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(INDIA)

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Energy and Peak Force Requirement in Potato Slicing

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An experimental study was conducted on Instron-6021 universal testing machine to investigate the influence of the cutting speed and the cutting angle on the energy and peak force requirements in potato slicing. The data were analysed using response surface methodology. The influence of the cutting speed was found to be insignificant. Both the peak force and the energy showed a definite minima with respect to the cutting angle.

Potato chips are probably the most popular form of processed products of potato. The operational steps involved in the industrial production of potato chips are: cleaning and washing, peeling, slicing, drying, chemical or hot water treatment for improving colour, frying, salting and flavour addition, and packaging[1]. The slicing of peeled potato is a major operation in chip production requiring considerable energy.

Paladini[2] conducted studies on cutting of extruded products and reported that the peak force is influenced by the cutting angle. He also discussed the considerations in designing cutter knives for extruders. Such published information on the force and energy requirements in slicing/cutting of potato is not available. The data on the influence of the speed and cutting angle on the force and energy requirements are needed for a rational design of cutting machinery, particularly the cutting knives.

This paper presents the results of a study conducted to examine the influence of the cutting speed and the cutting angle on the energy and the peak force required for potato slicing.

Materials and Methods

Experimental design: A 4^3 full-factorial design was used. The independent variables were: cutting speed, cutting angle and core diameter. The peak force and the energy were considered as the dependent variables. Each experiment was replicated five times. The design particulars are given in Table 1.

TABLE 1. VARIABLES IN POTATO SLICING

Variables	
Cutting angel, θ	0, 30, 45 & 60°
Cutting speed, S	0.25, 0.50, 0.75, 1.0 m/min
Core diameter, D	0.011, 0.016, 0.021 & 0.025 m
Replications	5

Testing machine and slicing attachment: The slicing tests were conducted on an Instron-6021 universal testing machine. The operation was controlled by a micro-processer through a control console. The

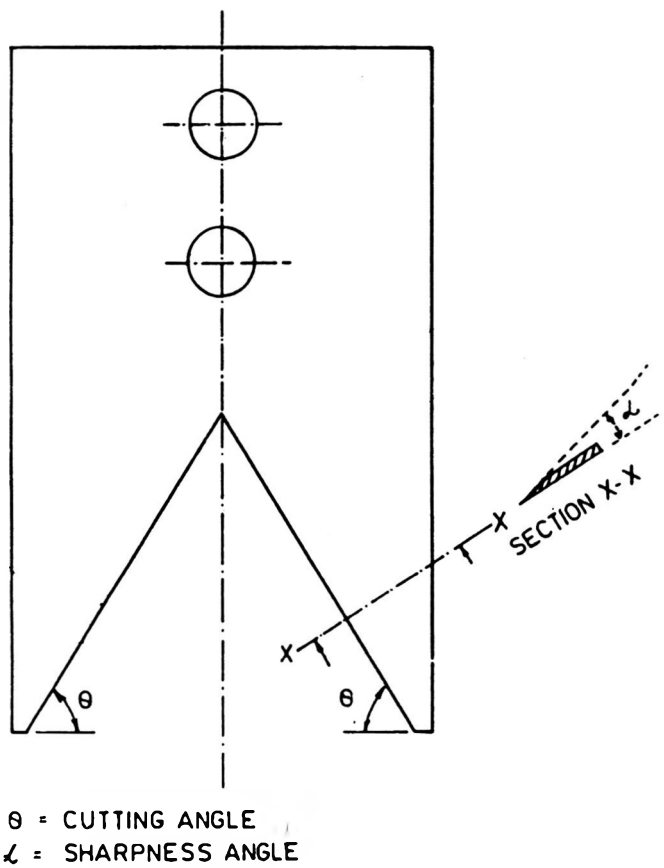

Fig. 1. The typical blade geometry.

Warner-Bratzler shear test cell of the Instron machine was modified by replacing its blade by cutting blades made of 1 mm thick GI sheet. The geometry of a typical cutting blade is illustrated in Fig. 1. Four blades having different cutting angles of 0, 30, 45 and 60° were made. The cutting edges of the blades were sharpened to a sharpness angle of about 15°.

