–	31.1

*Gruel preparation. Values are means of two determinations

insoluble precipitate had about 9 per cent of antitryptic activity compared to the soluble extract. However, the amount of antitryptic activity in the precipitate after heat treatment (21 μg trypsin inhibitor/g seed) or after preparation of unleavened bread (36 μg/g seed) did not increase compared to the precipitate obtained from uncooked seeds (60 μg/g seed) suggesting that the inhibitors are not just precipitated during heat processing. With tamarind seeds even after extracting five times with 0.15 M NaCl, the residue contained trypsin inhibitor equivalent to 740 units (μg of trypsin inhibition per g seed). The residue of roasted seeds had, however, lower inhibitor content (396 μg/g seed).

Even though the millets studied have relatively less anti-tryptic activity (40-65 μg trypsin inhibited/g seed) compared to the legumes (580-26000), the residual activity in the former was comparable to the latter, after food processing. Considering that the millets account for bulk of the vegetarian diet in many regions of the world, the inhibitors in millets will be of more relevance from the nutritional view point. Both the antitryptic and anti-chymotryptic activities in sorghum were completely resistant to heat treatment, whereas only the antichymotryptic activity in ragi was found to be stable. Since ragi is known to contain two distinct inhibitors, a trypsin/chymotrypsin inhibitor and a trypsin/amylase inhibitor[14], it is conceivable that the former is more resistant to processing.

Hamaker *et al*[15] showed that protein extractable by NaCl solution decreased in sorghum and maize on boiling for 20 min. While this treatment did not affect the *in vitro* digestibility of maize proteins, the quantity of indigestible protein increased from 19.3 to 35.2 per cent in sorghum. A detailed study on the digestibility of proteins on cooking in relation to trypsin inhibitor levels and decrease in solubility will be of value in assessing the impeding role of protease inhibitors in the digestion of proteins.

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Pichia membranaefaciens: A Benzoate Resistant Yeast from Spoiled Mango Pulp

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An isolate of *Pichia membranaefaciens* obtained from spoiled mango pulp exhibited high tolerance towards sodium benzoate. Growth occurred in yeast extract-peptone-dextrose broth containing 1500 and 3000 ppm of sodium benzoate at pH 4.0 and 4.5 respectively. However, this isolate showed less tolerance to potassium metabisulphite and potassium sorbate. In mango juice of pH 3.5 and 4.0, growth was noticed in the presence of 500 and 1500 ppm of sodium benzoate respectively with reduced cell population over a period of 8 days. Comparison of preservative resistance of this strain with another strain of *P. membranaefaciens* isolated from grape juice revealed that the character is specific only to the strain isolated from mango pulp.

Association of *Pichia membranaefaciens* with a wide range of fruits and their products has been reported[1-3]. Its role in the spoilage of table wines causing film formation and off-flavour production is well known[4]. In a study on the preservation of mango pulp, a strain of *P. membranaefaciens* which exhibited high degree of tolerance towards sodium benzoate was encountered. Yeasts possessing such character could cause problems during chemical preservation of fruit products. Hence, effect of common preservatives like sodium benzoate, potassium metabisulphite and potassium sorbate on the growth of this yeast was tested at different pH. Growth characteristic was also studied in mango juice of pH 3.5 and 4.0 in the presence of varying levels of sodium benzoate. This strain was compared with another strain of *P. membranaefaciens* isolated from grape juice for its resistance to preservatives. The results of these investigations are reported in this communication.

Materials and Methods

Pichia membranaefaciens strain MP-63 isolated from a spoiled mango pulp preserved with sodium benzoate was used in this study. Another strain of *P. membranaefaciens* BB-6-0 isolated from grape juice was used for comparison.

The preservative resistance studies were carried out in Yeast Extract-Peptone-Dextrose (YEPD) broth consisting of yeast extract, 0.5 per cent; peptone, 1.0 per cent and glucose, 2.0 per cent at pH 3.5, 4.0 and 4.5. To 50 ml of sterile double strength medium in 250 ml Erlenmeyer flasks, the required volume of freshly prepared and sterile 1 per cent (w/v) stock solution of respective preservatives were added before inoculation. The flasks were inoculated with 1 per cent inoculum prepared by adjusting the optical density of 24 hr old culture to 0.3. After thorough mixing, 10 ml of the medium was distributed in sterile 15 × 150 mm test tubes and incubated at 30°C. After 7 days, growth was measured at 600 mm by making suitable dilutic

Mango juice used for growth studies consisted of one volume of freshly extracted mango pulp mixed with equal volume of water which was finally adjusted to 14° brix by the addition of cane sugar. The pH was adjusted to 3.5 and 4.0 by using citric acid and the final acidity in these samples were 0.22 and 0.48 per cent respectively. About 2.5 l sterilised mango juice was inoculated with 24 hr old culture to give an initial count of approximately 1×10^3 cells/ml in the juice. After thorough mixing, 250 ml of juice was asceptically transferred to 500 ml sterile Erlenmeyer flasks and calculated amount of sodium benzoate was added to these flasks to give varying concentrations. The flasks were then kept in an incubator maintained at 30± 1°C. Total plate counts at 0 hr and subsequently at intervals of 24 hr were enumerated by plating the properly diluted samples in acidified yeast extract-peptone-dextrose agar medium. The plates were incubated at 30°C and viable counts were noted after 6 days.

Results and Discussion

It is evident from Table 1 that *P. membranaefaciens* strain MP-63 did not grow in the presence of either

TABLE 1. EFFECT OF PRESERVATIVES ON GROWTH OF *P. MEMBRANAEFACIENS* STRAINS MP-63 AND BB-6-0 IN YEPD BROTH

Preservative level (ppm)	Growth* of *P. membranaefaciens* (MP-63) at indicated pH.			Growth* of *P. membranaefaciens* (BB-6-0) at indicated pH.		
	3.5	4.0	4.5	3.5	4.0	4.5
		Potassium metabisulphite				
0	0.155	0.187	0.201	0.123	0.139	0.151
250	0.198	0.165	0.141	–	0.129	0.119
500	–	0.141	0.131	–	–	0.104
750	–	–	0.133	–	–	0.079
1000	–	–	0.106	–	–	–
		Sodium benzoate				
0	0.155	0.187	0.201	0.123	0.139	0.151
250	0.213	0.298	0.303	–	0.076	0.111
500	0.138	0.227	0.237	–	–	0.069
750	–	0.112	0.203	–	–	–
1000	–	0.057	0.151	–	–	–
		Potassium sorbate				
0	0.155	0.187	0.201	0.123	0.139	0.151
250	0.147	0.191	0.227	–	–	–
500	–	0.158	0.186	–	–	–
750	–	–	0.182	–	–	–
1000	–	–	0.117	–	–	–

*Average O.D. values of 1:10 diluted triplicate samples at the end of 7 days incubation.
– No growth

500 ppm of potassium metabisulphite or potassium sorbate at pH 3.5 but showed growth at 500 ppm of sodium benzoate. In contrast, as little as 250 ppm of these preservatives completely arrested multiplication of strain BB-6-0. At pH 4.0, strain MP-63 showed growth even at 1000 ppm of sodium benzoate but did not grow above 500 ppm of either potassium metabisulphite or potassium sorbate. However, strain BB-6-0 showed growth only at a lower concentration of either potassium metabisulphite or sodium benzoate (250 ppm). When tested at pH 4.5, none of the preservatives inhibited growth of strain MP-63 at 1000 ppm. At this pH, growth of strain BB-6-0 was controlled by either 1000 ppm of potassium metabisulphite or 750 ppm of sodium benzoate. Potassium sorbate was more effective to strain BB-6-0 than other preservatives. The above results indicated that the strain MP-63 exhibited higher resistance to benzoate than strain BB-6-0.

Since the strain MP-63 showed growth at 1000 ppm of sodium benzoate at pH 4.0 and 4.5 as shown in the previous experiment, its tolerance to higher levels of benzoate was tested. The results revealed (Table 2) that the strain MP-63 can grow up to 1500 ppm at pH 4.0. Growth was not inhibited even at 3000 ppm, the highest concentration tested at pH 4.5. Therefore, it is concluded that benzoate resistance is specific only to the strain MP-63.

Since the strain MP-63 showed high resistance to sodium benzoate in laboratory media, its growth pattern in mango pulp, the natural substrate from which it was isolated was studied in the presence of sodium benzoate. The pH of one lot of mango juice was adjusted to 4.0 because of its nearness to pH of ripe mango fruits which ranges from 3.8 to 4.2. Another lot was adjusted to pH 3.5 in order to see whether the growth of this yeast could be controlled at lower pH with less quantities of sodium benzoate. Viable count data (Fig. 1) showed that at pH 4.0, total cell count was reduced when mango juice contained 1000 and 1500 ppm of sodium benzoate. The

TABLE 2. EFFECT OF SODIUM BENZOATE ON GROWTH OF *P. MEMBRANAEFACIENS* STRAIN MP-63 IN YEPD BROTH

Sodium benzoate (ppm)	Growth* at indicated pH		
	3.5	4.0	4.5
0	0.163	0.205	0.215
1000	–	0.062	0.157
1500	–	0.033	0.051
2000	–	–	0.049
2500	–	–	0.040
3000	–	–	0.041

*Average O.D. values of 1:10 diluted triplicate samples at the end of 7 days incubation.
– No growth

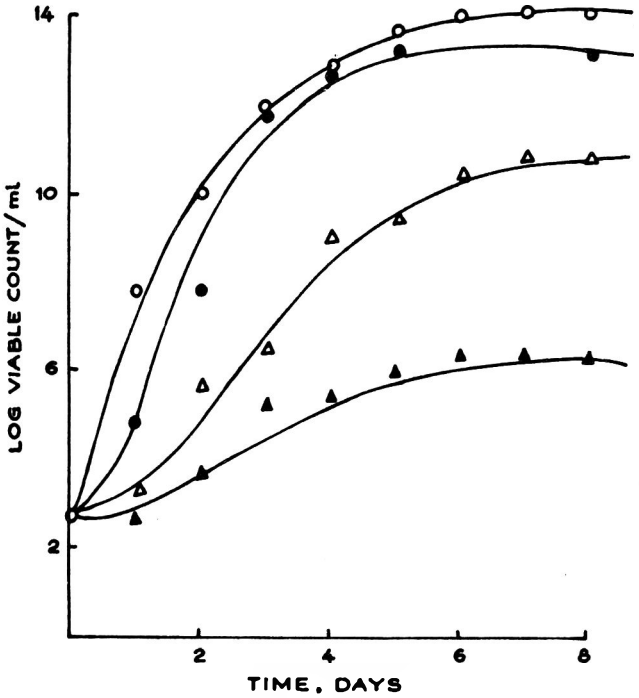

Fig 1. Effect of sodium benzoate on growth of *P. membranaefaciens* strain MP – 63 in mango juice of pH 3.5 at 30°C. Sodium benzoate, O ppm (o—o); 500 ppm (●—●) ppm (△—△); 1500 ppm (▲—▲)

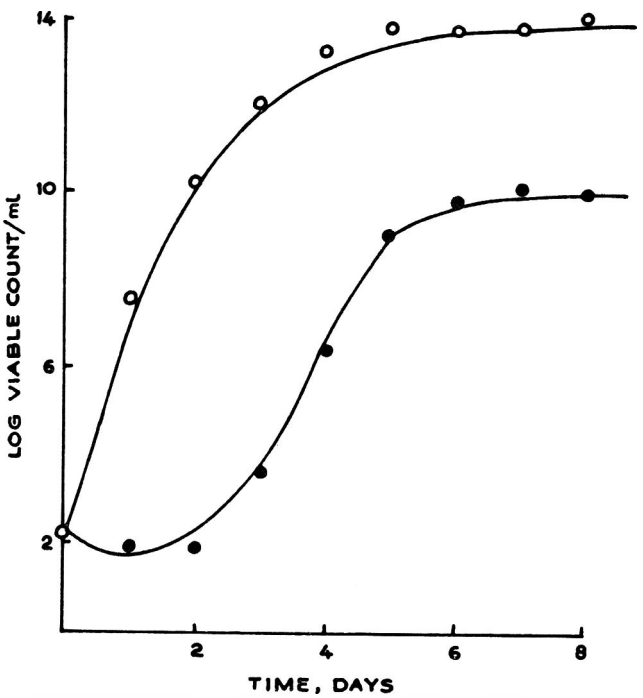

Fig 2. Effect of sodium benzoate on growth of *P. membranaefaciens* strain MP – 63 in mango juice of pH 4.0 at 30°C. Sodium benzoate, O ppm (o—o); 500 ppm (●—●); 1000 ppm (△—△); 1500 ppm (▲—▲)

maximum viable counts observed after 8 days growth in the presence of 0, 500, 1000 and 1500 ppm of sodium benzoate were 1.25×10^{14}, 1.16×10^{13}, 8.6×10^{10} and 2.6×10^{6} cells/ml respectively. Thus, a progressive decrease in cell count was observed as the concentration of sodium benzoate in mango juice increased from 0 to 1500 ppm. A delay of 24 hr for the initiation of cell multiplication was noticed with 1500 ppm of sodium benzoate, the highest concentration tested. At pH 3.5, growth was completely inhibited when 1000 ppm sodium benzoate was incorporated in mango juice. However, with 500 ppm, a lag period of 48 hr was observed (Fig. 2). The maximum viable counts at the end of 8 days growth period were 1.5×10^{14} and 1.26×10^{10} at 0 and 500 ppm of sodium benzoate respectively.

It is well known that the undissociated molecule of benzoic acid is responsible for antimicrobial property[5]. The benzoic acid in strongly acid solution is reported to be 100 times as efficient as in neutral solution. Hence, there is a relationship between pH and preservative action of benzoate. Most of the sensitive organisms can be controlled by benzoate at lower pH. However, some yeasts may develop resistance to benzoate. Warth[6] has shown that *Saccharomyces bailii* can adapt phenotypically to very high levels of benzoate and he attributed the development of such resistant character to homeostatis by which the yeasts develop a mechanism to excrete acids out of the cell. During this phenomenon, the organism utilises more and more energy to maintain the internal pH constant which finally results in reduction in cell growth. In addition to this, transport of metabolites is disrupted which also affects the cell growth. Probably the same mechanism might operate in *P. membranaefaciens* strain MP-63 resulting in high resistance to benzoate.

Although yeasts possessing such character are very rare, a strain of *S. bisporus* var. *bisporus* exhibiting high resistance to sorbic acid has been reported[7]. Similarly, a strain of *P. membranaefaciens* has been shown to possess resistance to benzoic acid up to 700 ppm[5]. Such benzoate resistant yeasts may be introduced into fruit products which mainly depend upon benzoate for their preservation. This study suggested that such spoilage could be controlled with reduced levels of preservatives at low pH.

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A Modified Process for Low Cost Palm Oil Extraction

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Three processing factors were related to the oil extraction efficiency and quality of palm oil in a 2^3 factorial experiment. The three factors were (i) short delays in processing harvested palm fruit bunches, (ii) fermentation of the fruit, and (iii) chopping of the palm fruit bunches removing spikelets for processing. Processing of the spikelets without delay or fermentation yielded the highest oil extraction efficiency (about 87 per cent) and the best quality oil with a free fatty acid content of 2.31 per cent and the lowest carotenoids level.

The oil palm fruit bunch has a central woody stalk to which clusters of spikelets bearing the fruits are attached. The palm fruit is a drupe with a fleshy outer layer called the mesocarp and a hard core called the nut. The nut comprises the endocarp (shell) which encloses the endosperm (kernel). Two types of oil are obtainable from the palm fruit, mesocarp oil (palm oil) and endocarp oil (palm kernel oil). Palm oil is an important agricultural product used for soup and table fats such as margarine in many countries. It is also used in the manufacture of soap and cosmetics.

The extraction of palm oil from freshly harvested palm fruit bunch involves five operations which are fruit loosening, sterilization, digestion, oil expression and clarification. These operations are carried out in both small scale and big industrial mills, the difference being in the sequence and equipment used. Fig. 1 shows the operational flow diagram for both types of mills. The process used in the small scale mills is time consuming, labour intensive and tedious in nature and although short delays and fermentation improve fruit recovery (loosening) from the bunch, they lead to production of poor quality oil[1]. The extraction process used in the big industrial mills requires equipment that is complicated and too expensive for small scale operations and palm oil extraction efficiencies of less than 80 per cent are still observed in many big industrial mills in Nigeria. The poor extraction efficiency has been attributed to unstrippable fruits in the residual fruit stalk[2].

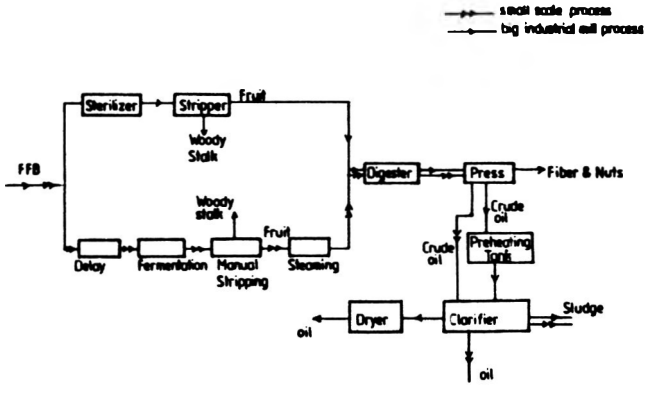

Fig. 1. Flow sheet for palm oil extraction.

The quality criteria for commercial transaction in palm oil are usually free fatty acids (FFA) and oxidation levels[3] and the quality requirements of different grades of palm oil are shown in Table 1. There is a need for the development of simple, low cost but efficient technology for producing good quality palm oil. The research reported in this paper was designed to investigate the effects of: (i) short delays in processing harvested palm fruit bunches, (ii) fermentation of palm fruit and (iii) chopping of the harvested palm fruit bunches removing spikelets for processing on oil extraction efficiency and the quality of palm oil produced.

Materials and Methods

Materials: The palm oil extraction machines used were:

1. A short hand axe for chopping the spikelets (spikes with fruits) from the bunch.
2. A sterilizer (steam cooker) made trom a 200 l drum into which was built a perforated elliptical plate resting on a stand at 30° slope. An opening was cut in the side wall and a discharge gate was inserted sloping 30° downwards. The sterilizer was fired with wood from beneath and palm fruits were processed in batches at atmospheric pressure.
3. A horizontal digester made by the Nigerian Institute for Oil Palm Research, Benin City

TABLE 1. QUALITY REQUIREMENTS OF PALM OIL

Quality parameters	Edible virgin oil	Standard quality oil	Special quality oil
FFA (as palmitic acid) (%)	<4.6	3.0 – 5.0	1.0 – 2.5
Total carotenoids (as β-carotene), (ppm)	500 – 2000	500 – 1000	<500

was operated with a 5.6 kW electric motor driven by a pulley system. The digester has beater arms arranged spirally along its shaft. This was used for mashing of the sterilized fruits and spikelets to facilitate extraction of crude oil by the press.

4. A hand hydraulic ram press designed for operation at a maximum pressure of 50 bar and was used for the expression of crude oil.
5. A batch type palm oil clarifier designed for clarification which was achieved by flotation during static settling of the palm oil. The clarifier consisted of a cylindrical drum and a conical bottom through which all contents were discharged.

The bunches and other components were weighed on a scale with a maximum capacity of 136 kg and an accuracy of 0.057 kg. A Mettler PC 200 balance was used to weigh out 5 g of palm oil to 0.01 g accuracy for titration. A SP8-400 Scanning PYE UNICAM UV/VIS spectrophotometer was used for the measurement of absorbance of carotenoids in palm oil at 0.5% concentration in hexane.

Methods: A 2^3 factorial experiment was designed to investigate the effect of bunch form (chopped and unchopped), delay time and fermentation time on the extraction efficiency and the quality of palm oil produced. Levels of the three factors were as shown in Table 2. The order of the experiments was randomized.

Forty whole fresh bunches of palm fruits collected from the crosses of *dura x tenera* and *tenera x tenera* oil palm fields of the Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Nigeria were used in this study. The bunches were divided and assigned lot numbers 1 to 8 for processing by combination of the three factors (Table 2).

TABLE 2. LEVELS AND FACTORS IN THE 2^3 FACTORIAL DESIGN

Levels	Bunch form (B)	Fermentation time (F) (days)	Delay time (D) (days)
Low	Chopped bunch (–)	0 (–)	0 (–)
High	Full (unchopped) bunch (+)	3 (+)	1 (+)

The *dura x tenera* fruits had an oil content of about 20.1 per cent, while the *tenera x tenera* fruits had an oil content of about 20.7 per cent by weight of the fresh fruit bunch[5]. The oil content is the potential yield of the bunch.

At the beginning of the experiment, spikes and fruits from four of the lots were chopped while the fruit bunches in the other four lots were cut into four parts. The cutting allowed for easy loading into the sterilizer and facilitated steam penetration. The samples to be fermented were heaped together and sprinkled with water while those subject to delay were spread out on the floor of the laboratory to avoid heat generation and allowed for ventilation. For runs with both fermentation and delay, the delay came before the fermentation.