Methodology: Commercial grade potato was procured from the local market and stored at 5°C in a low temperature cabinet. The required amount of potato was taken out for the experiment and was kept at room temperature (about 30°C) for 2 hr. Cylindrical cores of different diameters were taken out with the help of cork-borers of the required sizes. The cores were sliced through the slicing cell at the speed preset by the control console of the Instron machine. The resulting values of the peak force and the energy were displayed on the screen of the console.

Results and Discussion

The two-way analysis of variance of peak force (Table 2) and energy data (Table 3) indicated a very significant variation within replications (F-value of 15.51 and 17.32 for peak force and energy, respectively, against a table value of 3.40 at 99 per cent significance). As the replications for one test condition were taken from a single core of one potato tuber, the variation amongst the replications seems to be due to the variation in the mechanical properties within the same tuber. The variability in the mechanical properties within a single tuber has also been reported by Voisey *et al*[3]. However, this variation was not investigated further and only the average of the replications was considered for the following analysis of response surfaces.

The general form of the quadratic response surface for n variables is

$$Y = a_o + \sum_{i=1}^{n} a_i \cdot x_i + \sum_{i=1}^{n}\sum_{j=1}^{n} a_{ij} \cdot x_i \cdot x_j \quad \ldots(1)$$

Response surface models were obtained for the peak force and energy data through multiple regression technique. The resulting response surfaces were

$$F = -1.88 - 5.34E-02\,\theta + 1.98\,S + 936.22\,D + 3.48E-03\,\theta^2 - 1.79\,S^2 + 4467.24\,D^2 - 8.76E-02\,\theta S - 4.92\,\theta D + 49.94\,SD \quad \ldots(2)$$

$$E = 3.65E-02 - 4.42E-03\,\theta + 2.16E-02\,S - 4.11\,D + 8.26E-05\,\theta^2 + 5.44E-03\,S^2 + 786.75\,D^2 - 9.95E-04\,\theta S + 8.72E-02\,\theta D - 1.37\,SD \quad \ldots(3)$$

TABLE 2. ANOVA FOR PEAK FORCE RESPONSE SURFACE

Source of variation	Sum of squares	df	Mean square	F*
Included terms				
D, θ^2, θD	1768.50	3	589.5	71.26
Discarded terms				
θ, S, S^2, D^2, θ.5, S.D	63.03	6	10.505	1.27
Residual	446.72	54	8.273	
Total	2278.25	63		

*$F_{0.95}$ (3,54) = 2.78 $F_{0.95}$ (6,54) =2.27

All the terms of the response surfaces (Eq. 2 and 3) are not significant as was observed by checking the t-values corresponding to different regression coefficients. The response surfaces were simplified by sequential elimination of the term with lowest t-value and finding the new response surface with respect to the remaining variables. The significance of the combined contribution of the discarded terms was also evaluated at this stage[4]. The process of elimination was continued till the combined contribution of the discarded terms remained insignificant at 95 per cent confidence level. The following response surfaces resulted.

TABLE 3. ANOVA FOR ENERGY RESPONSE SURFACE

Source of variation	Sum of squares	df	Mean square	F*
Included terms				
θ, θ^2, D^2	1.36263	3	0.45421	105.52
Discarded terms				
S, D, S^2, θ.S, θ.D, S.D	0.01401	6	0.00234	1.06
Residual	0.11919	54	0.00221	
Total	1.49584	63		