Apart from the different treatments indicated in the experimental design, all eight lots were subsequently subjected to the same set of processing conditions which were, 90 min of sterilisation in a steam cooker; 20 min in the digester; oil expression using the hydraulic press, 15 min for crude oil cooking; and 60 min of static settling in the clarifier. The following sets of data were collected for each lot.

Per cent loose fruits was determined by weighing the loose fruits from each lot before processing. Per cent clean nut in press cake (nuts with no adhering tissues) was obtained by manual separation of the fibre and nut in the press cake and weighing separately. Absorbance of carotenoids in palm oil at 446 and 470 nm was determined in the spectrophotometer. Free fatty acid per cent (FFA%) was estimated by titrimetry according to the American Oil Chemists method[6]. Per cent oil recovered from the fresh fruit bunch was obtained by weighing the oil recovered from the bunch. Measurements were repeated three times and the means used for analysis.

Results and Discussion

The results obtained from the experiment are presented in Table 3. The analysis of the data was carried out using Yate's algorithm. The significances of main effects of factors and interactions were tested by the F-tests[7]. The three factor interaction in the absence of an independent estimate of error, was first used to test the significance of the two factor interactions. The non significant components of the two factor interactions at 10 per cent level were

TABLE 3. RESULTS OF THE 2^3 FACTORIAL EXPERIMENT

Experiment number	Factors			Loose Fruits (%)	Clean nuts, (%)	Absorbance at 446 nm	Absorbance at 470 nm	Oil recovered (%)	FFA, (%)
	B	F	D						
1	–	–	–	0.0	32.0	0.78	0.65	17.7	2.31
2	+	–	–	0.0	25.9	0.78	0.67	4.3	3.07
3	–	+	–	12.9	13.8	1.31	1.14	15.2	4.60
4	+	+	–	32.8	17.9	0.98	0.86	10.9	2.33
5	–	–	+	8.5	31.2	0.67	0.58	17.1	3.10
6	+	–	+	6.2	25.0	0.99	0.86	5.7	3.22
7	–	+	+	34.3	30.0	1.09	0.94	12.4	5.23
8	+	+	+	40.9	20.4	1.17	1.01	15.5	4.84

merged with the three factor interaction for getting an estimate of valid error to test main effects.

Fruit Loosening: The analysis presented in Table 4 show that delay for 1 day and fermentation for 3 days each independently assist in fruit loosening. This is believed to be due to the continued natural growth of the abscison layer at the point of fruit attachment to the stalk. Values of per cent fruit loosened obtained were, however, low (Table 3) when compared to a published figure of 55 per cent[5]. This may be because normally palm fruits are fermented for 7 to 14 days, whereas in this study they were fermented only for 3 days. A shorter fermentation period was used to observe if improved oil extraction efficiency attributed to fermentation could be obtained without significant reduction in oil quality since prolonged fermentation exposed the fruit oil to fungal infection.

Production of clean nuts: The production of clean nuts from the digester is both desirable and an indication of efficient digestion. Clean nuts free of cushioning caused by adhering mesocarp tissue are desirable during nut cracking in kernel recovery. The analysis presented in Table 5 indicate that none of the tactors considered had a significant effect at 5 per cent level on the production of clean nuts.

Oil recovery: The analysis presented in Table 6 indicate a significant interaction of fermentation and

TABLE 4. ANALYSIS OF EFFECTS OF PROCESSING FACTORS ON FRUIT LOOSENING

Source of variation	Effect, (%)	Sum of squares	D.F.	Mean squares
Average	63.29	–	–	–
Main effects				
Bunch form (B)	3.79	28.65	1	28.65
Fermentation (F)	24.30	1180.49	1	1180.49**
Delay (D)	11.30	353.51	1	353.51**
Interactions				
BF	4.96	49.50+	1	49.50
BD	–1.69	5.68	1	70.69
FD	–5.95	70.69+		
BFD	–0.50	0.49	2	3.09

+ interaction not included in error estimation,
** significant at 1% level.

TABLE 5. ANALYSIS OF EFFECTS OF PROCESSING FACTORS ON PER CENT CLEAN NUTS

Source of variation	Effect, (%)	Sum of squares	D.F.	Mean squares
Average	24.54	–	–	–
Main effects				
Bunch form (B)	4.46	39.74	1	39.74
Fermentation (F)	7.99	127.76	1	127.76
Delay (D)	4.27	36.42	1	36.42
Interactions				
BF	1.72	5.90		
BD	–3.44	23.70	4	26.04
FD	5.09	51.87		
BFD	–3.37	22.68		

TABLE 6. ANALYSIS OF EFFECTS OF PROCESSING FACTORS ON OIL RECOVERY

Source of variation	Effect, (%)	Sum of squares	D.F.	Mean squares
Average	12.37	–	–	–
Main effects				
Bunch form (B)	–6.50	84.57	1	84.57*
Fermentation (F)	2.33	10.88	1	10.88
Delay (D)	0.67	0.89	1	0.89
Interactions				
BF	5.88	69.09+	1	69.09
BD	2.38	11.35	3	5.03
FD	0.25	0.12		
BFD	1.34	3.61		

+interaction not included in error estimation,
*significant at 5% level.

TABLE 7. ANALYSIS OF EFFECTS OF PROCESSING FACTORS ON ABSORBANCE AT 446 nm.

Source of variation	Effect, (%)	Sum of squares	D.F.	Mean squares
Average	0.972	–	–	–
Main effects				
Bunch form (B)	0.016	5.28×10^{-4}	1	5.28×10^{-4}
Fermentation (F)	0.331	0.219	1	0.219*
Delay (D)	0.019	7.03×10^{-4}	1	7.03×10^{-4}
Interactions				
BF	–0.141	0.040		
BD	0.181	0.066+	1	0.066
FD	0.034	2.28×10^{-3}		
BFD	0.024	1.13×10^{-3}	3	0.0145

+ interaction not included in error estimation
* significant at 5% level

TABLE 8. ANALYSIS OF EFFECTS OF PROCESSING FACTORS ON FREE FATTY ACID LEVEL

Source of variation	Effect, (%)	Sum of squares	D.F.	Mean squares
Average	3.58	–	–	–
Main effects				
Bunch form (B)	–0.47	0.44	1	0.44
Fermentation (F)	1.25	3.13	1	3.13
Delay (D)	1.00	1.98	1	1.98
Interactions				
BF	–0.96	1.84		
BD	0.29	0.16	4	0.77
FD	0.48	0.45		
BFD	0.55	0.62		

bunch form and further analysis of the interaction revealed that:

1. fermentation decreased oil recovery from the chopped bunch by about 3.5 per cent while it increased that from the full bunch by about 8.2 percent at the levels investigated:
2. processing of the full bunch rather than the chopped bunch reduced the oil recovery from the unfermented fruit by about 12.3 per cent and that of the fermented fruit by 0.63 per cent.

Differences in the effects of fermentation on oil recovery from the two bunch forms may be because for the full bunch, only the fruits that are loosened are put in the digester and since fermentation increases fruit loosening it makes more fruits available for digestion. For the chopped bunch, all the fruits passing through the sterilizer are utilized in the digester; thus fruit loosening does not affect the quantity of fruits available for digestion. The reduction in oil recovered from the chopped bunch after fermentation, indicates that fermentation reduces the quantity of extractable oil in the fruits.

The highest value of per cent oil recovered by weight of the fresh fruit bunch (17.7) was obtained when spikelets were processed without fermentation. This gives an oil extraction efficiency of about 87 per cent when compared with the potential oil yield of about 20.4 per cent by weight of the fresh fruit bunch of the parent population.

Palm oil quality: Absorbance at 446 nm was always higher than that at 470 nm (Table 3), with the average ratio of absorbance at 446 nm to absorbance at 470 nm being 1.59 with a standard deviation of 0.0188. The effects of the factors on absorbance at both wavelengths were identical. Analysis of absorbance at 446 nm (Table 7) indicates that the level of carotenoids in the palm oil significantly increased after fermentation. Large concentrations of carotenoids impair bleachability of palm oil and products of carotenoid degradation cause rancidity. The results indicate that fermentation tends to lower the quality of the oil and processing of spikelets without fermentation gives low levels of oxidation. None of the factors investigated had a significant effect on free fatty acid level of palm oil produced (Table 8). Spikelets processed without delay and fermentation resulted in palm oil with a free fatty acid level (2.31 per cent corresponding to the special quality grade) (Table 1).

It is concluded that processing of spikelets from freshly harvested oil palm fruit bunch without delay or fermentation yields the highest oil extraction efficiency and the best quality oil.

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Instrumental Measures of Texture of Tomato Fruit and Their Correlation with Protopectin Content: Effect of Fruit Size and Stage of Ripeness

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Six force-deformation based characteristics of texture viz: deformation, epicarp strength, locular resistance, compliance and firmness were determined and correlated with protopectin content in tomato. Fruit size affected deformation, epicarp strength and toughness values but had no significant effect on locular resistance, compliance and firmness. Toughness was insensitive to ripening. Although all six force-deformation characteristics were significantly correlated with protopectin, locular resistance compliance and firmness showed the best correlation coefficients (r = > 0.80). Locular resistance, a measure of internal firmness, appears a good parameter for quick determination of texture in the tomato.

Texture of the tomato fruit is commonly measured in terms of firmness irrespective of methodologies[1-3]. Subjectively, firmness of the tomato is sensed by squeezing the fruit in the hand, thus utilizing the principle of deformation. Softening or loss of firmness during ripening is the result of the breakdown of the cell wall. Major components of the cell wall implicated in fruit ripening include pectic substances[4], cellulose[5] and polysaccharides[6]. However, the only cell wall change that is clearly related to tomato fruit ripening is the conversion of insoluble protopectin to soluble pectin[7]. Therefore, any attribute supposed to measure a textural change must be well correlated with protopectin solubilization. It is also appropriate that such an attribute must not be affected by fruit size. The present study was carried out to compare six textural characteristics obtained through puncture tests and also to correlate these with protopectin content in order to find the best simple characteristic that measures ripening in the tomato fruit.

Materials and Methods

Tomato fruits (*Lycopersicon esculentum* Mill cv. Vendor) at various stages of ripening were harvested and sorted into four ripeness classes: mature-green, turning, pink and firmripe corresponding to 0, 1, 3 and 5 ripeness scores respectively. Except in the trial which dealt with the effect of fruit size, only medium sized fruits of 100 ± 5 g were used for texture measurement.

1 mm diameter, 3 cm long probe was screwed on to a chuck. The chuck, in turn, was attached to a Model 1122 Instron Universal Testing Machine. Puncture was caused at a crosshead speed of 20 mm/min and a chart speed of 100 mm/min. Each fruit was punched twice on opposite sides of the apical scar and away from the locular ridge. Twenty-five fruits were similarly punched per treatment. From the force-deformation curves so obtained, six characteristics were calculated as follows: Deformation, the distance (mm) of probe travel from first contact with the tomato fruit surface to the bioyield point (Fig. 1); Epicarp strength, the force (N) at bioyield; Locular resistance, the residual force following bioyield; Firmness, the maximum slope of the force deformation curve[8]; Compliance, the deformation per unit epicarp strength. Toughness, the total energy consumed during puncture equals the area under the force-deformation curve.

Following puncture tests, a 25g sample was taken from the blossom end of each fruit and analysed for protopectin by the methods of Kanujoso and Luh[9] and McComb and McCready[10]. All data were subjected to analysis of variance. Also simple correlation between variables was carried out[11].

Results and Discussion

Fruit size affected deformation, epicarp strength and toughness but had no significant effect on locular resistance, compliance and firmness (Table 1). Brinton and Bourne[12] found that an increase in the diameter

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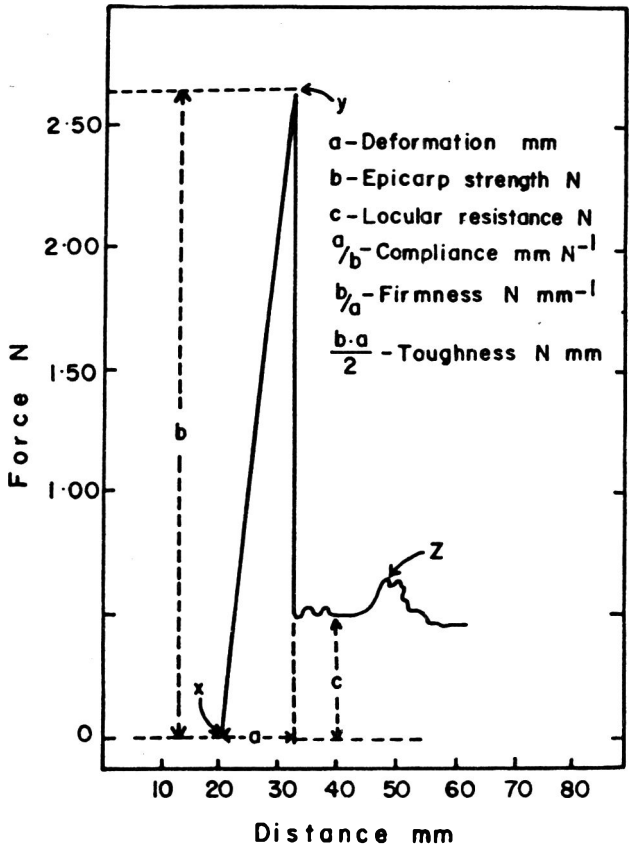

Fig. 1. Typical force-deformation curve obtained during puncture of individual tomato fruit. x = point of puncture probe contact with fruit surface; y = bioyield point; z = point of probe contact with fruit placenta.

of agar gel spheres can cause a change in deformation. Our results showed that a 75 per cent increase in fruit size caused a 50 per cent reduction in deformation. Small fruits had larger deformation, epicarp strength and toughness values than large fruits probably due to their greater epicarp elasticity. Since cell enlargement predominates the post fruit-set phase of fruit development, it appears that the pericarp of larger fruits had been stretched close to their limit during development and hence yielded more readily to puncture during tests.

According to the general expectation of changes occurring during ripening, epicarp strength, locular resistance and firmness decreased while deformation and compliance increased as ripening progressed (Table 2). However, compared with other characteristics, a more drastic drop was obtained for locular resistance between the turning and pink stages of ripening. Similar results were obtained by Holt[13]. Such drastic drop in locular resistance indicates that the ripening process progresses more rapidly within the tomato fruit than its external manifestation. Toughness was not affected by ripening. Insoluble protopectin content decreased with increasing ripeness.

All six force-deformation characteristics calculated had significant correlation (P = 1%) with protopectin content. Epicarp strength, locular resistance compliance and firmness correlatd with protopectin to the extent of r > 0.78 (Table 2). These values are higher than those (r <0.71) obtained by Deshpande *et al*[14] and Sayed *et al.*[15] The highest correlation (r=−.88) was between compliance and protopectin. Firmness was perfectly correlated with compliance. The high correlation coefficient of compliance with epicarp strength (r = −.91) indicates that among fruits of uniform size, epicarp strength is a goood alternative parameter especially where quick tests, requiring no calculation, are preferred. In this connection, we note that "firmness" determinations of many workers[2,16] were, in fact, epicarp strength values. However, when fruits of varying sizes are to be used, locular resistance is a more reliable parameter than epicarp strength for a quick test of texture in the tomato. Also, the fact that locular resistance is highly correlated with both protopectin and compliance indicates that, with respect to texture, the overall rates of external and internal manifestations of ripening are uniform in the tomato.

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TABLE 1. EFFECT OF FRUIT SIZE ON THE FORCE-DEFORMATION CHARACTERISTICS OF FIRM-RIPE TOMATO FRUIT

Fruit size	Fruit Wt[z] (g)	Deformation (mm)	Epicarp strength (N)	Locular resistance (N)	Compliance (mm N^{-1})	Firmness (N mm^{-1})	Toughness (N mm)
Small	85.10 ± 5	22.50[b] ± 2.10	2.91[b] ± 0.42	0.13[a] ± 0.011	7.73[a] ± 2.01	0.13[a] ± 0.021	32.74[b] ± 5.98
Large	138.95 ± 5	11.84[a] ± 1.30	1.58[a] ± 0.19	0.13[a] ± 0.009	7.47[a] ± 1.75	0.13[a] ± 0.018	9.32[a] ± 2.11

z Average of 25 fruits

Values in the same column with same superscript are not significantlv different according to Duncan's multiple range test (P =0.05)

TABLE 2. FORCE-DEFORMATION CHARACTERISTICS AND PROTOPECTIN CONTENT OF TOMATO FRUITS AT DIFFERENT STAGES OF RIPENESS AND THEIR CORRELATION

Factor	Deformation (mm)	Epicarp strength (N)	Locular resistance (N)	Compliance (mm N^{-1})	Firmness (N mm^{-1})	Toughness (N mm)	Protopectin (mg % AUA)*
Ripeness							
Mature-green	12.03^{a}	2.65^{c}	0.50^{d}	4.53^{a}	0.22^{c}	15.84^{a}	20.5^{c}
Turning	12.67^{a}	2.72^{c}	0.40^{c}	4.66^{a}	0.21^{c}	16.92^{a}	20.0^{c}
Pink	14.91^{b}	2.04^{b}	0.13^{b}	7.29^{b}	0.14^{b}	15.17^{a}	18.3^{b}
Firm-ripe	18.16^{c}	1.71^{a}	0.08^{a}	10.55^{c}	0.095^{a}	15.41^{a}	10.1^{a}
Correlation Coefficient (R)							
Protopectin	$-.61^{**}$	$.79^{**}$	$.83^{*}$	$-.88^{**}$	$.86^{**}$	$.53^{**}$	–
Compliance	$.72^{**}$	$-.91^{**}$	$-.84^{**}$	–	-0.99^{**}	$-.59^{**}$	$-.88^{**}$

* AUA = Anhydrouronic acid

** Significant at P = 0.01

Figures in the column with different superscripts are significantly different ($P \leq 0.01$) according to Duncan's multiple range test.

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Extension of the Storage Life of Fungicidal Waxol Dip Treated Apples and Oranges under Evaporative Cooling Storage Conditions

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Apples (cv. 'Royal Delicious') dip treated in wax emulsion containing 6% solids and a fungicide (Bavistin on a.i. basis 1000 ppm) and the untreated apples stored for 42 days under ambient conditions (21-28°C, RH 30-80%) showed 6.3 ± 0.5% and 9.3 ± 1.7% physiological losses in weight (PLW) and 36.0% and 59.4% shrivelling, respectively. The fungal spoilage for 42 days storage was 8.3% in untreated and 2.7% in treated apples. Apples, treated and untreated as above, when stored for 42 days under evaporative cooling storage conditions (22-24°C, RH 90 ± 5%) showed 2.9% and 3.4% PLW respectively. No shrivelling or spoilage occurred in both treated or untreated lots stored for 6 weeks under evaporative cooling conditions. Similar study carried out with coorg mandarins treated with 6% waxol with a fungicide (Bavistin on a.i. basis 1000 ppm) gave storage life of 20 days under evaporative cooling storage conditions as against a storage life of 5 days for untreated fruits stored at ambient conditions. Thus, evaporative cooling storage gave 6 times longer storage life for apples and 4 times longer storage life for mandarins than at ambient conditions.

The loss in the marketable quality of apples and oranges due to shrivelling, occurring as a result of transpirational loss of water during their post harvest handling and storage, under tropical ambient conditions is a major factor for the economic losses faced by producers and tradesmen. Application of fungicidal wax emulsion of appropriate strength has been shown to extend the storage life by 100 per cent[1]. In recent years, lot of interest has been shown in the development of evaporative cooled storage systems for fruits and vegetables in different institutions in the country[2,5]. Model structures of evaporative cooling (EC) storage systems have been developed in this Institute and their efficiency and the storage behaviour of potatoes were assessed[6]. The systems developed at this Institute enable to maintain inside the EC chamber a temperature one to two degrees centigrade higher than the ambient wet bulb temperature with a relative humidity of 90 ± 5 per cent. The present study was undertaken to assess the storage behaviour of apples and oranges dip treated in fungicidal wax emulsion.

Materials and Methods

Apples, cv. 'Royal Delicious' from Gopalpur orchard, Himachal Pradesh, arriving by truck to Mysore within a week after harvest, were obtained in the month of September. These were sorted and divided into 4 lots of 36 fruits each of which, 2 lots were treated with a fungicidal wax emulsion (6 per cent total solids and containing a fungicide Bavistin at 1000 ppm on a.i. basis) and the other two lots were kept as such and served as controls. Out of these 4 lots, one wax treated lot and one of control were stored at ambient temperature (21-28°C, RH 30-80%) per cent in a ventilated wooden crate in a wire mesh almirah. The other two lots (one waxed and the other unwaxed control) were stored in a evaporative cooled chamber, oranges were also treated similarly.

Description of evaporative cooling chamber: A metallic EC chamber measuring 45 × 45 × 45 cm (0.1 m^3 approx.) was fabricated with a 2 mm thick galvanised iron sheet with the top side open. A metallic tray of 46 × 46 cm with 4 conduit pipes of 3,6,9 and 12 inches length protruding to the inside of the tray were welded on the 4 corners of the tray, to serve as ventilation holes when this tray was used as lid. The four sides of this metallic chamber were covered with a cloth and the top ends of the cloth were immersed in water placed in the top tray. The whole chamber was placed in a metallic tray of 50 × 75 cm size containing water (bottom reservoir for the collection of excess water flowing down). The bottom ends of the cloth covering the side walls of the EC chamber were allowed to dip in the water placed in the bottom tray. By the downward gravitational flow of water, the cloth surrounding the metallic chamber is made to remain wet to allow evaporation continuously (Fig. 1). A wire mesh basket of size 30 × 30 × 30 cm (vol. 27 1) filled with fruits was kept inside this chamber, leaving adequate space all round the basket for the circulation

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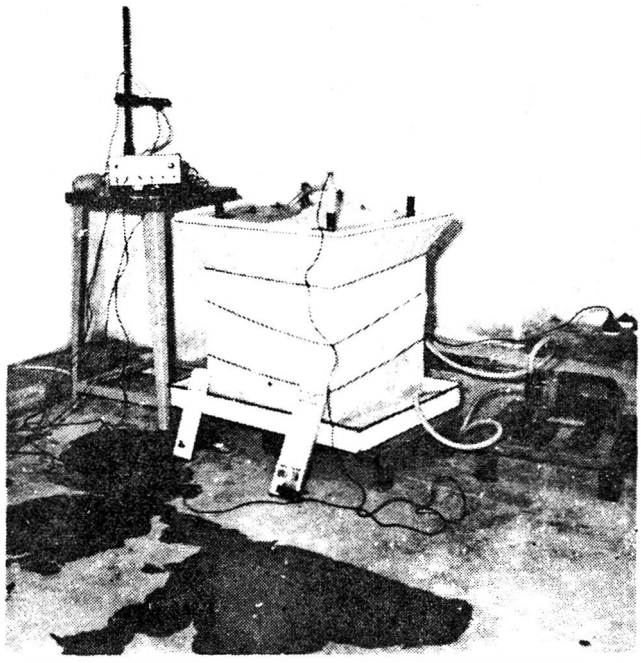

Fig. 1. Evaporative cool chamber – square metallic container

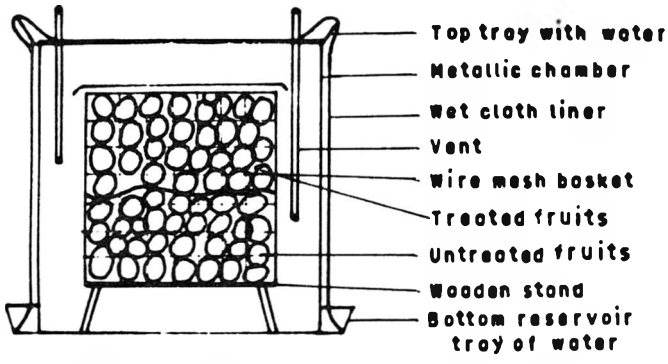

Fig. 2. Diagramatic representation of the metallic chamber used for evaporative cooling system.

of air (Fig. 2). The water collected in the bottom tray was put back periodically into the top one together with water lost due to evaporation, for recirculation, as described by Rama.[6]

Observations: The PLW was recorded at intervals for which 10 fruits per each treatment were weighed initially, earmarked and distributed along with the unmarked fruits. The loss in weight in these marked fruits was recorded at intervals and was reported as cumulative per cent loss. The per cent shrivelled fruits were recorded by visually noting the symptoms of shrivelling, the extent of which is judged by the market acceptability. Fungal wastage was recorded and expressed as per cent. Two EC chambers were used at full capacity; the number of replicates used for measuring shrivelling and microbial spoilage is based on 36 fruits.

Sensory evaluation: At the end of 6 weeks, the sensory quality of the fruits were evaluated. hedonically on a 5-point scale (maximum for best quality attribute and a value of 2 is considered fair) by a panel of 10 judges. Twelve fruits were kept for visual appearance comparison over the rest being used cut into six pieces, longitudinally giving one piece for each panelist. At least 3 pieces of each treatment coming from 3 different apples were given to each panelist.

Results and Discussion

The data on the PLW of apples during storage presented in Table 1 show that waxing reduced the PLW under both ambient and EC storage conditions; however, the differences in PLW between waxed and unwaxed apples were much less under EC storage than at ambient condition. At the end of 6 weeks under evaporative cool storage conditions, whether waxed or not apples had shown marked reduction in PLW, nearly 1/3 of those stored at ambient conditions. Under ambient conditions of storage for 6 weeks, waxol treated apples showed less number of shrivelled and spoiled fruits. On the other hand, under evaporative cooling storage conditions, there were no shrivelled apples irrespective of whether they were waxol treated or not. Further, there was no fungal spoilage also.

Data on the sensory quality of apples given in Table 2 show that control apples stored for six weeks at ambient conditions showed marked deterioration in all

TABLE 1. CUMULATIVE PERCENTAGE P.L.W. AND SHRIVELLING IN APPLES STORED UNDER EVAPORATIVE COOLING SYSTEM

Treatments	% P.L.W.			% spoilage		% shrivelling*	
	1 wk	4 wk	6 wk	4 wk	6 wk	4 wk	6 wk
Control (R.T.)	2.20	7.48	9.3 ± 0.53	2.8	8.3	41.6	59.4
Waxed (R.T.)	1.60	5.28	6.3 ± 0.47	0	2.7	19.4	36.1
Control (E.C.)	0.44	–	3.4 ± 0.12	0	0	0	0
Waxed (E.C.)	0.55	–	2.9 ± 0.19	0	0	0	0

R.T: room temperature, E.C.: Evaporative cooling
*No shrivelling at one week
Mean ± SEM

TABLE 2. SENSORY QUALITY OF APPLES STORED FOR SIX WEEKS UNDER EVAPORATIVE COOLING

Treatment	Appearance (shrivelling)	Colour	Texture	Taste	Aroma
Control (RT)	2.3 ± 0.21	3.5 ± 0.22	2.5 ± 0.22	2.3 ± 0.21	2.5 ± 0.22
Waxed (RT)	2.8 ± 0.17	3.5 ± 0.22	3.5 ± 0.34	2.8 ± 0.40	2.8 ± 0.36
Control (EC)	4.8 ± 0.20	4.2 ± 0.21	3.2 ± 0.31	3.3 ± 0.31	3.3 ± 0.42
Waxed (EC)	4.5 ± 0.20	4.0 ± 0.26	3.3 ± 0.33	2.7 ± 0.33	3.0 ± 0.26

Values are means ± SEM

TABLE 3. CUMULATIVE PERCENTAGE PHYSIOLOGICAL LOSSES IN WEIGHT AND PERCENTAGE WASTAGE IN ORANGES STORED UNDER EVAPORATIVE COOLING SYSTEM

Treatment	Storage condition	% P.L.W. at indicated storage period (days)		% Wastage after 20 days storage		
		12	20	Shrivelling	Fungal	Total
Control	R.T.	17.5 ± 1.09	**	25	29	54
Waxed	R.T.	9.8 ± 0.11	**	10	10	20
Control	E.C.	6.4 ± 0.29	9.5 ± 0.39	14	14	28
Waxed	E.C.	2.2 ± 0.11	3.4 ± 0.34	1	1	2

Values are means ± SEM; ** Heavily infected and hence discarded.

the quality attributes. While waxed apples stored at ambient conditions retained quality equal to that of EC stored apples with regard to texture, taste and aroma they were definitely inferior to the latter in appearance and colour. Therefore, considering the overall quality, the EC stored apples were superior to those stored at ambient conditions whether waxed or not. Among the apples stored under EC condition although waxed apples were at par for colour, texture and appearance, appear to be inferior to the control apples. It is not surprising since apples being climacteric type of fruits, lowering of temperature and also restricting the oxygen supply caused by waxol dip treatment might have subdued the climacteric peak, restricting the development of taste and aroma comparable to that of controls.

Data on storage behaviour of oranges, preserved in Table 3, show results similar to that observed for apples (Table 1) for PLW, shrivelling and fungal spoilage. Fungicidal waxol dip treated oranges, stored under EC storage conditions gave the best results, followed by control oranges stored under EC storage conditions.

Thus, the results of these studies clearly indicate immense possibilities for the extension of the storage life of waxol dip treated oranges and apples by storing them under evaporative cooled storage conditions.

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Interactions Among Meat, Fillers and Extenders in an Emulsion System

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The possibilities of interactions upon combining different meat systems with various types of fillers like hard wheat flour, corn starch and skim milk powder and extenders such as mustard flour, Promax 70, acid washed soy concentrate and sodium caseinate in emulsion systems were explored. Emulsifying capacity (EC), emulsion stability (ES) and water holding capacity (WHC) were selected as the functionality tests. The interaction among extenders and meat systems was found to be significant for EC and ES. Extenders also affected the ES. The weak meat systems resulted in the lowest WHC values. The extenders and fillers acted antagonistically in combination with the strong meat system while the effect with the other two meat systems was synergistic.

A major concern in the manufactures of emulsion type meat products has been to balance the quality and quantity of protein with processing functionality, nutritional value and cost. Lauck[1], Comer[2] and Comer and Dempster[3] studied various non-meat ingredients and components of meat blocks individually and in combination with each other. The functionality tests were made on individual components but performance was predicted or assessed on the product formed. In emulsified comminuted sausage type products, performance of non-meat ingredients was assessed in combination with proteins. However, the interactions between the non-meat ingredients and meats were not investigated. Porteous and Quinn[4] explored the possiblity of interactions on mixing extender proteins (Promine D, Torutein and spray dried skim milk) and meat in the functionality test measurements. Hard wheat flour, corn starch, sodium caseinate, mustard flour and their interactions were not investigated. Model commercial meat systems consisting of various meat protein sources and commonly used binder units have not been studied extensively.

The objective of this study was to evaluate the individual functional properties and to explore interactions among the three different meat systems and each of the extenders and fillers in various combinations.

Materials and Methods

The lean beef chuck, lean pork trim, pork hearts and pork back fat were obtained from the Department of Animal and Poultry Science. The beef tripe and mechanically deboned chicken were procured from commercial establishments. The lean beef chuck, lean pork trim, beef tripe and pork hearts were ground twice through a 4.8 mm plate and the pork back fat was ground once through a 12.7 mm plate. The ground materials were analyzed for proximate composition, weighed, vacuum packaged and stored frozen at –20°C for approximately one month before use.

Three different meat blocks were formulated using the various ingredients and classified as strong, medium and weak meat systems (Table 1) following the systems of Comer and Dempster[3] with slight

TABLE 1. COMPOSITION OF FORMULATIONS

Ingredients	% in meat block		
	Strong	Medium	Weak
Meat block			
Lean Beef chuck	50	0	0
Pork trimmings	20	45	0
Pork hearts	0	15	15
Mechanically deboned chicken	0	0	30
Beef tripe	0	15	30
Pork back fat	30	25	25
Total meat block	100	100	100
Other ingredients	*(% of meat block)*		
Seasoning	3.6	3.6	3.6
Filler*	3.0	3.0	3.0
Extender*	3.5	3.5	3.5
Abbad Water	45.9	45.9	45.9
Toral fresh homogenate (g/100 g meat block)	156	156	156

* Non-fat dry weight basis.

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TABLE 2. COMPOSITION AND FUNCTIONAL PROPERTIES OF EXTENDERS AND FILLERS

Ingredients	Moisture (%)	Crude (%)	Total N (%)	N solubility index	% Cold absorption	% Hot absorption	Water holding capacity (g/g)	Emulsifying capacity (l/g total N)	Dextrose equivalent (g/100g)
Mustard flour	3.44	30.22	5.12	40.24	145.0	260.0	3.40	5.10	13.19
Promax 70	5.65	0.89	11.05	27.57	482.5	485.0	4.30	1.65	12.73
Sodium caseinate	6.80	0.26	14.26	97.05	–	–	–	19.50	–
Corn starch	9.51	0.00	0.11	0.00	72.5	90.0	4.10	–	92.78
Hard wheat flour	11.56	1.36	2.32	20.68	90.0	335.0	4.23	2.10	76.49
Skim milk powder	3.97	0.15	5.89	100.00	–	–	–	17.60	49.39

modifications. The total fresh weight was 156 g using the general formula: 100 g meat block, 3.0 g filler and 3.5 g of extender source on non-fat dry weight basis, 3.6 g seasoning and 45.9 g added water (including moisture contributed by non-meat ingredients). The seasoning contributed 200 ppm sodium nitrite, 300 ppm sodium erythorbate and 3.5 g salt to the formulation. Six different non-meat ingredients obtained from a commercial source were analyzed for proximate composition and functional properties. They were categorized as either extenders (mustard flour, Promax 70, and sodium caseinate) or fillers (corn starch, hard wheat flour and skim milk powder) on the basis of Canadian Food and Drug Regulations[5] using dextrose equivalent and total nitrogen values (Table 2).

The moisture, crude fat and crude protein contents were determined using standard AOAC procedures[6]. The cold absorption values were determined by a modification of AACC[7] method 56-20 as modified by Comer[2] and hot absorption values were determined by the method of Comer[2]. The nitrogen solubility index (NSI) was determined by the method of Inklaar and Fortuin[8] and expressed as percentage of total nitrogen in the sample. The dextrose equivalent determinations were made using the methods of Sophianopoules[9] and expressed as percentage of reducing sugars in the sample.

The WHC of non-meat ingredients was measured using the method of Smith *et al*[10] and that of meat and mixtures of meat and non-meat ingredients by the procedure of Acton[11]. The salt soluble protein content of the extracts used for emulsifying capacity determinations were estimated by the Kjeldahl method using AOAC[6] procedures, expressed as the percentage salt soluble nitrogen of the total nitrogen in the samples, and referred to as nitrogen solubility (NS). The EC was measured by the method of Porteous and Quinn[4]. The ES was determined by the method of Porteous[12].

The samples were prepared on the basis of 200 g of meat block (Table 1) for each of the meat systems. The components of the meat block, excluding back fat, plus extenders and fillers were weighed in the required amounts mixed manually in weighing boats using a spatula and transferred quantitatively into a Waring blender. The samples were blended for 30 sec, remixed and blended for an additional 30 sec. The blended samples were transferred into polyethylene bags, sealed and refrigerated overnight at 4°C.

The combinations of meat systems, extenders and fillers were studied using a completely randomized 3^3 factorial design[13]. There were two replicates and two determinations for each parameter. The second replicate was conducted after the first one had been completed. An average of the two determinations from each replicate was used in the statistical analyses. All the results were subjected to analysis of variance using the general linear programme (GLM) of the Statistical Analysis System (SAS). All tests of statistical significance were made at the probability level of $\alpha = 0.05$. A multiple linear regression was performed on the values for NS, EC and ES.

Results and Discussion

Analytical and functional property data for the non-meat and meat ingredients are shown in Tables 2 and 3. The total nitrogen contents of the non-meat ingredients varied from 0.11 per cent for corn starch to 14.26 per cent for sodium caseinate. The nitrogen solubility index (NSI) which gives an indication of the water soluble protein ranged from zero for corn starch to 100 for skim milk powder. The cold and hot absorption data reflected the water-binding properties of the protein and carbohydrate components of the extenders and filler sources. Due to the presence of protein, hard wheat flour had higher cold absorption values than corn starch and absorbed greater amounts of moisture upon heating. The cold and hot absorption characteristics of the vegetable protein products are

TABLE 3. COMPOSITION AND FUNCTIONAL PROPERTIES OF MEAT INGREDIENTS

Ingredients	Moisture (%)	Crude fat (%)	Total N fat (%)	Emulsifying capacity (l/g total N)	Water holding capacity (g/100 g)
Beef chuck	71.9	10.6	19.7	3.8	6
Lean pork trim	53.8	28.8	16.6	4.4	31
Pork hearts	75.5	9.5	15.3	6.4	4
Beef tripe	82.9	5.0	13.4	2.8	16
Mechanically deboned chicken meat	61.6	28.4	11.4	4.4	–
Back fat	7.9	90.2	2.1	–	–

dependent upon the effect of processing procedures on the insoluble protein[2].

The moisture and protein contents varied from 7.9 and 2.1 per cent in back fat to 82.9 and 19.7 per cent in beef chuck respectively (Table 3) The fat content ranged from 5.0 per cent in beef tripe to 90.2 per cent in back fat. Mechanically deboned chicken meat did not show any WHC because of the presence of very little myofibrillar proteins. The pork trimmings had a low moisture content and therefore gave the highest per cent WHC among the meat ingredients used. The NSI values for the non-meat ingredients reflect the handling and processing treatments. Freezing generally lowers the NSI of meat ingredients while heat treatment has a similar effect upon NSI of vegetable protein products. The emulsifying efficiency of the salt soluble protein was greater at lower concentrations; hence the high EC values. The soluble protein concentrations obtained in this study and the EC values for lean beef and tripe were similar to those reported by Carpenter and Saffle[14]. The EC values for hard wheat flour are not considered reliable for comparison purposes since a very small volume of oil is titrated and the NSI value itself is unreliable[2]. Corn starch did not form an emulsion since it obtained negligible amounts of soluble nitrogen.

Nitrogen solubility (NS): The analysis of variance in Table 4 shows that there was a significant ($P < 0.05$) three factor interaction among the meat systems, extenders and fillers. In order to evaluate the significant three factor interaction, the mean NS values for the various treatments were plotted as histograms (Fig. 1). The NS values for the strong meat system were improved by sodium caseinate in the presence of both corn starch and hard wheat flour while skim milk powder resulted in a lower NS. This could be attributed to the reduced solubility of sodium caseinate due to the calcium present in skim milk powder[15]. Promax 70 lowered the NS value of the strong meat system in the presence of each of the three extenders which may be due to its lower total nitrogen content. Promax 70 gave almost the same NS values for both hard wheat flour and skim milk

TABLE 4. ANALYSIS OF VARIANCE DATA FOR VARIOUS COMBINATIONS OF MEAT SYSTEMS, EXTENDERS AND FILLERS

Source	d.f.	Mean squares: % N solubility in 1.0 M NaCl solution	Mean squares: Emulsifying capacity (l/g total N)
Total:	53	110.28	2.49
Replicates	1	0.17	0.17
Treatments	26	209.44*	4.20*
Meat systems	2	608.05	19.24*
Fillers	2	2.72	1.07
Extenders	2	1523.16*	25.86*
Meat systems × fillers	4	103.28*	1.36
Extenders × fillers	4	19.22	1.10
Meat systems × extenders	4	94.39*	2.90*
Meat systems × extenders × fillers	8	41.47*	8.42
Error	26	15.36	0.87

* $P < 0.05$

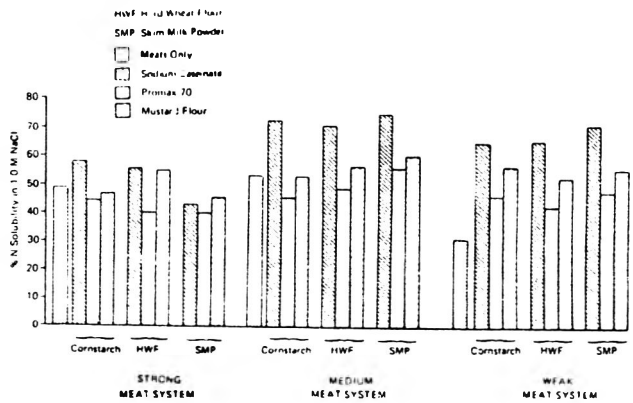

Fig. 1. Effect of various combinations of meat systems, fillers, and extenders on the nitrogen solubility in 1.0 M NaCl (n = 2).

powder and higher for corn starch. This may be due to the reduced solubility of proteins as a result of complex formation among the proteins present in meat system, extender and filler. Mustard flour improved the NS of strong meat systems in the presence of hard wheat flour while corn starch and skim milk powder acted antagonistically. Hard wheat flour in combination with mustard flour improved the NS of the strong meat system which may be due to the higher NSI of the mustard flour.

Sodium caseinate improved the NS value of the medium meat system in the presence of corn starch, hard wheat flour and skim milk powder. The combination of Promax 70 with both corn starch and hard wheat flour lowered the NS while with skim milk powder the NS was slightly improved. The NS of a mixture of medium meat system and mustard flour in the presence of corn starch resulted in no improvement while the incorporation of hard wheat flour and skim milk powder resulted in higher values.

All the treatment combinations of weak meat systems with the extenders and fillers improved the NS of the meat system with a maximum improvement for combinations of sodium caseinate with fillers and a minimum for Promax 70.

The NS values were the highest for medium meat system (59.7) followed by the weak (55.8) and strong (47.7) ones when averaged over the extenders and fillers. Among the extenders, sodium caseinate gave the highest NS values (64.0) followed by mustard flour (53.5) and Promax 70 (45.7) when averaged, over meat systems and fillers. The average of NS values for fillers, pooled over the meat systems and extenders were within a close range, i.e. 54.2, 54.1 and 54.8 for the corn starch, hard wheat flour and skim milk powder respectively.