*$F_{0.95}$ (3,54) = 2.78 $F_{0.95}$ (6,54) = 2.27

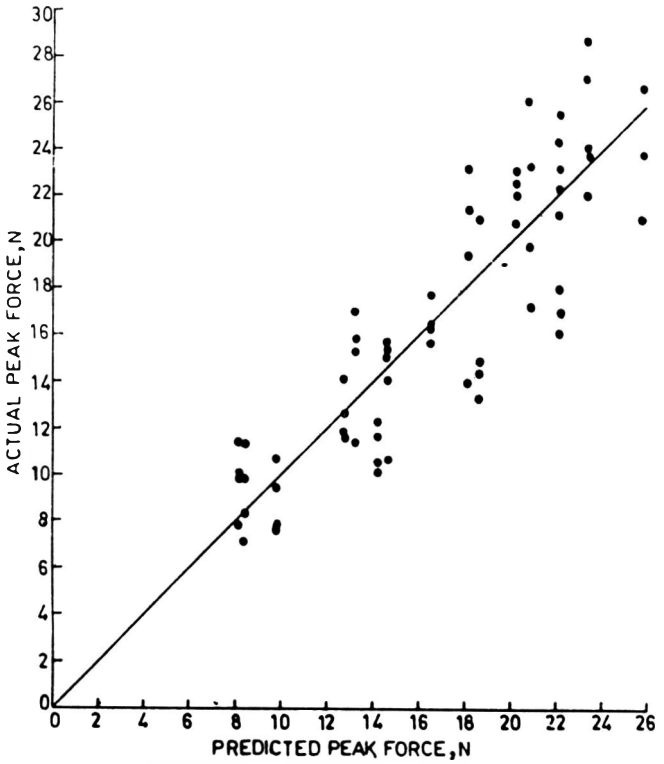

Fig 2. Performance of the peak force response surface

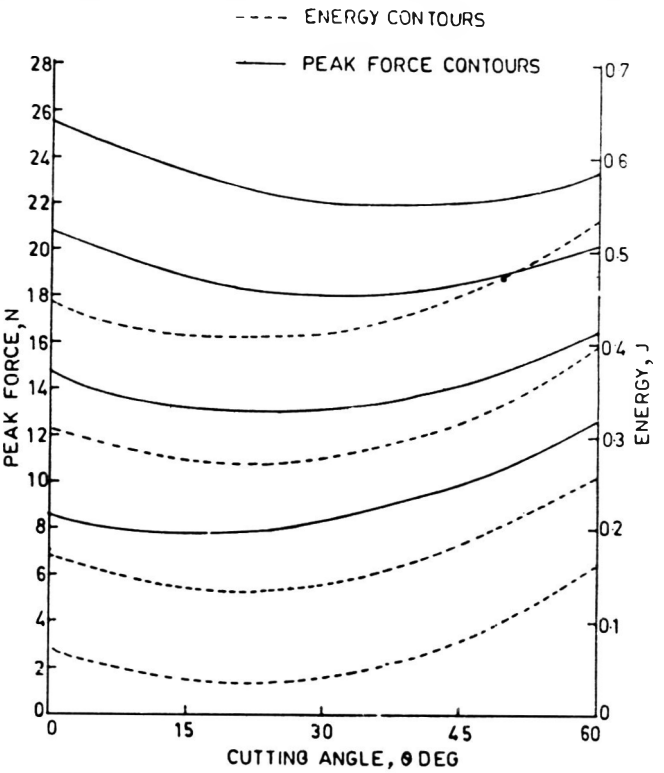

Fig 4. Peak force and energy response surface

$$F = -5.0957 + 1230.91\ D + 2.652E{-}03\ \theta^2 - 7.972\ \theta\ D \quad \ldots(4)$$

$$E = -1.8078E{-}02 - 3.4486E{-}03\ \theta + 8.26E{-}05\ \theta^2 + 731.113\ D \quad \ldots(5)$$