The NS values in 1.0 M NaCl solution indicated that combinations of the strong meat system with the extender and filler sources gave the lowest values. The proteins of hard wheat flour and skim milk powder may have formed a complex with those of Promax 70 and mustard flour, resulting in these lower solubility values. This revealed that the fillers alone did not have a significant effect upon the NS but when combined with meat systems played a significant role.

Emulsifying capacity (EC): The analysis of variance data in Table 4 shows that the meat system extender interaction was significant ($P > 0.05$). In order to evaluate this interaction, the means averaged over the fillers were plotted in the form of histograms (Fig. 2). The amount of oil emulsified by the medium and weak meat systems in combination with sodium caseinate was higher than the meats alone while with the strong meat systems the effect was antagonistic, lowering the

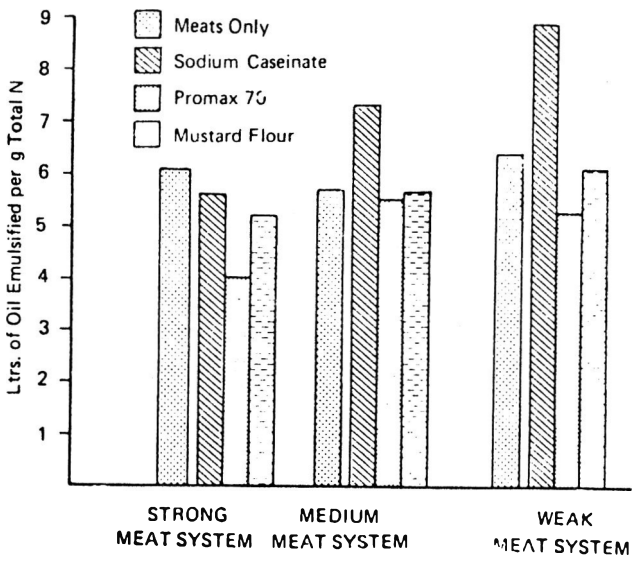

Fig. 2. Effect of various combinations of meat systems and extenders on emulsifying capacity (n = 6)

EC. Promax 70 performed antagonistically with all three meat systems decreasing their EC. Mustard flour also showed an antagonistic effect similar to Promax 70 although the values were higher in the former. The EC of the medium meat systems alone was approximately same as with mustard flour and was slightly lower in the case of weak meat systems. It was observed that the meat system extender interaction was dependent upon the amount of meat protein in the system.

EC was 6.79, 6.19 and 4.98 l of oil/g total N for the weak, medium and strong respectively when averaged over the extenders and fillers. The EC of extenders when averaged over the meat systems and filler sources was the greatest for sodium caseinate (7.31), followed by mustard flour (5.70) and Promax 70 (4.96). The fillers did not have a significantly ($P > 0.05$) different effect on EC, but the value was highest (6.26) for skim milk powder when compared to corn starch (5.93) and hard wheat flour (5.78). This could be attributed to the higher NSI of skim milk powder. The EC results showed similar effects to those of NS. The myofibrillar proteins may compete with the non-meat proteins and result in lowering the EC of the system.

Emulsion stability (ES): The analysis of variance (Table 5) reveals that only the two factors interaction between meat systems and extenders was significant ($P < 0.05$) (Fig. 3) Sodium caseinate gave the highest ES for the medium system and the lowest for the strong system. Promax 70 resulted in similar ES values for both strong and medium meat systems, and considerably lower values for the weak one. Mustard flour performed the best with the strong system giving

TABLE 5. ANALYSIS OF VARIANCE DATA FOR VARIOUS COMBINATIONS OF MEAT SYSTEMS, EXTENDERS AND FILLERS

Source	d.f.	Mean squares: % Emulsion stablity	Mean squares: % Water holding capacity
Total:	53	38.70	107.50
Replicates	1	2.20	21.40*
Treatments	26	70.22*	215.55*
Meat systems	2	8.03	2077.56*
Fillers	2	72.27*	355.17*
Extenders	2	698.57*	6.17
Meat systems × fillers	4	4.00*	78.22*
Extenders × fillers	4	8.09	28.67*
Meat systems × extenders	4	41.59*	18.31*
Meat systems × extenders × fillers	8	6.66	28.22*
Error	26	8.58	2.75

* $P < 0.05$

almost the same ES as Promax 70 but considerably lower ES with both the weak and medium meat systems. The lower ES values in the case of Promax 70 and mustard flour can be attributed to lower NS values. Mustard flour resulted in comparatively less viscous emulsions as compared to Promax 70 and sodium caseinate which gave thicker emulsions. The non-significant main effect of meat systems and a significant interaction effect between meat systems and extenders indicated that meat systems by themselves did not play a significant role in ES but contributed significantly ($P < 0.05$) in combination with the extenders. The extenders gave a significant ($P < 0.05$) effect on ES with a maximum average value for sodium caseinate (86.2) and similar values for Promax 70 (75.8) and mustard flour (75.1) when pooled over the meat system and fillers. There was a significant main effect of fillers giving almost the same values for corn starch (78.0) and hard wheat flour (77.8) when averaged over the meat systems and extenders. Skim milk powder resulted in a considerably higher ES (81.4) which is in accordance with its greater EC. The greater amounts of salt soluble nitrogen resulted in the emulsification of larger amounts of oil and a more viscous emulsion.

A multiple linear regression analysis was performed on the NSI, EC and ES data for the various treatments to determine whether EC or NSI had the most effect on ES keeping ES as an independent variable (z) and EC (y) and NSI (x) as dependent variables. The regression equation was as follows:

$z = 62.2 + 0.13\,(x) + 1.67\,(y)$

The variables were found to be positively correlated with coefficient of determination $r^2 = 0.39$. The regression coefficients for NSI and EC indicate that the latter is thirteen times more effective than the former in its influence on ES.

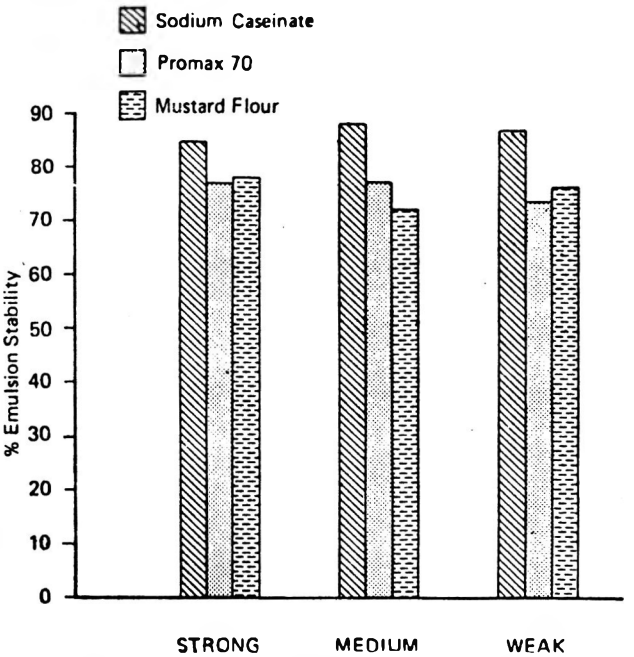

Fig. 3. Effect of various combinations of meat systems and extenders on emulsion stability (n = 6).

Water holding capacity (WHC): Analysis of variance in Table 5 shows that the three factor interaction among meat systems, extenders and fillers was significant ($P < 0.05$). The data used for evaluating this interaction is presented in Fig. 4. Water holding capacity values for the strong meat system were improved by sodium caseinate in the presence of corn starch, hard wheat flour and skim milk powder. Hard wheat flour in combination with each of Promax 70 and mustard flour resulted in an antagonistic effect upon the WHC of the strong meat system. This could be attributed to the interaction of meat and extender proteins to alter molecular and physical forces responsible for the binding of water. Synergism of sodium caseinate and mustard flour was greater when combined with skim milk powder than with the hard wheat flour (Fig. 4) All the combinations of extenders and fillers gave a synergistic effect in the case of medium meat systems. Synergism of sodium caseinate and Promax 70 was the greatest for skim milk powder and lowest for corn starch. Mustard flour produced a greater synergistic effect in combination with skim milk powder in comparison to that with hard wheat flour and corn starch.

In the case of the weak meat system, all the treatment combinations gave synergistic effects on the WHC of meats. Combinations of extenders with corn starch produced minimum WHC values while that with skim milk powder gave the maximum WHC. There were slight differences among the WHC values obtained for combinations of corn starch and hard

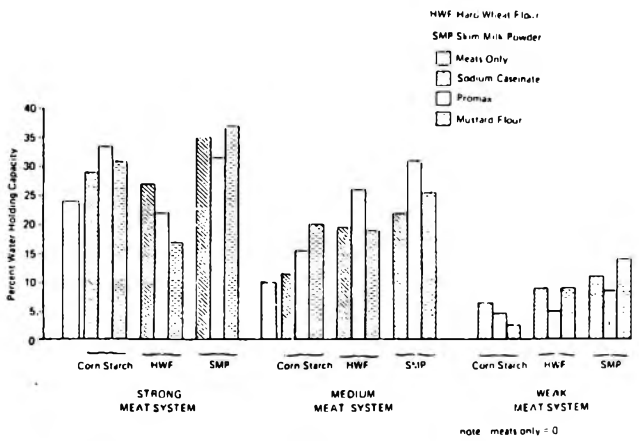

Fig. 4. Effect of various combinations of meat systems, fillers and extenders on water holding capacity (n = 2).

wheat flour with Promax 70. The combinations of hard wheat flour with sodium caseinate and mustard flour gave the same WHC while with Promax 70 it was lower. On the whole, the combinations of the weak meat systems with extenders and fillers resulted in the lowest WHC values when compared to the strong and medium systems. Extenders did not play a significant ($P > 0.05$) role in the WHC of various treatment combinations by themselves but contributed to the significant three factor interaction. There were significant differences in WHC values among the fillers. Skim milk powder gave the highest mean WHC values of 23.9 while the corn starch (17.1) and hard wheat flour (15.6) gave lower values. The differences among the filler sources may be attributed to a sparing effect of skim milk powder on the amount of muscle proteins due to higher NSI as suggested by Schut.[16].

The combinations of various meat systems with extenders and fillers interacted synergistically or antagonistically, depending upon the types of material used. All extenders except sodium caseinate acted antagonistically with the strong meat systems, resulting in lower NS and EC values. The synergism shown by sodium caseinate in combination with the strong meat system was not as great as in the case of medium and weak meat systems.

The weak meat system produced minimum WHC values. The medium meat system performed the best when EC, ES and WHC were considered. Mustard flour resulted in the lowest ES in case of medium meat system. It appears from this study that the nitrogen solubility (NS) in a 1.0 M salt solution was an important factor in predicting the EC and ES of a mixture of meats, extenders and fillers.

Acknowledgement

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Effect of Levels of Fillers and Extenders on the Functionality of a Meat System

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Combination of a medium meat system with Promax 70, sodium caseinate and hard wheat flour was used to study various levels of extenders and fillers using a response surface design. A 2.5:3.0 (% of meat block) of hard wheat flour and extenders produced an optimal overall response of emulsifying capacity (EC), emulsion stability (ES) and water holding capacity (WHC) for both the extenders.

Bawa *et al*[1] explored the interactions among three different meat systems viz. strong, medium and weak with various fillers like corn starch, hard wheat flour and skim milk powder and extenders such as mustard flour, Promax 70 and sodium caseinate in emulsion systems. The medium meat system was found to perform the best in combination with fillers and extenders when emulsifying capacity (EC), emulsion stability (ES) and water holding capacity (WHC) were considered. Of the fillers evaluated, hard wheat flour is the most widely used, while skim milk powder is not a common filler. Therefore, the medium meat system and hard wheat flour were selected to study the effect of various levels in combination with Promax 70 (soy concentrate produced using acid wash technique) and sodium caseinate.

The objective of this work was to evaluate various levels of hard wheat flour as filler with each of the two extenders Promax 70 and sodium caseinate in a medium meat system for obtaining the best overall functionality.

Materials and Methods

Materials used and the method of sample preparation were the same as reported by Bawa *et al*[1] except that only a medium meat system, hard wheat flour (HWF), Promax 70 (PMX) and sodium caseinate (SC) were used. Methods for the determination of EC, ES and WHC were also the same as reported earlier[1].

Fillers and extenders were used at 0, 0.88, 3.0, 5.12 and 6.0 per cent of the meat block on a non-fat dry weight basis (Table 1) and were evaluated using a central composite rotatable design[2]. The four points [(0.88, 0.88), (5.12, 0.88), (0.88, 5.12), (5.12, 5.12)]

TABLE 1. CENTRAL COMPOSITE ROTATABLE DESIGN

Filler Hard wheat flour	Medium meat system									
	Extenders									
	Promax 70					Sodium caseinate				
	0.00	0.88	3.00	5.12	6.00	0.00	0.88	3.00	5.12	6.00
0.00			x					x		
0.88		x		x			x		x	
3.00	x		x		x	x		x		x
			5 points					5 points		
5.12		x		x			x		x	
6.00			x					x		

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constituted a 2 x 2 factorial design. The central five points gave approximately equal precision within a circle of radius 3.00. The four points [(0, 3), (6, 3), (3, 0) and (3, 6)] were included to form a central composite rotatable design with $\alpha = 3$. In all, there were 13 points replicated twice to give a total of 26 combinations.

The average of the two determinations was used for statistical analysis. The data were analyzed using the General Linear Model (GLM) option of the SAS programme (SAS Institute Inc., Raleigh, North Carolina) and analysis of variance procedures were used for non-meat proteins separately. The regression equations were plotted using the three dimensional plotting programme, SCI PO_4, and differentiated to obtain the levels of fillers and extenders giving the optimum response for each of the variables. All tests of statistical significance were made at $P = 0.05$.

Results and Discussion

Emulsifying capacity (EC): The analysis of variance (Table 2) reveals that either of the two extender proteins when studied individually were found to have significant ($P < 0.05$) interaction effect at the levels used. Hard wheat flour x extender interaction was significant ($P < 0.05$) for sodium caseinate and non-significant for Promax 70. The interaction for hard wheat flour levels was significant for Promax 70 while for extender levels, it was significant for both Promax 70 and sodium caseinate. Thus, it indicated that the levels of hard wheat flour and Promax 70 had significantly different effects on emulsifying capacity when combined with the medium meat system, but the effect of a level of one of them with various levels of the other was almost uniform. Sodium caseinate, because of its high NSI and protein content resulted in almost double the EC (8.15 l of oil/g total N) as compared to Promax 70 (4.72 l of oil/g total N) when averaged over the various level combinations. Increasing the level of hard wheat flour used for a particular level of sodium caseinate generally resulted in lowering of the EC. A similar effect was also observed for Promax 70 with an optimum at around 3.0 per cent of the meat block. However, at the 0.88 per cent level of sodium caseinate, the EC increased as the percentage of hard wheat flour increased from 0.88 to 5.12 per cent. These results were evaluated using three dimensional response surface diagrams (Fig 1 and 2) for Promax 70 and sodium caseinate respectively. It can be seen from Fig 1 that the higher levels of hard wheat flour had a negative effect while the lower levels resulted in a slight improvement as shown by a positive slope of 0.1727 for hard wheat flour. Promax 70 led to a uniform decrease in EC as the level was increased from 0 to 6 per cent, but the rate of decrease was slightly more at higher levels. The differentiation of the regression equation gave a maximum EC of 5.33 for a combination of 1.58 per cent hard wheat flour and 0.091 per cent Promax 70. Fig 2 revealed that the increase in levels of both hard wheat flour and sodium caseinate resulted in improved EC but the rate of increase in EC was about four times for sodium caseinate as compared to hard wheat flour. The rate of increase in EC for either one of them was slower at higher levels than at lower levels. The combination giving a maximum EC of 11.5 was 0.20 per cent hard wheat flour and 8.43 per cent sodium

TABLE 2. ANALYSIS OF VARIANCE DATA FOR THE EFFECT OF VARIOUS COMBINATIONS OF A MEDIUM MEAT SYSTEM WITH LEVELS OF HARD WHEAT FLOUR AND EXTENDERS (PROMAX 70 OR SODIUM CASEINATE)

		Mean squares					
		Promax 70			Sodium caseinate		
Source	d.f.	EC	ES	WHC	EC	ES	WHC
Total	25	0.36	4.69	30.66	2.15	35.18	34.58
Replicates	1	0.94*	24.27	3.85	0.11	8.35*	9.85
Treatments	8	8.89	8.45*	61.63*	104.53*	104.53*	93.03*
Hard wheat flour	1	1.44*	1.18	256.14*	0.64*	17.95*	404.10*
Extender	1	2.39*	0.78	150.32*	43.35*	17.45*	0.002
Hard wheat flour x hard wheat flour	1	0.73*	36.47*	52.70*	0.033	2.94	232.01*
Extender x extender	1	0.30*	10.44*	12.38	3.26*	676.89*	0.738
Hard wheat flour x extender	1	0.003	3.10*	1.13	2.43*	58.27*	10.13
Lack of fit	3	0.28*	5.21*	6.79	1.00	20.90*	32.41*
Replicate x treatment	8	0.088	2.77*	25.22	0.05	3.61*	8.72
	8	0.033	0.41	8.5	0.077	0.77	5.10

* $P < 0.05$ EC: Emulsifying capacity. ES: Emulsion stability. WHC: Water holding capacity.

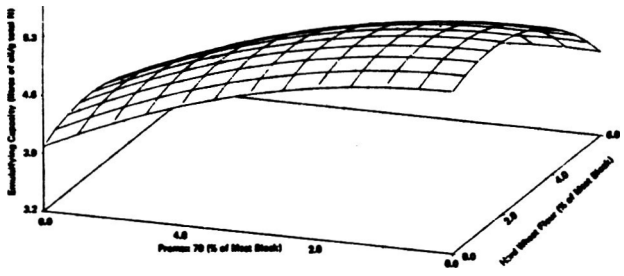

Fig. 1. Response surface diagram for the effect of various levels of hard wheat flour and Promax 70 on the emulsifying capacity of a medium meat system. EC = 5.19 + 0.17 (HWF) + (–0.001) (PMX) + (–0.05) $(HWF)^2$ + (–0.03) $(PMX)^2$ + (.004) (HWF) (PMX).

caseinate. The EC values seemed to be additive in the case of sodium caseinate which is in agreement with earlier work of Nilsson and Persson[3], but for Promax 70 an interaction resulting in an antagonistic effect was observed. This may be due to the concentration effect of salt soluble nitrogen in the extracts[4].

Emulsion stability (ES): Analysis of variance (Table 2) reveals that there was a significant ($P < 0.05$) hard wheat flour x extender interaction effect for both of the extenders. Levels of hard wheat flour within themselves had a significant ($P < 0.05$) interaction effect in the case of Promax 70. Hard wheat flour and extender levels had a significant effect on the ES of the medium meat system for sodium caseinate while a non-significant ($P < 0.05$) effect was observed in the case of Promax 70. This indicated that the levels of hard wheat flour and Promax 70 did not affect the ES. The ES for various level combinations ranged from 66.6 to 71.41 per cent in the case of Promax 70 while for sodium caseinate the range was 68.49 to 85.34 per cent. Sodium caseinate (79.98 per cent) resulted in a greater emulsion stability as compared to Promax 70 (69.51) when averaged over the various combinations. The 0/3 per cent and 3/3 per cent combinations of

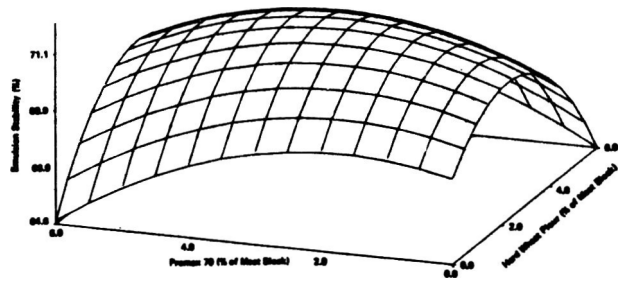

Fig. 3. Response surface diagram for the effect of various levels of hard wheat flour and Promax 70 on the emulsion stability of a medium meat system. ES = 67.88 + 1.75 (HWF) + (PMX) + (–0.38) $(HWF)^2$ + (–0.19) $(PMX)^2$ + 0.14 (HWF) (PMX).

hard wheat flour with sodium caseinate and the 3/3 per cent combination of hard wheat flour with Promax 70 gave higher ES values when compared to all other combinations.

It can be seen from Fig 3 that an increase in levels of hard wheat flour increased the ES at a decreasing rate but after reaching a maximum, the ES was then negatively affected. Promax 70 resulted in a slight improvement in ES until about the 2 per cent level with a uniform decrease thereafter. The differentiation of the regression equation gave a maximum ES of 71.1 per cent for a combination of 2.78 per cent hard wheat flour and 2.65 per cent Promax 70. Fig 4 reveals that the increase in levels of both hard wheat flour and sodium caseinate resulted in an improved ES. The combination giving maximum ES of 85.5 was 1.80 per cent hard wheat flour and 3.39 per cent sodium caseinate. Promax 70 lowered the stabilizing effect at higher level combinations with hard wheat flour while sodium caseinate resulted in greater ES values. Similar results have been reported by Nilsson and Persson[3] using Promine D and sodium caseinate.

Water holding capacity (WHC): The analysis of variance (Table 2) reveals a significant ($P < 0.05$)

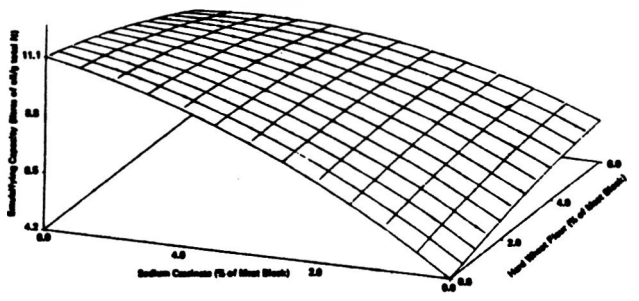

Fig. 2. Response surface diagram for the effect of various levels of hard wheat flour and sodium caseinate on the emulsifying capacity of a medium meat system. EC = 4.18 + 0.42 (HWF) + 1.79 (SC) + (–0.02) $(HWF)^2$ + (–0.11) $(SC)^2$ + (–0.12) (HWF) (SC).

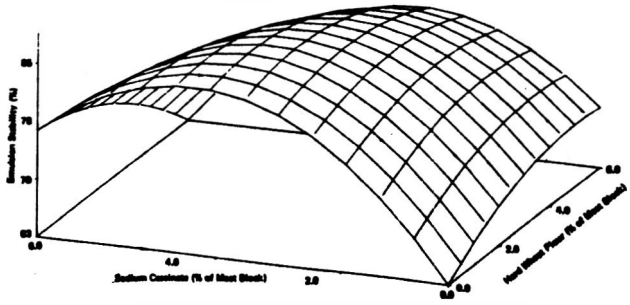

Fig. 4. Response surface diagram for the effect of various levels of hard wheat flour and sodium caseinate on the emulsion stability of medium meat system. ES = 63.04 + 3.13 (HWF) + 11.60 (SC) + (–0.30) $(HWF)^2$ + (–1.55) $(SC)^2$ + (–0.60) (HWF) (SC).

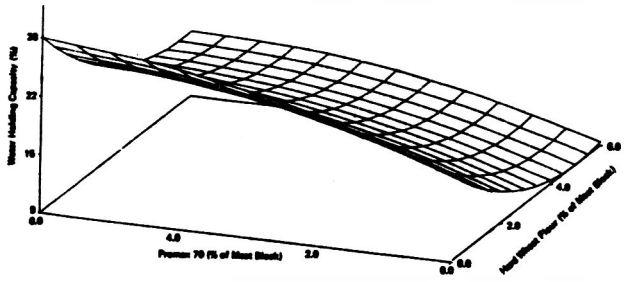

Fig. 5. Response surface diagram for the effect of various levels of hard wheat flour and Promax 70 on the water holding capacity of a medium meat system. WHC = 19.0 + (–4.04) (HWF) + (PMW) + 0.40 $(HWF)^2$ + (–0.21) $(PMX)^2$ + (–0.08) (HWF) (PMX).

hard wheat flour level interaction for both the extenders. Hard wheat flour had a significant effect on the WHC for both the extenders; however, the extender effect was significant ($P < 0.05$) in the case of Promax 70 and non-significant ($P > 0.05$) for sodium caseinate. Promax 70 resulted in a higher WHC (17.23 per cent) when compared to sodium caseinate (13.23) because of higher cold and hot absorption values[1]. The increase in levels of hard wheat flour for various levels of Promax 70 decreased the WHC at a faster rate. An increase in the levels of Promax 70 for various levels of hard wheat flour resulted in an improvement of the WHC (Fig. 5) which can be explained by the high cold absorption values for Promax 70[1].

The increase in levels of sodium caseinate for various levels of hard wheat flour did not change the WHC except when it was 5.12 per cent of meat block. The increase in level of sodium caseinate from 0.88 to 5.12 resulted in a slight increase in WHC from 11.0 to 14.0 per cent. The increase in level of hard wheat flour for various levels of sodium caseinate showed an effect (Fig. 6) similar to that for Promax 70 which is in accordance with the observations by Schut[5] who showed that WHC decreases with increased levels of additives in meat emulsions. Increased levels of sodium caseinate had a negligible effect on WHC due to complete solubility. The differentiation of

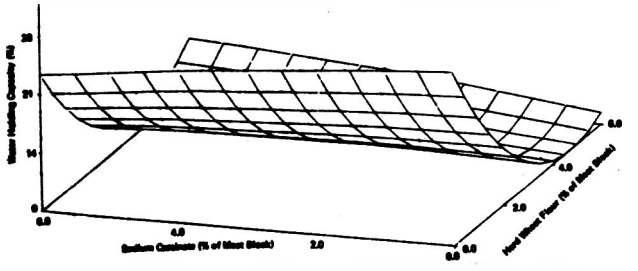

Fig. 6. Response surface diagram for the effect of various levels of hard wheat flour and sodium caseinate on the water holding capacity of a medium meat system. WHC = 27.83 + (–8.48) (HWF) + (–0.44) (SC) + 0.89 $(HWF)^2$ (–0.05) $(SC)^2$ + 0.25 (HWF) (SC).

TABLE 3. PREDICTED RESPONSE FOR EMULSIFYING CAPACITY (EC) EMULSION STABILITY (ES) AND WATER HOLDING CAPACITY (WHC) FOR VARIOUS COMBINATIONS OF EXTENDERS AND HARD WHEAT FLOUR (HWF) BASED ON REGRESSION EQUATION

H.W.F: Promax 70	HWF: Na caseinate	EC (l Oil/ g total N)	ES (%)	WHC (%)
2.5:3.0	–	5.02	71.1	17.7
3.0:3.0	–	5.16	71.1	16.7
3.5:3.0	–	4.88	70.9	15.9
3.0:3.5	–	4.86	71.0	17.4
3.0:2.5	–	5.05	71.1	15.9
–	2.5:3.0	8.57	85.3	12.3
–	3.0:3.0	8.53	85.1	10.9
–	3.5:3.0	8.47	84.8	9.9
–	3.0:3.5	8.89	85.0	10.9
–	3.0:2.5	8.11	84.5	10.9

regression equations gave a maximum WHC of 16.29 per cent for a combination of 5.67 per cent hard wheat flour and 6.15 per cent Promax 70 and 9.47 per cent for a combination of 3.98 per cent hard wheat flour and 5.45 per cent sodium caseinate.

From these results, it can be seen that the levels of each component required to maximize the response for EC, ES and WHC were different for each variable studied. A lack of fit test (Table 2) was found to be significant ($P < 0.05$) for both the proteins in all measurements, except for WHC when Promax 70 was used in the formulation. The interaction effect between replicates and treatments was significant ($P < 0.05$) for ES of both the extenders. This could be a 0.05) for ES of both the extenders. This could be a result of the normal variability in the meat ingredients and in the preparation of homogenates which make it difficult to reproduce stability results with high precision[6].

Sodium caseinate gave a higher EC and better EC when compared to Promax 70 in the presence of hard wheat flour in a medium meat system; it was the reverse for WHC. The responses for EC, ES, and WHC were calculated for hard wheat flour/sodium caseinate or hard wheat flour/Promax 70 combinations of 2.5/3.0, 3.0/3.0, 3.5/3.0, 3.0/2.5 and 3.0/3.5 per cent of meat block (Table 3). It was found that combinations of 2.5/3.0 gave the best overall responses when all variables were considered.

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Cold Shock Reactions in Tropical Fishes

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Cold shock reactions in several species of marine, fresh water and brackish water fishes were studied. Stiffening was found to occur more rapidly at 0°C than at room temperature in all the species studied. A comparative study of the biochemical characteristics of cold shock reactions at 0°C and rigor mortis at room temperature was carried out. Significant differences in pH, ATPase activity & glycogen content were observed between muscles stiffened by the above two processes. An intense thermal shock was observed in fishes exposed to 37°C.

Tropical fish has been found to exhibit a cold shock reaction similar to that of *rigor mortis* by several workers.[1,2] Locker and Hagyard were the first to observe a cold shortening in terrestrial animal muscle.[3] Curran *et al*[4] in an extensive study conducted recently have shown that in tilapia (*Oreochromis aureus/Niloticus hybrid*) cold shock stiffening occurred after 2 hr at 0°C and after 7 hr at 22°C. It was also reported by Disney *et al*[5] that iced tilapia exhibited a rigor like shortening which developed within one hour. Uniced fish did not enter rigor mortis until 2-5 hr after capture.

Rigor in a decisive way pre-determines the shelf life of fresh fish. When rigor is extended in time, either through a delay in its onset or by its duration, this correspondingly defers the attack by the spoilage bacteria[6]. On the other hand, an intense contraction during *rigor mortis* may affect the connective tissue and increase gaping in fish[7]. Also, stiffening caused by *rigor mortis* poses problems in filleting of fish especially when carried out at sea. A comparative study on *rigor mortis* and cold shock reactions will significantly contribute towards improving the handling practices of many species of tropical fishes.

The objective of this study was to investigate the problem of cold shock reactions in several species of marine, fresh water and brackish water fishes and to compare cold shortening with *rigor mortis* set in fish at room temperatures.

Materials and Methods

Representative samples from fresh water, brackish water and marine fishes were used for the study. Fresh water fishes including mrigal (*Cirrhinus mrigala*), rohu (*Labeo rohita*) and silver carp (*Hypophthalmichthys molitrix*) and common carp (*Ciprinus carpia*) were collected from the fresh water ponds maintained by the College of Fisheries, Panangad. Fishes were caught by cast net and were brought alive to the laboratory.

Brackish water fishes like mullet (*Liza parsia*), cat fish (*Tachysurus* sp), therapon (*Therapon jarbua*) Long rayed silver biddy (*genres filamentosus*) and spotted butter fish (*Scatophagus argus*) were harvested using dragnet from the brackish water ponds maintained by College of Fisheries, Panangad. Fishes were brought alive to the laboratory for further study. Marine fishes including six banded trevally (Caran x sexfasciatus), lactarius (*L. lactarius*) and Malabar anchovy (*Thrissocles malabaricus*) were collected from the Chinese nets operated at Fort Cochin and also from trawlnet operated by the vessel Matsya – I. In the latter case, ice storage studies were conducted on board the vessel.

Ice storage of fish was carried out by mixing fish with ice in the proportion 1:2 by weight in an ice box. The temperature of 15°C was achieved in the lower chamber of a refrigerator. Temperature was accurately maintained with the help of a freezer temperature monitor. Room temperature was 28 ± 1°C. The temperature 37°C was obtained in an incubator provided with a thermostat.

pH was determined on a 2:1 water fish homogenate using a glass electrode. ATPase was assayed according to Noguchi and Matsumoto [8] and reported as μ mole Pi/min/mg protein. Glycogen was determined as per

the procedure of Hassid and Abraham[9]. Fishes were stored in bulk quantity and biochemical tests were carried out in duplicate on representative samples drawn from the lot and average values reported.

Stiffening: A simple method described by Cutting[10] and modified by Curran *et al*[4] was used for the determination of stiffening. In this method, the sag of the tail was noted when the fish are held vertically by the head with the tail pointing upwards. The assessments were carried out at intervals of 10 min following death. Curran *et al*[4] described, five stages of stiffening viz. (1) flaccid, (2) tail bent half way, (3) tail bent slightly, (4) tail straight, and (5) whole fish very rigid. In the present study, fish was considered in rigor when tail was straight (Stage 4).

Results and Discussion

Table 1 illustrates the time taken by different species of fish for undergoing cold stiffening at 0°C and *rigor mortis* at room temperature. Stiffening occurred in fish more rapidly at 0°C than at room temperature. Among the fresh water species, this phenomenon was more prominent in mrigal. In iced mrigal, stiffening occurred within 10 min after death whereas in samples kept at room temperature, the time of onset of rigor was 120 min.

Cold shock reactions in brackish water fishes varied considerably between species. Onset of stiffening was significantly advanced in mullet stored in ice than those exposed to room temperature. Iced storage resulted in rapid onset of stiffening in all the species of marine fish studied. This effect was greatest in lactarius (*L. lactarius*).

TABLE 1. COMPARISON OF TIME TAKEN FOR THE ONSET OF COLD SHORTENING AND *RIGOR MORTIS* IN DIFFERENT SPECIES OF TROPICAL FISHES

Species	No.of fishes examined	Length (cm)	Weight (g)	Time (min) taken for onset of stiffening	
				Iced	Room temp.
Fresh water fishes					
Mrigal	6	21	120	10	120
Rohu	6	20	100	40	120
Silver carp	6	36	573	90	170
Common carp	6	20	170	30	60
Brackish water fishes					
Mullet	6	24	155	30	60
Cat fish	6	25	120	40	60
Therapon	6	12	70	40	60
Long rayed silver biddy	8	16	62	20	30
Spotted butter fish	6	14	83	30	50
Marine fishes					
Six banded trevally	12	12	12	20	30
Lactarius	6	17	75	10	70
Malabar anchovy	12	14	40	10	40

TABLE 2. TIME (MIN) TAKEN FOR THE ONSET OF STIFFENING IN FISH AT DIFFERENT TEMPERATURES

Temperature	Rohu	Milk fish	Mullet	Silver carp
0°C	40	20	30	90
15°C	100	10	35	70
Room temp.	120	90	40	150
37°C	30	15	30	60

Three replications were carried out and the average value reported.

In order to understand the effect of different temperatures on the onset of stiffening in tropical fishes, an experiment was conducted by placing the same species under different temperatures and noting the time taken for the onset of rigor. Results are given in Table 2. Rapid stiffening was observed in all the species of fish stored at 37°C. Stiffening occurred within minutes of exposure to 37°C. Further studies are required to reveal the true nature of this phenomenon of thermal shock reactions in tropical fishes. Similarly, rapid stiffening was observed with samples exposed to 0°C. Table 2 shows the time taken for the onset of stiffening in different species of fish exposed to 15°C; it was greatest in those samples exposed to room temperature.

Muscle pH was highest in fresh fish (Table 3). A slight lowering of pH was observed in fish that had undergone stiffening at 0°C. pH was further lowered at the onset of *rigor mortis* in samples stored at room temperature. This pattern of change in pH is in good agreement with the results obtained for white fish muscle at the onset of *rigor mortis*[11]. The study reveals that cold shortening occurs without significant fall in muscle pH and thus brings out the distinction between these two phenomena.

ATPase activity of fresh fish muscle was compared with that of muscle at the time of cold shortening and the onset of *rigor mortis*. The results are shown in Table 3. Muscle ATPase activity at the time of stiffening in fishes stored in ice was higher than that in muscle of fish immediately after death. Higher ATPase activity was found in fish kept at room temperature. Similar results were obtained for mullet, milk fish and silver carp. ATPase activity in fish muscle gives an index of the extent of denaturation of myofibrillar proteins.

Cold shortening differs from *rigor mortis* in ATP content of the muscle. *Rigor mortis* in fish is induced

TABLE 3. BIOCHEMICAL CHANGES IN FISH MUSCLE IMMEDIATELY AFTER DEATH AND AT THE START OF THE STIFFENING

Storage condition	pH	ATPase activity (μM Pi/min/ mg prot)	Glycogen (mg/g tissue)
		Mullet	
Death*	7.38	0.365	3.50
0°C	7.20	0.384	1.68
Room temp.	6.82	0.456	1.44
		Chanos	
Death*	6.26	0.409	8.95
0°C	6.24	0.460	7.87
Room temp.	5.90	0.429	7.10
		Silver Carp	
Death*	7.12	0.259	6.12
0°C	7.01	0.271	6.04
Room temp.	6.81	0.292	5.22

*Immediately after death

with a decrease in ATP level of muscle[12]. The intensity of *rigor mortis* was found to be dependent upon the amounts of ATP decomposed per unit time[13]. However, in the case of cold stiffening, it was observed that shortening occurred while about 40 per cent ATP still remained in the muscle. Due to this similarity in ATP level, cold shortening was found to resemble thaw rigor more closely than normal post rigor changes. The ATP level remains rather constant during cold stiffening because of a fast resynthesis of ATP from adenosine diphosphate by the glycolytic process[14].

Highest glycogen content was observed in muscles of fish immediately after death (Table 3). Lowering of glycogen was observed in muscles of fishes stored at 0°C and at room temperature. Manohar[11] has reported similar rapid decrease in glycogen content of white fish muscle held in ice.

Cold shock phenomenon is explained by the influence of temperature on the membrane system of the sarcoplasmic reticulum. Below about 15°C, decreasing temperature causes an increasing inactivation of the ATP driven calcium pump which transports Ca^2 ions from the sarcoplasma into the sarcoplasmic reticulum. Therefore, Ca^{2+} ions are released from the sarco-tubular system, they activate the myosin ATPase and consequently, initiate the onset of stiffening.[14]

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J. Fd. Sci. Technol., 1988, Vol. 25, No. 2, 92–93

PRE-FORMED ENTEROTOXINS OF *STAPHYLOCOCCUS AUREUS* IN INFANT FOODS

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One hundred and two samples of infant foods of different brands were analysed for the presence of pre-formed enterotoxins of *Staphylococcus aureus*. Only two samples contained pre-formed enterotoxin type B, at a level of 0.70 and 0.34 μg per 20 g of the products as determined by Rocket-immuno-electrophoresis. Both the samples were also positive for pre-formed TDNase. However, no relationship could be established between TDNase and enterotoxin as out of 14 TDNase positive samples only 2 contained preformed enterotoxins.

Staphylococci elaborate a series of proteins which produce typical enterotoxigenic reactions in humans and certain animals[1-3]. Because of their extraordinary heat stability, these toxins may be carried over from the raw materials to the finished heat processed foods[4-6]. The ability of these enterotoxins to reactivate after heat treatment has also been shown[7]. Food poisoning outbreaks due to preformed staphylococcal enterotoxins present in spray dried milks has been reported[8,9]. Considering the high incidence of enterotoxigenic *S. aureus* in infant foods[10] the present study was conducted to examine the incidence of pre-formed staphylococcal enterotoxins in infant foods.

A total of 102 different samples of infant foods, comprising 10 brands of infant milk foods and 3 brands of cereal weaning foods were collected from milk plants as well as from the local markets of Karnal. All the samples were extracted for pre-formed enterotoxins of staphylococci by carboxymethyl cellulose extraction procedure of Bennett and Mc Clure[11]. The identification of the enterotoxins was done by microslide gel double diffusion test[12]; quantitation was carried out by single radial immuno-diffusion technique[13] and rocket immuno-electrophoresis technique[14]. Results were expressed as μg of pre-formed enterotoxin per 20 g of the product. For all these identification and quantitation purposes, a standard set of antisera (A to E) obtained from Dr. M.S.. Bergdoll, Food Research Institute, Wisconsin was used. Baby food samples were also subjected to the detection of pre-formed thermostable deoxyribonuclease (TDNase) using the procedure of International Dairy Federation (IDF).[15]

Two of the 102 infant food samples belonging to one batch each of brands II and V of infant milk foods showed the presence of preformed enterotoxin type B (Table 1). The levels of pre-formed enterotoxin were 0.70 and 0.34 μg per 20 g of the product as determined by RIE. The presence of these enterotoxins in infant foods has to be considered seriously, in view of their hazardous nature. Although the detected levels of the enterotoxins were low, these may be considered biologically active since less than 1.0 μg of enterotoxin per 100 g of food has been shown to cause food poisoning[16]. This has great implication in the practice of bottle feeding as in the absence of any regulatory control, enterotoxins may escape detection and reach the consumer resulting in food poisoning outbreaks.

Due to the complex extraction procedures involved in direct detection of enterotoxins, the search for a reliable and simple technique is being made in different laboratories world over. In view of the correlation between TDNase test and enterotoxin production[17-19] all the samples were subjected to TDNase test. The results indicated that the two samples showing presence of enterotoxins also exhibited pre-formed TDNase activity. At the same time, this correlation was found to be evasive, as out of 14 TDNase positive samples only two samples were found to contain pre-formed enterotoxin. It may thus be inferred that TDNase test may not be fully relied upon as an indicator of the presence of pre-formed enterotoxin.