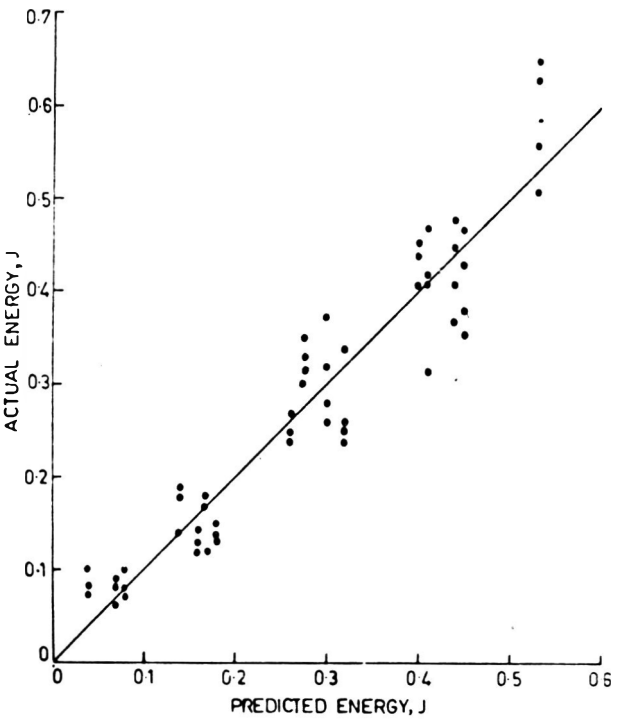

Fig 3. Performance of the energy response surface

The performances of these response surfaces in describing the experimental data are illustrated in Fig. 2 and 3, respectively. It may be noted that the cutting speed had no significant influence on the peak force and the energy. The cutting speed, therefore, does not seem to be a critical factor for the cutting knife design. Fig. 4 illustrates the response surface contours for the peak force and energy. Both the peak force and the energy have a definite minima with respect to the cutting angle. Paladini[2] reported a decrease in the peak force with an increase in the cutting angle, but did not mention the minima. The energy contours corresponding to different core diameters are parallel and the minima is observed for cutting angle between 15 to 25°. The peak force contours show a little convergence towards higher cutting angles and the optimum cutting angle with respect to peak force increases as the core diameter is increased.

Mathematically, the optima for the response surface can be obtained by differentiating as

$$dF/d\theta = 2 \times 2.652E{-}03\ \theta - 7.972\ D = 0 \quad \ldots(6)$$

and

$$dE/d\theta = 2 \times 8.26E{-}05\ \theta - 3.4486E{-}03 = 0 \quad \ldots(7)$$

Hence, the optimum cutting angle with respect to peak force, θ_f^*, and with respect to energy, θ_e^*, are $\theta_f^* = 1503\ D$ and $\theta_e^* = 20.88°$, respectively.

As the two optimum cutting angles are not identical, a decision about the objective criterion (peak force vs. energy) will have to be taken in designing the cutter

knives. Minimization of peak force shall ensure longer life for the cutting knife, while the minimization of energy will reduce the energy cost of the cutting machinery.

It is concluded that: (i) The energy and the peak force are not influenced by the cutting speed. (ii) The energy requirement is minimum for a cutting angle of about 21°. (iii) The cutting angle corresponding to the minimum peak force is dependent on the potato core diameter.

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Effect of Musk Melon *(Cucumis melo)* Seed Supplementation on the Nutritive Value of Wheat Chapati

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Musk melon seed flour was acceptable upto 20% level in chapatis. The supplementation of musk melon seed flour to wheat chapatis resulted in an increase in protein by 4.46% and fat content by 9.98% thus improving the energy content. Lysine remained the first limiting amino acid in the supplemented chapatis. Liver nitrogen, plasma proteins and PER in supplemented chapati group were higher than the wheat chapati group but the differences were not significant.

Diets consumed by the majority of the population in India are cereal based and contain only small amounts of pulses, vegetables, milk and other animal foods. Cereals provide 80 per cent of total calories and 70 per cent of protein. The most common cereal consumed in Northern India is wheat and about 85-90 per cent of it is in the form of chapatis[1]. It would, therefore, be advantageous to make chapati as nutritious as possible especially for children who need energy-protein rich diets.

Musk melon (*Cucumis melo*) seeds are a rich source of protein as well as oil and if used in dietaries can improve protein and energy intake. The seeds contain 23-36 per cent protein, 40-47 per cent oil and are also rich in calcium, phosphorus and iron[2,3]. In the present investigation, an attempt has been made to supplement wheat chapati with musk melon seed flour and to determine its effect on energy and quantity and quality of protein of wheat chapatis.

Materials and Methods

The musk melon seeds were cleaned, washed thoroughly with water and sun-dried, ground to a fine powder and sieved through 20 mesh. The flour obtained was stored in air-tight containers.

For making chapatis, 100g flour was mixed with water and kneaded thoroughly for 10 min. with measured amount of water to make a dough of moderately stiff consistency. It was covered with wet muslin cloth and kept for 30 min at room temperature. The dough was kneaded again for 5 min before making chapatis. In supplemented chapatis, wheat flour was replaced by 20 to 30 per cent musk melon seed flour. The dough was divided into 5 equal portions and each portion was rounded off between the palms of the hands, rolled into chapati of 6 inch diameter and baked on a hot iron plate (*Tawa*) till done.

Chapatis were prepared from wheat flour and wheat flour supplemented with musk melon seed flour at 20, 25 and 30 per cent levels. Preliminary evaluation showed that chapatis containing 30 per cent musk melon seed flour were not acceptable. All the quality attributes in these chapatis were adversely affected, the maximum effect being on texture. Sensory evaluation of chapatis was done by a panel of 10 judges in terms of colour, appearance, texture, aroma and taste, doneness and overall quality. The wheat chapatis and most acceptable combination i.e. those at 20 per cent level of supplementation were dried in a dehydrator at 50±1°C and finely ground and stored.

Wheat flour, musk melon seeds, musk melon seed flour, wheat chapati and wheat-melon (80:20) chapatis were analysed for proximate principles, calcium and iron using AOAC methods[4]. For tryptophan estimation, samples were hydrolysed using barium hydroxide followed by determination according to Opienska *et al*[5]. Cystine was analysed using the method of Evans and Bendemer[6] after acid hydrolysis. The rest of the amino acids were analysed using an automatic amino acid analyser after hydrolysis with 6N HCl at 110°C for 24 hr. Amino acid scores for four critical amino acids were calculated using the suggested requirement pattern for preschool children by WHO[7].

To determine the protein efficiency ratio (PER) wheat chapati and wheat-melon chapati diets were fed to weanling rats at 8.5 per cent protein level. Skim milk powder was used for reference protein diet. Non-nutritive cellulose and refined groundnut oil were added to adjust the level of crude fibre and fat in the diets to 5 and 10 per cent, respectively. The average

weight of rats at the beginning of the experimental period was 36g and each group consisted of 10 rats of both sexes in equal number. The protein efficiency ratio was determined as described by Evans and Witty[8].

At the end of the experimental period, animals were sacrificed and blood and liver tissues collected. Liver nitrogen was determined according to AOAC[4]. Plasma proteins were estimated using the biuret method[9]. Plasma lysine and methionine were estimated using thin layer chromatography[10]. Plasma tryptophan and cystine were determined by the methods used for food analysis[5,6]. The results were statistically analysed using 'Analysis of Variance'.

Results and Discussion

Amount of water required for making dough from whole wheat flour, 20 and 25 per cent supplemented flour was 85,70 and 60 ml/100 g, respectively, thus indicating a decrease in amount of water required for dough making for supplemented flours.

The chapatis prepared from wheat and wheat supplemented with musk melon seed flour were evaluated for their sensory characters and the results are presented in Table 1. The results indicated that 20 per cent musk melon flour supplemented chapatis were comparable to whole wheat chapatis whereas chapatis supplemented with 25 per cent musk melon had lower scores for texture, aroma and taste and overall quality as compared to whole wheat chapatis and also those supplemented at 20 per cent level. Thus increased supplementation of musk melon flour resulted in decreased trend in quality of chapatis.