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TABLE 1. QUANTIFICATION OF PRE-FORMED STAPHYLOCOCCAL ENTEROTOXINS IN INFANT MILK FOODS

Brand	Batches analysed	No. of samples +ve	Type of enterotoxin (MGDD)	Enterotoxin concn (μg/20g) SRID	RIE
II	15	1	B	0.62	0.70
V	10	1	B	–*	0.34

* No zone obtained (< 0.50 μ g)

MGDD: Microslide Gel Double Diffusion, SRID: Single Radial Immunodiffusion, RIE: Rocket Immuno-electrophoresis

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J. Fd. Sci. Technol., 1988, Vol. 25. No. 2, 94–96

EVALUATION OF LAC DYE FOR HEPATOTOXIC EFFECTS – TO USE AS A FOOD COLOURING AGENT

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The effect of lac dye on some functionally important enzymes of the liver (glutamic oxaloacetic transaminase and glutamic pyruvic transaminase) and serum (alkaline phosphatase, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase) at 0 (control), 0.2, 1.0, 2.0, 10.0 and 20.0 mg per day for a period of 6 and 24 hr., 7 days, 1 month and 3 months as well as 6 months to adult, male, albino rats is reported. The study indicates that lac dye does not produce any statistically significant change in any of the enzyme activities. Hence, the dye may not have any hepatotoxic effect.

Lac dye or laccaic acid is a brilliantly red coloured dye obtained from the resinous secretion of a small insect called *Laccifer lacca* or *Kerria lacca*. In the initial purification steps of stick lac to obtain shellac, lac dye is produced as a water soluble waste product. Chemically lac dye has been reported to be isolated from stick lac, which contains 10 per cent of the dye. The sodium and potassium salts of laccaic acid are completely soluble in water[1]. Structure of laccaic acid was first suggested by Mayer and Cook[2] but later on, seven ether esters of laccaic acid were obtained based mainly on NMR data[3].

Though colour in food is an important sensory attribute, most of the synthetic colours presently in use have been found to produce teratogenicity[4], carcinogenicity[5], sarcomas of the peritoneum and intestinal tract[6], increased incidence of several sub-clinical conditions[7] and growth retardation[8]. Some of the dyes are also reported to alter the immune mechanism in man[9]. Hence, as a part of the food safety measure, many of the synthetic colours are restricted from being used and as a result, use of natural dyes, after proper toxicity evaluation are being recommended.

The present paper reports the effect of lac dye on the hepatic glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities as well as serum alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities-all of which are directly or indirectly related to hepatic function.

For the present study, adult, male albino rats of Charles Foster strain of body weight of about 110-120 g were used. They were maintained on a diet consisting of wheat flour (840 g, protein content approximately 11 per cent); casein (40 g, protein content approximately 80 per cent; groundnut oil (70 ml); vitamin mixture[10] (10 g) and salt mixture[11] (40 g). Water was given *ad libitum*. The animals were kept at 12 hr light and 12 hr dark with room temperature set to 26±2°C.

The crude lac dye was obtained through the Shellac Export Promotion Council (Ministry of Commerce, Govt. of India) from the Indian Lac Research Institute (Indian Council of Agricultural Research, Govt. of India). It was dissolved in a mildly alkaline solution and kept overnight for complete extraction. The dye was then shaken in a mechanical shaker and centrifuged at 4000 x g for 30 min. The supernatent was collected, filtered and the filtrate was evaporated to dryness in a lyophilizer. The recovery of the pure dye from the crude one was 20-22 per cent. Purified dye when run in HPLC analyser showed a major peak of pure laccaic acid with other minor peaks[12].

Doses were selected according to Prevention of Food Adulteration Act, No. 30, 1955 of Govt. of India, which stipulates the maximum use of an additive in food to be 200 mg per kg of diet or beverage. Since each rat was found to consume approximately 10 g of diet per day, the maximum permissible dose worked out to be 2.0 mg dye per rat per day. Two stock solutions of 4.0 mg dye per ml and 40.0 mg of distilled water were prepared. The dye was fed *per os* at doses of 0 (control), 0.2, 1.0, 2.0, 10.0 and 20.0 mg per rat per day to six different groups of rats. The periods of treatment were (1) acute; 6 and 24 hr (2) sub-acute; 7 days, 1 month and 3 months and (3) chronic, 6 months.

After the completion of the treatments, the animals were sacrificed by instant decapitation. Trunk blood was collected and the serum was obtained by centrifuging the whole blood at 3000 x g for 10 min in a refrigerated centrifuge. Livers were dissected out, wiped free of blood and kept in crushed ice till they were weighed. A 10 per cent homogenate of the livers was prepared in 0.32 M sucrose solution and centrifuged at 3000 x G for 30 min. The cell debris free supernatent was used as the enzyme source. Activities of hepatic GOT and GPT were assessed according to the method of Reitman and Frankel[13]. Serum alkaline phosphatase activity was estimated as per the method

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TABLE 1. EFFECT OF LAC DYE ON HEPATIC GOT AND GPT ACTIVITIES

Dose mg/rat/day	Acute treatment 6 hr	Acute treatment 24 hr	Sub-acute treatment 7 days	Sub-acute treatment 1 month	Chronic treatment 3 months	Chronic treatment 6 months
			Hepatic GOT[a]			
0	17.0 ± 2.1	20.1 ± 1.4	16.7 ± 0.1	16.5 ± 0.3	15.4 ± 0.9	13.0 ± 0.3
0.2	15.9 ± 1.7	19.2 ± 1.4	17.3 ± 0.3	16.4 ± 0.3	15.4 ± 0.7	12.4 ± 0.1
1.0	15.7 ± 1.7	20.5 ± 1.6	17.4 ± 0.6	17.2 ± 0.5	15.2 ± 0.6	12.1 ± 0.1
2.0	15.5 ± 1.4	21.7 ± 0.3	17.0 ± 0.2	16.8 ± 0.2	15.2 ± 0.5	11.6 ± 0.4
10.0	15.7 ± 1.9	19.0 ± 1.3	17.4 ± 0.3	17.2 ± 0.3	15.2 ± 0.4	12.0 ± 0.1
20.0	17.2 ± 0.5	18.7 ± 0.8	16.9 ± 0.5	16.8 ± 0.6	15.8 ± 0.5	10.7 ± 0.2
			Hepatic GPT[a]			
0	11.1 ± 1.30	12.1 ± 0.4	11.9 ± 0.8	13.5 ± 0.6	13.1 ± 0.9	13.6 ± 0.02
0.2	10.5 ± 0.20	13.7 ± 0.6	12.6 ± 0.7	13.1 ± 0.2	13.3 ± 0.6	13.6 ± 0.40
1.0	11.0 ± 0.02	12.5 ± 1.0	12.6 ± 0.8	13.3 ± 0.2	13.0 ± 0.2	13.5 ± 0.10
2.0	11.6 ± 1.60	12.9 ± 0.8	12.4 ± 0.5	13.2 ± 0.1	13.2 ± 0.1	13.7 ± 0.20
10.0	9.8 ± 0.10	12.3 ± 0.4	12.2 ± 1.4	13.2 ± 0.2	13.3 ± 0.3	13.6 ± 0.20
20.0	12.7 ± 0.20	12.3 ± 0.5	12.0 ± 0.2	13.4 ± 0.1	13.1 ± 0.2	14.4 ± 0.30

a. μg Na-pyruvate liberated / mg protein / hr.
Mean ± SEM of 3 independent determinations.
Experimental not significantly different from controls.

TABLE 2. EFFECT OF LAC DYE ON SERUM ALKALINE PHOSPHATASE, SGOT AND SGPT ACTIVITIES

Dose mg/rat/day	Acute treatment 6 hr	Acute treatment 24 hr	Sub-acute treatment 7 days	Sub-acute treatment 1 month	Chronic treatment 3 months	Chronic treatment 6 months
			Serum Alkaline Phosphatase[a]			
0	4.8 ± 0.1	6.4 ± 0.5	4.6 ± 0.3	5.2 ± 0.3	4.7 ± 0.4	6.4 ± 0.4
0.2	4.2 ± 0.2	6.3 ± 0.2	4.7 ± 0.8	5.0 ± 0.8	4.9 ± 0.2	6.6 ± 0.4
1.0	4.0 ± 0.2	5.5 ± 1.0	4.3 ± 1.5	4.8 ± 1.1	5.0 ± 0.2	6.8 ± 0.3
2.0	4.8 ± 0.5	6.7 ± 0.3	5.2 ± 0.3	5.3 ± 0.3	4.6 ± 0.7	6.4 ± 0.5
10.0	4.6 ± 0.3	6.4 ± 0.6	4.9 ± 0.1	4.8 ± 1.4	5.1 ± 0.4	6.3 ± 0.7
20.0	4.5 ± 0.4	6.3 ± 0.2	5.1 ± 0.7	5.1 ± 0.2	4.9 ± 0.4	6.4 ± 0.4
			SGOT[b]			
0	2.4 ± 0.3	2.4 ± 0.7	3.1 ± 0.2	3.1 ± 0.3	3.1 ± 0.2	3.8 ± 0.4
0.2	2.6 ± 0.4	2.2 ± 0.6	3.2 ± 0.9	2.7 ± 0.7	3.2 ± 0.3	3.7 ± 0.9
1.0	2.7 ± 0.5	2.5 ± 0.2	3.2 ± 0.2	2.9 ± 0.5	2.6 ± 0.3	3.9 ± 0.7
2.0	2.5 ± 0.3	2.6 ± 0.8	2.9 ± 0.5	2.9 ± 0.3	2.8 ± 0.4	4.1 ± 0.9
10.0	2.7 ± 0.3	2.9 ± 0.6	3.1 ± 0.3	2.9 ± 0.2	2.9 ± 0.5	3.9 ± 0.4
20.0	3.0 ± 0.7	2.6 ± 0.5	3.3 ± 0.3	2.8 ± 0.5	3.9 ± 0.3	3.9 ± 0.2
			SGPT[b]			
0	1.2 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	1.9 ± 0.1	0.8 ± 0.1	1.5 ± 0.3
0.2	1.2 ± 0.3	1.6 ± 0.3	1.9 ± 0.1	1.9 ±0.2	0.9 ± 0.2	1.7 ± 0.3
1.0	1.2 ± 0.2	1.3 ± 0.3	1.5 ± 0.6	1.5 ± 0.3	0.9 ± 0.1	1.6 ± 0.4
2.0	1.3 ± 0.3	1.5 ± 0.3	1.3 ± 0.9	1.9 ± 0.2	0.8 ± 0.1	1.5 ± 0.8
10.0	1.3 ± 0.4	1.6 ± 0.3	1.5 ± 0.5	1.7 ± 0.2	0.9 ± 0.1	1.5 ± 0.3
20.0	1.2 ± 0.3	1.5 ± 0.3	1.6 ± 0.7	1.5 ± 0.3	0.9 ± 0.2	1.3 ± 0.4

[a] μ g PNP liberated/mg protein/hr. [b] μg Na-pyruvate liberated/mg protein/hr.
Mean ± SEM of 3 independent determinations. 'Experimental' not significantly different from controls.

of Urrego and Epstein[14]. Protein was measured according to the method of Gornall *et al*[15]. Statistical analysis of the data was done using students 't' test taking $P < 0.05$ as significant level.

Results presented in Table 1 reveal that acute, sub-acute and chronic administration of lac dye virtually had no effect on the activities of hepatic GOT and GPT when compared to respective controls ($P > 0.90$ to >0.05) at all doses. Observations on the activities of the serum enzymes i.e. serum alkaline

phosphatase, SGOT and SGPT (Table 2) also indicate that there is no significant change in the activities of these enzymes after lac dye feeding.

Transaminations catalysed by GOT and GPT in the liver, are key reactions in maintaining a balance of the amino acid pool in the system[16,17] and also serve as important links between the metabolism of protein and amino acids with that of carbohydrates[18]. Therefore, absence of any change in the activities of both these enzymes indicate that lac dye may not interfere with these cellular processes. This is further confirmed by normal activities of serum enzymes *viz.* alkaline phosphatase, SGOT and SGPT, indicating normal functioning of the liver in the dye fed animals. As a matter of fact, the activities of these enzymes are known to increase in the serum whenever there is any damage to the liver[19,20]. Thus, from the present investigation it appears that lac dye might not have any toxic effect on the hepatic tissue. This study gets further support from some previous observations, reported from this laboratory indicating that lac dye has no deleterious effect on certain other hepatic components in experimental animals[21-25]. Multi-generation studies have also indicated the dye to be non-toxic[26]. A recent report also confirms lac dye to have no mutagenic activity at least in the mammalian system and also does not possess any tumour promoting activity[12].

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J. Fd. Sci. Technol., 1988, Vol. 25, No. 2, 97–98

HAEMATOLOGICAL AND SERO-ENZYMOLOGICAL CHARACTERISTICS OF ALBINO RAT INDUCED BY DIPHENYL BIS-AZO BINAPHTHIONIC ACID (CONGO RED)

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The effects of sublethal dose (70 mg/kg body wt) of Congo red (CI 22120) on haematology and sero-enzymology were studied in albino rat at 30 and 60 days after oral administration. The RBC count and packed cell volume decreased significantly and it was associated with a rise in WBC count. Hb content also increased after 60 days of treatment. Mean corpuscular volume and mean corpuscular haemoglobin concentration too, increased markedly. ESR exhibited increased values while clotting time was normal after long term treatment. Succinic dehydrogenase showed a significant decrease, whereas malic dehydrogenase and lactic dehydrogenase were elevated; however, there was no significant change in pyruvic dehydrogenase. Serum transaminases (GOT and GPT) were elevated after azodye administration.

Prevention of Food Adulteration Rules, India[1] has placed the dye, Congo red (CI 22120; Diphenyl dis-azo binaphthionic acid, DDBA) in the list of non-permitted (in edibles) dyes. Joint FAO/WHO Experts' Committee on Food Additives[2] includes it in the group C II according to toxicological categorisation which reflects that no long term effects of the dye are known.

Whereas literature on haematological responses to azodyes is available[3,4], there is paucity of information regarding Congo red in mammals. Recently, a few reports limited to aquatic animals have been published from our laboratory[5,6]. The present communication deals with haemato-enzymological responses of albino rats fed DDBA for 30 and 60 days.

Thirty healthy female rats (*Rattus rattus*, albino) from the departmental rattery in the weight range 125-140 g were used. The animals were divided into two groups of 20 and 10. The animals of the 1st group were administered 70 mg/kg body wt of DDBA (Sandoz India Ltd., in distilled water) orally by force-feeding on alternate days. The 2nd group served as control. All the rats were maintained in laboratory conditions (Temp. 22 ± 2°C) on pellet diet and water *ad libitum*. The rats were sacrificed after 30 and 60 days to obtain blood samples and serum for analysis.

Fresh blood was used to determine red blood corpuscles (RBC), white blood corpuscles (WBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC)[7] as also clotting time (CT)[8] and erythrocyte sedimentation rate (ESR)[9]. The enzymes, succinic dehydrogenase (SDH, EC: 1.3.99.1) and pyruvic dehydrogenase (PDH, EC: 1.2.4.1)[10], malic dehydrogenase (MDH, EC: 1.1.1.37)[11], lactic dehydrogenase (LDH, EC: 1.1.1.27), glutamic oxalacetic transaminase (GOT, EC: 2.6.1.1.) and glutamic pyruvic transaminase (GPT, EC: 2.6.1.2)[12] were determined in serum. The data were statistically analysed by student's 't' test for evaluating differences.

Haematological and sero-enzymological data are presented in Tables 1 and 2, respectively. The data reveal a significant decrease in RBC and PCV but an increase in Hb content, WBC, MCV and MCHC. CT increased in short term feeding but became normal after 60 days. ESR was however, enhanced both at 30 and 60 days after treatment. Serum enzymes LDH, MDH, SGOT and SGPT were elevated significantly whereas SDH activity decreased. Though there was a slight decrease in PDH, it was not significant.

A significant tall in RBC reflects erythropenia in dye fed rats which appears as a sequel to direct injurious action of the toxicant on rats[13]. Further, increase in MCV, MCHC, ESR and PCV may also be

TABLE 1. HAEMATOLOGICAL ALTERATIONS IN ALBINO RATS ADMINISTERED CONGO RED

	Control	30 days	60 days
RBC (x 10^6/mm^3)	6.89 ± 0.09	6.03 ± 0.07**	4.91 ± 0.05**
WBC (x 10^3/mm^3)	10.40 ± 0.85	17.00 ± 0.87*	32.60 ± 1.46**
Hb (%)	15.63 ± 0.16	14.77 ± 0.26	19.30 ± 0.38**
PCV (%)	44.22 ± 1.87	36.87 ± 1.20*	33.46 ± 1.21*
MCV (μm^3)	64.18 ± 0.56	61.20 ± 1.00	68.10 ± 0.70*
MCHC (g/dl)	35.35 ± 0.36	40.05 ± 0.70**	57.69 ± 1.20**
Clotting time (sec)	130.70 ± 2.95	155.00 ± 0.50**	134.33 ± 2.02
ESR (mm/hr)	1.34 ± 0.09	2.44 ± 0.02**	2.88 ± 0.03**

All the values are mean ± S.E. (10 estimations)* $P < 0.05$;** $P < 0.01$.
PCV: Packed cell volume; MCV: Mean corpuscular volume; MCHC: Mean corpuscular haemoglobin concentration; ESR: Erythrocyte sedimentation rate.

TABLE 2. SERO-ENZYMOLOGICAL PROFILES OF ALBINO RATS ADMINISTERED CONGO RED

Enzyme	Control	30 days	60 days
LDH (unit/ml)	73.53 ± 0.35	84.10 ± 0.19**	107.18 ± 1.78**
PDH (mg formazon/ml)	1.64 ± 0.09	1.64 ± 0.07	1.43 ± 0.01
SDH (mg formazon/ml)	2.42 ± 0.04	2.11 ± 0.07*	1.69 ± 0.09**
MDH (Sigma unit/ml)	32.30 ± 0.10	45.70 ± 0.06**	38.20 ± 0.10**
GOT (Sigma Frankel unit/ml)	20.55 ± 0.02	30.69 ± 0.02**	41.31 ± 0.04**
GPT (Sigma Frankel unit/ml)	10.82 ± 0.02	21.05 ± 0.04**	52.77 ± 0.04**

All values are mean ± S.E. (5 estimations); *P <0.05; **P< 0.01.
LDH: Lactic dehydrogenase, PDH: Pyruvic dehydrogenase; SDH: Succinic dehydrogenase; MDH: MALIC dehydrogenase
GOT: Glutamic oxalacetic transaminase; GPT: Glutamic pyruvic transaminase.

the cause of anaemia[14]. However, increase in Hb content is of unknown pattern. Variations in clotting time are considered to be due to the disturbances in clotting factor(s) induced by toxicant[13]. The results are in agreement with our previous studies made with metanil yellow[3]. Leucocytosis in response to DDBA may be a consequence of direct stimulation of immunological defence against the exogenous substance or due to tissue damage under chemical stress. It may be possible that the toxicant evoked a change in the homeostatic mechanism of the rat expressed as leucocytosis[3,4].

Lactic, succinic, pyruvic and malic dehydrogenases are considered to be most important enzymes in tissue respiration. Significant changes in these enzymes of serum reflect the disturbed enzyme levels of liver affecting energy metabolism. Depleted SDH activity confirms the damage to mitochondria which would lead to variation in several other enzymes of tricarboxylic acid cycle (TCA)[15]. Altered SDH and MDH would impair the oxidative releasing processes which ultimately may lead to cellular degeneration[16]. Increased LDH in serum revealed the altered anaerobic tissue respiration. Our findings are in consonance with the observations of Chenoweth and Ellman[17] who demonstrated that enzymes of metabolism may come out of the tissue into circulating fluid in intoxicated animals. The elevation in serum transaminases (SGOT and SGPT) may be attributed to tissue damage, or to their increased synthesis or to decreased transmination reactions. Increase in both the enzymes of serum is a common finding in liver disease[18].

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J. Fd. Sci. Technol., 1988, Vol. 25, No. 2, 99–100

IMPROVEMENT OF CHIP COLOUR BY REMOVAL OF COLOUR REACTANTS FROM POTATO TUBER SLICES BY WATER WASHING

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Reducing sugars, free amino acids and total phenolics of potato tuber slices of variety 'Kufri Chandramukhi' were lowered significantly when slices were washed with plain water. First washing was more effective in removing phenolics and free amino acids whereas effective removal of reducing sugars was observed only after the third washing.

Sun-drying of potato slices for preparing snacks is very popular in our country. These dried slices are served as potato chips after deep-fat-frying. The quality of chips varies due to discolouration or darkening of slices during sun-drying and browning during frying. In the darkening of slices, phenolic compounds are implicated[1] and the browning which develops during frying is due mainly to a reaction between reducing sugars and free amino acids.[2]. It has been earlier observed by the authors that the colour of potato chips, prepared from variety 'Kufri Chandramukhi', is improved by repeated washings of raw potato slices with plain water prior to blanching[3]. Therefore, it was of interest to investigate whether this improvement was due to removal of colour reactants viz. reducing sugars and free amino acids and also phenolics from potato tuber slices during washing.

Potato tubers of variety 'Kufri Chandramukhi' were obtained from Central Potato Research Station, Modipuram, Dist. Meerut. After peeling, tubers were cut into thin slices (0.16 to 0.18 cm) with a hand rotating slicing machine. Immediately, the slices were immersed in plain water (500 g slices in 1.5 l of water). The water containing slices, were agitated gently for 5 min and then drained off. The process of washing was repeated for a second and a third time. For each treatment (number of washings), three replicates of 500 g slices each were taken. After giving the desired number of washings, the excess water was drained off by placing the slices on a single layer of muslin cloth for one min. The slices were then processed for chemical analysis.

Sampling for chemical analysis: From each replicate, a total of 21 slices were obtained (comprising 7 small, 7 medium and 7 large slices), cut into one cm wide strips and mixed thoroughly to obtain a composite sample.

Extraction and estimation of colour reactants: Total phenolics, free amino acids and reducing sugars were extracted twice from 10 g composite sample by refluxing for 1 hr with isopropanol and then making up the volume to 100 ml. The phenolics were estimated by Folin-Denis reagent[4]. The free amino acids and reducing sugars were first obtained in aqueous solution after evaporating isopropanol and estimated by ninhydrin[5] and arsenomolybdate[6] reagents respectively. Chlorogenic acid, glycine and glucose were used as standards for phenolics, amino acids and reducing sugars respectively.

Results given in Table 1 indicate that the contents of phenolics, free amino acids and reducing sugars, can be brought down significantly by water washings and would partially explain our earlier report regarding improvement in colour of potato chips by repeated water washings[3]. The loss of free amino acids, however, may not be desirable from the nutritional point.

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TABLE 1. EFFECT OF WASHING ON POTATO SLICES

No. of washings to slices	Total phenolics		Free amino acids		Reducing sugars	
	(mg/100 g)	Reduction (%)	(mg/100 g)	Reduction (%)	(mg/100 g)	Reduction (%)
Fresh	47	–	447	–	163	–
Once	42	10.7	295	34.0	134	17.8
Twice	41	12.8	265	40.7	112	31.3
Thrice	39	17.0	272	39.1	81	49.7
S.E.M.	1.7		42		27	
CD (0.05)	3.8		97		63	

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J. Fd. Sci. Technol., 1988, Vol. 25, No. 2, 101–102

VOLATILE AND NON-VOLATILE ACIDS PRODUCED BY *CLOSTRIDIUM SPOROGENES* ISOLATED FROM PROCESSED CHEESE

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Five strains of *Clostridium sporogenes* isolated from canned processed cheese were grown in peptone yeast extract glucose broth and examined for volatile and non-volatile acids. Among volatile acids, acetic acid (9.8-46.6%) and butyric acid (18.2-28.8%) were found in all the culture filtrates while isobutyric (0-18.8%), isovaleric (0-27.8%), valeric (0-11.3%), isocaproic (0-31.1%) and caproic acid (0-0.9%) were found in some of the cultures. None of the strains produced propionic acid. Lactic acid (60-100%) and succinic acid (0-40%) were the non-volatile acids.

The importance of clostridia as spoilage and pathogenic organisms is well known[1-3] in canned dairy products. *Clostridium sporogenes* is probably the most common cause of spoilage in processed cheese[4], although other clostridia have also been implicated. These micro-organisms are identified based on several physiological and biochemical characteristics. Among these, the estimation of products of fermentation is considered more reliable for their identification and has attracted much attention[5]. The advantages of gas-liquid chromatography for detecting volatile and non-volatile end products as an aid in the identification of the microorganisms have been very well brought by several workers[5-7]. This investigation pertains to the GLC estimation of volatile and non-volatile acids produced by five isolates of *Clostridium sporogenes* isolated from commercial processed cheese samples.

The cultures were grown in peptone-yeast extract-glucose (PYG) broth of the composition: peptone, 0.5g; trypticase, 0.5g; glucose, 1.0g; yeast extract, 1.0g; resazurin solution (0.1 per cent), 0.4 ml; salt solution, 1.0 ml; vitamin K, 0.02 ml; cysteine-HCl, 0.05g and water, 100 ml. The salt solution contained calcium chloride (anhydrous), 0.2g; magnesium sulphate (anhydrous), 0.2g; dipotassium phosphate, 1.0g; monopotassium phosphate, 1.0g; sodium bicarbonate, 10.0g; sodium chloride, 2.0g and water, 1000 ml. The incubation was carried out at 37°C for 24 hr.

Analysis of volatile and non-volatile acids was carried out on a Packard 804 model GLC apparatus using 10 per cent FFAP (Pye Unicam Ltd) packed in a 1.8 m long and 3 mm i.d. glass column and flame ionization detector. The column temperatures were 180°C for volatile acids and 155°C for non-volatile acids with injection port temperature 220°C. The gas flow rate was 40 ml/min N_2. The preparation of the sample for volatile and non-volatile acids was based on the methods suggested by earlier workers[8-11]. GLC analysis was carried out according to Gupta and Murugesan[12]. Positive controls comprised volatile and non-volatile acids. Distilled water and uninoculated medium treated identically served as negative controls.

Acetic, butyric and lactic acids were invariably produced by all the strains of *C. sporogenes*: smaller quantities of isobutyric, isovaleric, valeric and isocaproic acids were also detected. Lactic acid and succinic acid were the two non-volatile acids (Table 1). Although the growth of *C. sporogenes* in PYG and processed cheese is not comparable, this study gives an indication that the taint produced by various strains of this organism in processed cheese may partially be due to the production of these volatile and non-volatile acids. In this study, all the five strains produced butyric acid although Lewis *et al*[7] and Holdeman *et al*[8] reported the absence of butyric acid in some of the strains they examined. None of the isolates examined produced propionic acid. It is known that in obligate anaerobes propionic acid is produced either from lactic or succinic acids[13]. But in the present study although lactic acid was formed in large quantities, none of it appeared to be routed towards the production of propionic acid. This finding merits further investigation.

TABLE 1. PRODUCTION OF VOLATILE AND NON-VOLATILE ACIDS BY VARIOUS STRAINS OF *C. SPOROGENES*

Type of acid	Isolate No.				
	5	15	57	120	136
Volatile (%)					
Acetic	10.2	11.2	9.8	46.6	28.5
Isobutyric	16.6	18.8	12.2	7.3	0
Butyric	18.2	28.8	28.5	37.9	71.5
Isovaleric	16.9	27.8	26.9	9.1	0
Valeric	7.6	6.2	11.3	0	0
Isocaproic	31.1	7.1	10.3	0	0
Caproic	0	0	0.9	0	0
Non-volatile (%)					
Lactic	100	60	100	95.5	93.6
Succinic	0	40	0	4.5	6.4

Propionic acid was absent in all the cultures examined. Values are means of three replicates.

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J. Fd. Sci. Technol., 1988, Vol. 25, No. 2, 103-104

MICRO-ORGANISMS ASSOCIATED WITH PRAWN PICKLE SPOILAGE

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A study of microorganisms in three samples of commercial prawn pickles showing signs of spoilage indicated that bacteria might have been responsible for spoilage. Some of the bacterial isolates were acid and salt resistant.

Pickled prawns are considered a delicacy and are generally expected to have a shelf-life of about 6 months at ambient temperature. Preparation of prawn pickle involves such steps as blanching, heating in oil and cooking which are expected to destroy most of the micro-organisms associated with it. The product generally has a pH of about 4.5 and contains salt, spices and vinegar which add to its preservative quality. In addition to these, preservatives like sodium ascorbate and sodium benzoate are added to increase the shelf-life.

In this communication, we report about micro-organisms encountered in some commercial samples of prawn pickles showing signs of spoilage. To our knowledge, this appears to be the first study on microbial spoilage of prawn pickles.

Three commercial prawn pickle manufacturers (designated A, B and C) delivered sealed samples of pickles showing signs of spoilage for laboratory analysis. The sealed bottles were opened and 50 g samples were drawn aseptically for microbiological examination. The samples were then examined for odour and texture. pH of the samples were determined using a digital pH meter and salt content as described in AOAC manual[1]. Total bacterial count was done on plate count agar, halophile count using halophilic agar and yeast and mold count using mycological agar containing penicillin and streptomycin. All these analyses were carried out as described by Speck[2]. Briefly, 50 g sample was homogenised with 450 ml phosphate bufferred saline and 0.1 ml aliquots of tenfold dilutions thereof were inoculated to respective media by surface spread method. Plates were incubated at room temperature (29 ± 2°C) and observed upto seven days. Representative colonies appearing on plate count agar were picked up for purification, characterisation and identification upto generic level using the technique of LeChavallier *et al.*[3]

Eleven representative isolates were chosen for study of salt and pH tolerance. Salt tolerance was studied in nutrient broth of pH 7.4 containing the following concentrations of NaCl (per cent) 0, 0.5, 3, 6, 10, 20 and 30. The broth was dispensed in 10 ml quantities and sterilised by autoclaving. Aliquots (0.1 ml) of test cultures grown in nutrient broth (unamended) overnight at room temperature (29 ± 2°C) were inoculated to two tubes each containing salt concentrations mentioned above and incubated at room temperature for one week. Growth was recorded at daily intervals as degree of turbidity.

For study of pH tolerance, nutrient broth of pH ranging from 2.8 to 7.6 at intervals of 0.8 was prepared by adding varying amounts of 0.2 M K_2HPO_4 and 0.1 M citric acid as described by Salle[4]. Two tubes each containing 10 ml nutrient broth of a particular pH value were inoculated with 0.1 ml of test culture grown overnight in nutrient broth (unamended) at room temperature. Tubes were incubated at room temperature for a week and growth recorded as degree of turbidity.

All the three samples of pickle showed slight off-odour, soft texture and gas formation. As indicated in Table 1, the samples had pH in the range 4.43-4.58 and salt content in the range 3.6-3.8 per cent. Sample II showed exceedingly high number of bacteria including halophiles. Yeast and mold count were nil in samples II and III and very marginal in sample I suggesting that these are not responsible for the spoilage and perhaps bacteria are the probable cause of spoilage.

A perusal of literature indicated that there is lack of information on micro-organisms associated with prawn pickles. Pradhan *et al.*[5,6] reported on microbiology of spoiled mango pickles and noted that many acid and salt tolerant micro-organisms were responsible for spoilage. Results in Table 2 show the bacterial types isolated from different pickle samples and their pH and salt tolerance. In spoilage of prawn pickle,

TABLE 1. BIOCHEMICAL AND MICROBIOLOGICAL CHARACTERS OF SPOILED PRAWN PICKLES

Manufacturer code	Sample No.	pH	Salt (%)	Total plate count/g	Halophile count/g	Yeast and mold count/g
A	I	4.47	3.8	9×10^6	1.4×10^4	10
B	II	4.58	3.7	1×10^9	3.0×10^7	Nil
C	III	4.43	3.6	2×10^6	2.6×10^4	Nil

Values are means of 3 determinations

TABLE 2. ACID AND SALT TOLERANCE OF REPRESENTATIVE ISOLATES

Sample	Representative isolate	Lowest pH for growth	Highest salt concn showing growth (%)
I	*Staphylococcus*	3.6	10
	Bacillus	4.4	3
	Yeast	2.8	10
II	*Staphylococcus*	2.8	10
	Pseudomonas	2.8	0.5
	Bacillus	4.4	3
	Arthrobacter	4.4	0.5
	Acinetobacter	5.2	0.5
III	*Bacillus*	4.4	10
	Staphylococcus	4.4	10
	Micrococcus	5.2	6

Values are means of 3 determinations

perhaps, acid tolerant micro-organisms are more important than salt tolerant ones since the salt content (4-5 per cent) is low. It is to be noted that some bacteria like *Staphylococcus* and *Pseudomonas* could grow at pH values as low as 2.8. However, the poor salt tolerance of the latter makes it unlikely to proliferate in prawn pickle. The presence of such bacteria as *Pseudomonas*, *Arthrobacter* and *Acinetobacter* (accounting to about 5 per cent of the flora) which had low salt tolerance but good acid tolerance in sample II suggests that there were micro-environments where salt penetration had not taken place. Perhaps, bacteria like *Bacillus*, *Staphylococcus* and *Micrococcus* which showed both acid and salt tolerance were responsible for spoilage of the pickles noted here. Yeasts and molds did not play a major role in the spoilage of prawn pickle samples. This is in contrast to the report that both bacteria and molds and yeasts are involved in spoilage of mango pickles[5]. This calls for a detailed study of the microbiology of prawn pickles. The source from which these acid and salt tolerant bacteria are derived also needs to be identified.

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EFFECT OF POTASSIUM ON NITROGEN AND CARBOHYDRATE CONTENTS OF TEA LEAVES (*CAMELLIA SINENSIS* (L) O. KUNTZE) AND QUALITY OF MADE TEAS+

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High dose of potassium i.e. 180 kg/ha applied to tea bushes, accelerates the conversion of theanine to glutamic acid, which is finally incorporated into proteins. Potassium also appears to help in the conversion of water-soluble carbohydrates to polysaccharides and results in decrease of theaflavins and thearubigins of made teas, hence affecting its quality.

The quality of made teas is dependent upon its chemical constituents, hence that of green leaves. In view of the very high content of polyphenols and their dominating role in liquor characters of tea[1] the role of other components, namely amino acids, carbohydrates etc. has not been explored extensively. The most abundant amino acid in tea leaf is theanine, which constitutes upto 60 per cent of the total amino acids, the other major amino acids being glutamic and aspartic acids[2]. Theanine has been reported to contribute towards tea quality[3]. No information is available on the effect of potassium on nitrogen and carbohydrate contents in tea plants and quality of made tea from tea bushes receiving high doses of potassium. The present communication reports the effect of potassium on the amino acids, carbohydrates, protein and crude fibre contents of green tea leaves and on theaflavin and thearubigin contents of made tea, since the high quality in tea has been correlated with high levels of theaflavins and adequate amounts of thearubigins[4-5].

The tea shoots were plucked from May to November at fortnightly intervals from tea bushes of clone 'TV 2' (*Camellia sinensis* var. assamica) receiving 0, 45 and 180 kg potash (as muriate of potash) with 135 kg nitrogen as ammonium sulphate and 45 kg phosphate in all treatments in the Borbhetta Experimental Farm of Tocklai Experimental Station, Jorhat, Assam. The experiment was laid in randomised block design. From dried leaves, water soluble carbohydrates were determined by the method reported earlier[6]. Reducing sugars were estimated following the method of Nelson[7]; non-reducing sugars were calculated by difference. Crude fibre was determined by the A.O.A.C. method[8]. After separation by paper chromatography, amino acids were determined using ninhydrin reagent as described by Bhatia and Deb[9]. Total nitrogen was determined by micro-kjeldahl method. Soluble nitrogen (amino acid N + caffeine N) was determined by the method reported by Wood *et al.*[3] Protein nitrogen was derived from the difference between total nitrogen and soluble nitrogen. Protein content was obtained by multiplying protein nitrogen with 6.25. Black tea was prepared from green tea shoots following Robert's method[10]. Theaflavin and thearubigin contents were estimated by the method of Roberts and Smith[4]. Teas were evaluated by two different taster's panels, one located at Calcutta and the other at Tocklai.

The quantitative changes of the major amino acids in tea shoots under different levels of potassium are given in Table 1, which shows that theanine concentration decreased and that of alanine, aspartic acid and glutamic acid increased as the potassium dose was raised from 0 to 45 kg/ha. Further increase in potassium dose from 45 to 180 kg reduced the levels of theanine, glutamine, valine and total amino acids, whereas there was no significant change in the contents of aspartic acid, glutamic acid and alanine. The decrease in theanine levels at the two levels of

TABLE 1. CHANGES OF AMINO ACID LEVELS IN TEA SHOOTS WITH POTASSIUM APPLICATION*

Amino acid levels	Concn (mg/100 g dry) at indicated levels K_2O (kg/ha)			C.D. at 5% level of Signif.
	0	45	180	
Alanine	25.3	28.5	29.3	2.12
Aspartic acid	124.2	150.1	150.9	7.68
Glutamic acid	234.3	283.6	185.0	14.82
Serine	26.4	28.5	28.3	2.02
Threonine	16.2	16.9	16.7	1.89
Glutamine	45.0	47.5	42.7	4.49
Tyrosine	16.3	17.4	16.6	1.20
Leucine/isoleucine	14.6	15.5	15.1	0.86
Phenylalanine	14.1	15.4	14.8	1.10
Valine	14.2	15.3	13.7	1.32
Theanine	1470.0	1357.0	1267.0	74.32
Total	2001.4	1975.5	1881.0	78.42

*Mean of 14 determinations during the plucking season May-Nov.

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TABLE 2. EFFECT OF POTASSIUM ON CARBOHYDRATES, CRUDE FIBRE IN TEA SHOOTS AND THEAFLAVINS AND THEARUBIGINS IN MADE TEAS.

Constituents	Concn (mg/100 g dry) at indicated levels K_2O (kg/ha)			C.D. at 5% level of signif.
levels	0	45	180	
Reducing sugars	0.204	0.164	0.142	0.016
Non-reducing sugars	1.904	1.839	1.723	0.077
Total sugars	2.144	2.003	1.865	0.074
Crude fibre[a]	8.30	9.40	9.80	–
Protein[a]	13.68	14.09	14.93	–
Theaflavins	2.574	2.336	2.317	0.102
Thearubigins	16.078	15.805	15.379	0.755

[a]Average of three replications.

potassium application was quite significant. It is known that biosynthesis of theanine takes place from glutamic acid and ethylamine catalysed by L-glutamate ethylamine ligase and breakdown of theanine results in an increase in glutamic acid content in tea shoots[11]. Therefore, it is likely that potassium accelerates the degradation of theanine to glutamic acid, which is either involved in the biosynthesis of protein or is incorporated into catechins of tea as reported by Konishi and Takahashi[12]. Since there was no additional accumulation of glutamine in potassium treated bushes, it is apparent that glutamic acid is not being stored as glutamine.

Reducing and non-reducing sugars gradually declined when potassium dose was raised from 0 to 45 and 180 kg/ha, but crude fibre content increased progressively (Table 2). Concomitantly protein content also gradually increased with increasing concentration of potassium. Similar changes in the water soluble carbohydrates have been reported in orchard grass, Italian Rye grass, chilli leaves and sunflower[13-16]. Potassium deficient plants are reportedly rich in reducing and non-reducing sugars. It has been suggested that in the absence of adequate potassium, carbon skelton that would normally go into protein synthesis accumulates as carbohydrates[17]. The decreases in reducing, non-reducing and total sugar contents in tea shoots with potassium application may be due to the condensation of simple sugars to form polysaccharides as evidenced by increased crude fibre content or their conversion to amino acids or their translocation to root zone for storage as starch. The increase in protein content in the leaf with the application of potassium has been reported besides accumulation of alanine, aspartic acid and glutamic acid[18].

The fall in the concentration of theaflavins and thearubigins in teas with increased dose of potassium indicates that quality in tea is slightly affected. Tasters' evaluation also corroborates the chemical assessment of quality of tea. However, no significant difference in Tocklai tasters' evaluation was found, whereas Calcutta tasters' panel showed a significant difference in the value of tea manufactured from potassium fertilized tea bushes as compared to control samples.

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STUDIES ON INFESTATION OF ACID-STABILIZED RICE BRAN WITH FLOUR BEETLE (*TRIBOLIUM CASTANEUM* HERBST)

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Rice bran stabilized by treatment with concentrated hydrochloric acid at 4.0 and 5.5% levels was highly unfavourable for the multiplication of red flour beetle, *Tribolium castaneum* Herbst. When adults were released into the treated bran for 15 days, the population at the end of 45 days were respectively 3 and 1% of that observed with control bran. Infestation studies with egg, larval and adult stages indicated that the acid treatment had a detrimental effect on larvae particularly the younger ones. Larval survivors in treated bran failed to reach the adult stage even after 45 days.

Rice bran valued for its oil for industrial and edible purposes may be stabilized by inactivating the lipase by heat treatment[1], low temperature storage[2,3], gamma irradiation or by using chemicals[2]. Recently, a chemical method for stabilization of rice bran by treating with concentrated hydrochloric acid at 40 l/tonne of bran has been reported[4]. As the acid treated bran can be stored for a longer period than untreated bran, it was intended to know whether the treatment has any effect on insect infestation. The flour beetle, *Tribolium castaneum* Herbst is the most common pest of rice bran and it can breed on rice bran of oil contents 0 per cent and above[5]. *T. castaneum* at different life stages were released on bran treated with HCl at 2.5, 4.0 and 5.5 per cent levels to study their development and population growth and the results are presented.

Rice bran was stabilized with 2.5, 4.0 and 5.5 per cent HCl (v/w) as described elsewhere[4]. The pH of water extracts of the above samples were 4.9, 3.75 and 3.1 respectively whereas for control bran it was 6.2. The bran samples were divided into 15 and 75 g aliquots to study the effect of acid treatment on the development and population growth of *T. castaneum*.

Life stages of *T. castaneum* were obtained from established cultures maintained on whole wheat flour fortified with dried yeast (5 per cent) at 25°C and 70 per cent RH. Unsexed, 2 week old adults, 30 per replicate, were allowed to breed in bran samples (75 g each) in 7.5 × 11 cm size bottles, covered with muslin cloth. There were three replicates each for control and treated samples. At the end of 15 days, the surviving adults were noted and discarded. The progeny produced was counted and discarded every week for 4 weeks.

One day old eggs, 50 per replicate, were released to 15 g bran samples (4 replicates each) in 2.5 × 15 cm test tubes. Insects surviving at 30 days and total population at the end of 45 days were recorded. To another batch of 15 g bran samples, ten-day-old larvae, 20 per replicate, were released in 2.5 × 15 cm test tubes covered by muslin cloth. Insects surviving as larvae, pupae, and adults were noted at the end of 15 days. The immature stages were returned to their containers for further observations whereas the adults were discarded. Counts were again made at 30 days. There were four replicates each for control and treated bran samples. Throughout the experiments, the test bottles and tubes containing bran samples were kept at the rearing temperature and RH. except during data collection. Analysis of variance and Duncan's multiple range test[6] were carried out after appropriate transformation of the data into angles or square roots.

In experiments with adults, there was significant reduction in multiplication of *T. castaneum* in acid stabilized bran as indicated by the size of the population at the end of 45 days (Table 1). Acid treatment even at 5.5 per cent level did not kill the parent adults. Nevertheless, the final population was notably reduced to 3 and 1 per cent of untreated controls respectively at 4.0 and 5.5 per cent acid treatment. Together the population at these levels was composed only of immature stages (mostly larvae) indicating the inhibitory effect on the development of the immature stages.

When eggs were released significant mortality occurred during development; hence survivors at the end of 30 days were much less than control at the two higher levels of acid treatment (Table 2). Also the insect population at 45 days was negligible (less than 1 per cent) in acid treated bran as compared to that in normal bran. Furthermore, in 4.0 and 5.5 per cent acid treated bran, it was observed that the immature stages constituted about 50 and 100 per cent respectively among the survivors at 30 days. The data reveal that the insects succumbed at the larval stage and the survivors could not reach the adult stage and remained mostly as larvae. This was more obvious when 10-day old larvae were tested.

The acid in the bran may act as a contact and/or stomach poison. Some of the fatty acids like acetic acid and formic acids and its esters are known to act as weak contact insecticides[7]. One of the possibilities for the susceptibility of younger larvae could be due to

TABLE 1. MULTIPLICATION OF *T. CASTANEUM* IN NORMAL AND ACID TREATED RICE BRAN

HCl level in rice bran (%)	Total No. of adults		Population at 45 days*		% immature stages in the population
	Released	Alive at 15 days**	Average	Range	
0	90	84	1568	1358-1833	49.5
2.5	90	76	1817	1560-2005	88.7
4.0	90	74	49	33-58	100
5.5	90	80	1	0-3	100

*Average for 3 replicates; **Parent adults discarded at 15 days.

TABLE 2. DEVELOPMENT OF PRE-ADULT STAGES OF *T. CASTANEUM* IN NORMAL AND ACID STABILIZED RICE BRAN

Acid level in rice bran (%)	Experiment with eggs			Experiment with larvae	
	No. of surviving at 30 days	Immature stages among survivors	Population at 45 days	% of survivors at 30 days	% immature stages among survivors
0	29.0 ± 3.2^a	0.3 ± 0.4^a	393.8 ± 142.1^a	90 ± 7.1^a	10.4 ± 7.5^a
2.5	31.3 ± 2.2^a	0^a	327.0 ± 111.7^a	40 ± 14.7^b	18.7 ± 16.4^a
4.0	7.3 ± 0.5^b	5.5 ± 1.0^b	3.8 ± 1.0^b	35 ± 9.1^b	63.6 ± 25.1^b
5.5	3.8 ± 2.8^b	3.8 ± 2.8^b	1.5 ± 1.5^b	21 ± 11.1^b	71.0 ± 19.8^b

Means ± S.D. of four replicates.
Means of the same column not followed by a common superscript differ significantly ($P < 0.01$).

corrosive nature of HCl in bran. Insect eggs are well protected from the entry of chemicals including insecticides by the chorion and eggs are inactive like pupae, whereas the adults are provided with thick and selerotized cuticles. But, in the case of larvae, as they develop from one instar to the other, the cuticle becomes less permeable as their cuticles thicken[7,8]. Hence, it may be expected that the soft cuticles of younger larvae are easily vulnerable to the corrosive action of the acid in the bran. Once the cuticles are damaged the larvae loose control over water balance, immunity to pathogens, etc, which finally lead to death.

The acid in the diet on ingestion may also act as a protoplasmic poison[7]. Krishna and Saxena[9] reported that in *T. castaneum* both in adults and larvae the pH within the gut varies from 5.2 to 6.0 in all its regions except in the hind part of midgut of the larvae where the pH is 6.8 to 7.0. And the optimum pH for most enzymes in the digestive system is around 5.4 except for amylase, pH being 6.0[10]. Therefore, it is possible that an abnormal diet of bran containing HCl with less than 4.0 pH is not digested by the newly hatched larvae resulting in death or irregularities in development. However, Krishna and Saxena[9] observed that a change in dietetic pH does not affect the pH of the gut of older larvae and adults of *T. castaneum*. The acid may also act as a phagodeterrant inhibiting the feeding reflexes of larvae and adults. Delay in development of *T. castaneum* larvae is not unusual, particularly when exposed to unfavourable diets.[11]

The exact reasons for the susceptibility of larvae and the resultant check on the rate of multiplication of *T. castaneum* are not known at this stage. Nevertheless, the data reveal that stabilization of bran by acid treatment at or above 4.0 per cent level of HCl has the advantage of keeping the bran free from infestation by the red flour beetle. Further studies with other stored product insects in stabilized bran are in progress.

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BOOK REVIEWS

Food Analysis: Principles and Techniques, Vol. 4 Separation Techniques: Edited by Dieter W Gruenwedel and John R. Whitaker, Marcel Dekker, Inc. 270, Madison Avenue, New York, N Y 10016, 1987; pp: 480; Price: $ 99.75 US & Canada; $119.50 (All other countries).

Among the series of Volumes on Food Analysis, Principles and Techniques, this book is the fourth volume devoted exclusively to Separation Techniques. As claimed by the Editors, this volume on Separation Techniques really meets the need for an up-to-date detailed treatment of the methods of modern food analysis.

There are seven chapters describing the different separation techniques written by leaders in the field and each chapter is treated with consummate expertise to apply analytical techniques to problems associated with quality assurance, product development and food safety.

The first chapter on distillation written lucidly details simple, batch and continuous fractional, flash, steam, vacuum, molecular, azeotropic extractive distillations and sublimation. The flexibility of distillation has greatly increased its applications for research and many industrial uses. The chapter has also listed a typical industrial application of each type of distillation.

An emerging separation technique viz Membrane separation processes has been dealt in great detail in chapter 3 from an engineering point of view and is very informative. The basic properties of membranes and the driving forces and fluxes involved in membrane processes are briefly discussed in this chapter. Structure and properties of synthetic membranes with SEM photographs, their preparation and applications in tabular form has been included. Membrane separation processes used industrially viz. microfiltration, ultrafiltration, reverse osmosis, osmosis, dialysis, electro-dialysis and gas evaporation have been discussed individually with clarity. Concentration polarisation is the bane of membrane processes and is a factor governing the design of membrane separation equipment and systems. The designs of different membrane modules with figures given in the chapter is very useful and informative. The industrial applications of microfiltration, ultrafiltration, reverse osmosis and electrodialysis have also been briefly described.

The other chapters viz. 2, 4, 5, 6 and 7 deal with the different types of chromatographic separations. Ion exchange and affinity chromatography, Exclusion processes: Molecular-Sieve, adsorption and Gel filtration chromatography, Thin layer chromatography, High pressure liquid chromatography and Gas chromatography, are described in these chapters with adequate theory, description of the components involved in each, sample preparation and diverse applications in food analysis. Each chapter is endowed with references very useful for the reader to refer to the original papers for further information. It is gratifying to note that these chapters are a comprehensive mine of information on analysis of food components gathered in a single book which otherwise would have been a difficult task for a researcher to collect.

This volume of Food Analysis on Separation Techniques is a must for analysts and scientists in the field of food science, chemistry, biochemistry, nutrition, environmental chemistry and microbiology.

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R.R.L., TRIVANDRUM

Food Engineering and Process Applications, Vol 1 and 2: Edited by M. Le Maguer and P. Jelen Elsevier, Applied Science Publishers, London, 1986; pp: 1255; Price:

The two volumes are compilation of selected papers presented at the Fourth International Congress on Engineering and Food held at Edmonton, Canada between July 7 and 10, 1985. Volume 1 deals with papers relating to transport phenomena including properties; volume 2 deals with studies and reviews on selected aspects of unit operations of food engineering. Both the volumes are indexed for cross reference.

The engineering of processing of produce of agriculture for food concerns with science and technology of Agricultural and Chemical Engineering, and Food Science. As such, these volumes contain contributions from all these three areas. The foci of food engineering applications for countries such as India and those of the developed West are different in many respects; but the basics of the technology remain substantially the same. Therefore, this publication in spite of its accent on products, processes, designs and operations dealing with perspectives and end-uses not very common to India, is of considerable value as

a scientific document that details some of the latest of the technology base.

The first volume which is of 642 pages compiles 55 papers organized into sections on rheology; physical, thermodynamic and transport properties, kinetics and modelling, heat and mass transfer, and irradiation processing. The first section which is on rheology contains 8 papers dealing with liquid foods, gels, meat and microbial suspensions including an excellent contribution on rheology of starch-protein matrix. Sections on physical and thermodynamic properties and transport properties respectively cover 13 and 6 papers including measurements and correlations, mathematical models, and measurement techniques, all mainly attending to high moisture systems. The contribution dealing with mathematical treatment of percolation theory for prediction of thermal conductivity is particularly interesting. The section on kinetics and mathematical modelling contains 11 papers out of which three papers on kinetics (differential scanning colorimetry, ascorbic acid degradation in apples, growth of *cl. perfringens* in meat) and two papers on modelling of separation processes (cyclone, and solid-liquid extraction) are of very good quality. Heat and mass transfer operations account for 7 papers including a very good one on reactivation of baker's yeast as influenced by drying conditions. Aspects of thermal processing are dealt within 8 papers. Two papers one each dealing with particulate foods in water fluidized beds, and infra-red radiation process are particularly noteworthy. The last section which is on irradiation processing contains two papers of general interest.

Volume 2 which is of 613 pages deals with unit operations of freezing (7 papers), extrusion (8 papers), membrane processing and other separation techniques including expression (10 papers), aspects of genetic engineering and biotechnology (9 papers), energy in processing of foods (4 papers), industrial engineering and process control (9 papers), industrial engineering and process control (9 papers), and a section on food engineering in developing countries (4 papers). The presentations contained are relatively more applied. The section on freezing and extrusion are quite focussed, and in a fashion present the state-of-art. Both the sections are strongly recommended as starting points for further study. All in all, both the volumes contain good source material and should be read by all those engaged in teaching and research in food engineering.

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Development of Fermentation Technology at the Indian Institute of Science, Bangalore, India (1911-1952) by Dr. T.N. Ramachandra Rao, 1987; pp: 285; Price: Rs. 125 (Xeroxed copy).

The present work is a collection and compilation of research work on various aspects of Fermentation Technology carried out at the Indian Institute of Science, Bangalore, during the period 1911-1952 (although in the introduction, the period is referred to as 1914-1952). Dr. T.N.R. Rao, who has brought out this excellent volume states "One of my objectives has been to place before the younger generation of post-independence scientists in India, a vivid picture of the early working conditions in idea imition, method of working and achievement of final objectives. An Indianness is thinly visible all through this effort". This objective is well achieved by the painstaking, consistent, labour of love-effort of Dr. Rao in rummaging and bringing together from quite a wide variety of official and non-official (personal contacts) sources, relevant material pertaining to research work in diverse fields grouped together as "fermentation technology". The entire presentation centres more on the personalities of Dr. Gilbert John Fowler, Prof. M. Sreenivasaya and Dr. M.K. Subrahmanyam, their students and research associates and the research work carried out by them at Indian Institute of Science, Bangalore. Dr. Rao has a firm conviction, and the reviewer agrees with him, that the work of those years became the "foundation for the new technology (Bio-technology)".

Dr. Rao has divided this publication under five headings – (1) Introductory (2) Dawn of Fermentation Technology (3) Science Climate and Research Planning (4) Development of Fermentation Technology and (5) Postscript. Although all the subjects presented in this volume do not strictly fall under fermentation technology as is understood by the term currently, one can readily appreciate the justification for including comprehensively all work carried out by the three eminent scientists of the period. In fact, it provides an excellent historical perspective not only for the development of research in fermentation technology and allied subjects, but of the development of the Indian Institute of Science itself and the development of research in biological sciences in the country in general. Dr. Rao has seen this aspect and provided enough connected links in organisational matters and development of scientific research in various parts of the world and eminent scientists who contributed to it. This makes the volume not only readable with sustained interest,

but equally useful as a reference. In the introductory chapter, there is an account of the history of development of scientific research in India, the history of the Indian Institute of Science at Bangalore, the establishment of the Department of Applied Chemistry, the earliest work on citric acid at Bangalore, Madras and Calcutta (1911-1914), Dr. G.J. Fowler's contribution, the beginning of work on acetone-butanol fermentation, ethyl alcohol from mahua flowers, fermentation of cellulose, fermentation of toddy and acetic acid production. Prof. M. Sreenivasaya joined the Institute in 1919 and worked at the Institute for 34 years till 1953 when he retired from Indian Institute of Science, Bangalore. However, he continued his work at Central Drug Research Institute at Lucknow for another 3 years before returning to Bangalore to lead a retired life. His early work was on lac production and life cycle of lac insect, spike disease of sandal, enzymatic studies on chemistry and structure of proteins, biochemistry of indigo dyevat, nutrition of rice moth and their use as test animals for nutritional and vitaminic studies etc. The CSIR was set up in 1944 and the work in Fermentation Technology got a push through research schemes for industrial work on molasses, microbial type cultures and production of industrial enzymes. Actually, the late Sir J.C. Ghosh was Director of the Indian Institute of Science, took Prof. Srinivasaya under his department of General chemistry of which he was also the Head, and provided space for starting a section of Fermentation Technology in 1941-42. This reviewer had the good fortune to work with Prof. Srinivasaya in this new Section as one of the 18 students who became the Team for undertaking various research projects in fermentation Technology. Dr. Rao has enumerated in the IV Chapter on development of fermentation Technology, the research programmes on production of yeast, industrial enzymes, antibiotics, biochemical studies on type cultures, power alcohol production, production of lactic acid, citric acid, acetone-butanol, micro-biological formation of sulphur and the cytogenetics of yeast and fungi (started by Dr. M.K. Subrahmanyam and his associates).

Dr. M.K. Subrahmanyam's contribution to research in cytogenetics of yeast started in 1944 when he joined Prof. Sreenivasaya upto 1960 has been well reported. Studies on the nutrition of silk worms carried out during 1950-54 on the meristematic cells of plants during 1957-70 has been included in the post-script section of this volume which.concludes with a report on the Institution of Prof. M. Sreenivasaya Awards, a complete list of students and colleagues of Prof. Sreenivasaya and a brief up-dated biodata of some of the students who responded to Dr. Rao's invitation to send this information to him.

All in all, this is an excellent work and should serve as a model for producing comprehensive historical information of past scientific research and contributions by eminent scientists who served institutions for long periods in a dedicated effort to organise, plan and stimulate research in different disciplines at such national institutions as the Indian Institute of Science, Bangalore. This reviewer and surely all those who were privileged partners in this adventurous period, will join Dr. Rao in the tribute he pays to Prof. M. Sreenivasaya – Guru Dakshina he calls it – for shaping their scientific career, stimulating their thoughts and ideas for carrying out innovative research and for the impact he made on their minds so effectively. Prof. Sreenivasaya was no easy person to get along with and yet his scintillating mind and provocative approach to research problems commanded the respect and regard for the depth of his knowledge, wide reading and adaptation of ideas from various fields in his work.

The reviewer commends this volume most strongly to all new entrants to the field of biological research including bio-technology in various institutions and universities and also to all research workers and those interested in the application of fermentation technology research to industry. This is an invaluable document for all libraries of scientific institutions. It is hoped that the Indian Institute of Science will publish this as its own publication and encourage scientists in other disciplines to prepare similar documents recording the historical development of research during the early years which has contributed to the development of its present status.

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Packaging: Specifications, Purchasing and Quality Control: Third Edition, Revised and Expanded by Edmund A. Leonard, Marcel Dekker, Inc. 270, Madison Avenue, New York, N.Y. 10016, 1987, pp: 240; Price: $37.50 (US & Canada), $45.00 (All other countries).

In India, since the last few years, packaging as a means of value addition has been receiving tremendous attention. As a consequence, packaging users have become more quality conscious and are looking for better ways of drafting specifications, quality control schedules and purchase contracts. But there are very few publications on the subject. For the Indian reader, this revised and enlarged edition of the

book will be very useful, particularly at this point of time.

The author of the book is a very distinguished packaging technologist with broad interests and wide experience. In the ten chapters of the book, the author has discussed, though briefly, the various steps in the development of packaging specifications, Q.C. schedules and purchase documents. Being a Professor in Food Science, the author illustrated his explanations with examples mostly from food packaging.

In the first chapter, – Functional and Marketing Criteria in Specifications, the author starts with definitions of a few common terms and a list of six steps in the development of package specification and covers primarily those entries relevant to marketing objectives, product protection, consumer function and graphic treatment. He emphasizes that specifications should be flexible for adoption to change and the necessity to preserve them, even though they are out-dated, for a future review of the development. He shows the necessity of inclusion of functional/ performance clauses besides the material and structural descriptions. He rightly points out that packaging graphics do not originate from aesthetic motives but from communication functions.

In the Second chapter, Manufacturing and Cost Criteria in Specifications, he discusses the structure (a major determinant of cost), and purchasing side of the package specifications. It is known that efficient packaging operations can tolerate only a certain amount of deviations in the structural dimensions and material qualities and also that closer the tolerances, higher are the costs. A discussion on their impact on the productivity and costs should have been included.

In the third chapter, Buyer Supplier Relation, he emphasizes the communication function between the supplier and the buyer for a smooth flow of materials, and for agreement on deviations from the specifications. In this, the role of technical personnel is also discussed. The relationship is highlighted with a discussion on the production of a customer conceived new package by the supplier. A format of purchase order is also given in the text.

In the fourth chapter, Quality Control Principles are discussed under seven headings. The part played by inspection in a quality control program for packaging is discussed in brief and in general, often overlapping test methods. Under appropriate test methods, the relevance of Mullen test on corrugated fibre-board the importance of evaluating finished packages for barrier properties and the necessity of participating in the round robin tests are emphasised. Under representative samples, a good discussion on sampling procedures and lot size and the implication on the acceptable quality levels with examples of cans, closures and laminates is included. Classes of defects and attributes and the scoring system are discussed under quality standards. The importance of quality control over quality audit and relevance of controlled quality are dealt briefly under the adjustment of manufacturing process. The deficiency and importance of specifications and standards are explained in the last section.

The fifth chapter is on Glass Container Specifications and Quality Control. In this, the elements of specifications for a glass container from the material of construction, the structural details of the body, the finish, the metal closure, the paper label and the corrugated fibre-board shipper are very well discussed with an example of 1 – quart Mayonnaise Package.

In the chapter on Metal containers specifications and quality control only tin cans were discussed. The discussion is very crisp and thorough and touches upon the empty weight of the can, the plate temper, body structure including the beads, tin coating weights, lacquer coatings, the end profiles and the corrugated fibreboard box using an example of 20 ounce bean soup can.

In the chapter on Plastic Package Specification and Quality Control, only the specifications for blow moulded bottles and blister packs are discussed with an example of one pint bath oil bottle and a blister pack for 2 inch hinges.

The eighth chapter considers paper, board and flexible package specifications and quality control and also includes that of plastic flexible packages, not dealt in the previous chapter. The discussion on the specifications and Q.C. of co-extruded films including infra-red methods of determining the thickness of individual layers and of metallised plastic films and papers will be very useful to the Indian readers at this time of the invasion of the co-extruded and metallised films in food packaging in India.

Recognising the rapidly growing diversity in shipping modes, a new chapter on unitised constructions for distribution (only pallets) is added. In this pallets and their positioning in the rail car and trailer, different types of shrink wrapping and stretch band and slip sheet are briefly touched upon. A good discussion on testing for transport-worthiness with right emphasis on vibration scanning, which is often neglected, is included.

The last chapter on computerised specifications highlights how the common computer word-processing and data processing would be useful in retrieving, in modifying and for selection of unit load interlocking

patterns. With the present day availability and awareness of computer systems and their applications in India, the information given in this chapter appears rudimentary.

The appendix on Technical and Trade Associations gives only those located in USA. Inclusion of at least some important associations in other parts of the world would have served the purpose better.

At the end, a useful bibliography on each chapter was included, but contains references only upto 1983 even though the book was revised and expanded in 1987.

The book can serve as a good text on basic principles of specifications and quality control and is particularly useful to the purchase managers who do not have a good technical background in packaging.

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

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 Notes

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