Chemical analysis revealed that wheat contained 12.34 per cent protein whereas musk melon seeds had 23.78 per cent protein and, 35.68 per cent crude fat (Table 2). The values for musk melon seeds were nearly the same as reported by Lazos[11] but slightly lower than those reported by Teotia and Ramakrishna[3] probably due to varietal and ecological differences. The results further indicated that preparation of musk melon seed flour resulted in an increase in protein and crude fat content with a corresponding decrease in crude fibre content. The supplementation of musk melon seed flour to wheat chapatis resulted in an increase in all the constituents including calcium and phosphorus.

Musk melon seed flour contained slightly higher amounts of lysine and threonine than wheat but significantly higher amount of tryptophan (Table 3). The amino acid scores for the four critical amino acids which are likely to be deficient in cereal based diets are given in Table 4. The data indicate that lysine was

TABLE 1. SENSORY EVALUATION OF WHEAT AND MUSK MELON SUPPLEMENTED CHAPATIS

Parameter	Wheat chapati	Wheat-melon chapati (80:20)	Wheat-melon chapati (75:25)
Colour	6.80 ± 0.61	6.80 ± 1.06	6.60 ± 0.77
Appearance	6.86 ± 0.28	6.83 ± 0.46	6.53 ± 0.53
Texture	6.50 ± 0.28	6.70 ± 0.33	5.60 ± 0.77
Aroma & Taste	6.70 ± 0.46	6.60 ± 0.14	6.10 ± 0.12
Doneness	6.60 ± 0.95	6.80 ± 0.33	6.00 ± 1.07
Overall Quality	6.70 ± 0.73	6.80 ± 0.78	6.00 ± 0.84

Values are means ± S.D.

Key to score: Strongly favourable 7, Favourable 6, Mildly favourable 5, Undecided 4, Mildly unfavourable 3, Unfavourable 2 and Strongly unfavourable 1.

TABLE 2. CHEMICAL COMPOSITION OF WHEAT FLOUR, MUSK-MELON SEEDS AND CHAPATIS

Constituents	Wheat flour	Musk-melon seeds	Musk-melon seed flour	Wheat chapati	Wheat-melon chapati (80:20)
Protein (N × 6.25) (%)	12.34	23.78	33.65	12.34	16.80
Ether extract (%)	2.23	35.68	44.96	1.07	11.05
Crude fibre (%)	0.55	37.70	6.21	0.55	1.68
Ash (%)	1.64	,3.59	4.65	1.50	2.07
Phosphorus (%)	0.36	0.81	1.28	0.35	0.53
Calcium (mg/100g)	47	82	116	45	56

Values are means of triplicate analysis.

TABLE 3. AMINO ACID COMPOSITION OF WHEAT CHAPATI, WHEAT-MELON CHAPATI AND MUSK-MELON SEED FLOUR (G/16G N)

Amino acid	Wheat chapati	Wheat-melon chapati	Musk-melon seed flour
Lys	2.60	2.65	3.38
His	2.01	2.04	1.94
Arg	3.83	8.32	15.04
Asp	4.58	5.20	7.01
Thr	2.53	2.55	3.19
Ser	4.49	4.10	4.75
Glu	26.34	22.43	15.04
Pro	7.84	6.03	2.90
Gly	3.01	3.56	4.70
Ala	3.30	3.15	3.92
Cys	1.02	1.46	1.65
Val	4.15	3.62	3.51
Met	1.53	0.88	0.47
Ileu	3.07	2.91	0.33
Leu	6.43	6.01	6.37
Tyr	1.30	1.90	1.95
Phe	5.66	4.23	4.64
Try	1.11	1.32	1.40

TABLE 4. AMINO ACID SCORES FOR FOUR CRITICAL AMINO ACIDS IN WHEAT CHAPATI, WHEAT-MELON CHAPATI AND MUSK-MELON SEED FLOUR*

Amino acid	Wheat chapati	Wheat-melon chapati	Musk-melon seed flour
Lysine	44.82**	45.68**	58.27**
Methionine + cystine	102.00	93.60	80.80
Threonine	74.40	75.00	93.82
Tryptophan	100.90	120.00	127.00

*Amino acid scores were calculated using requirement pattern for pre-school children suggested by WHO.
**Limiting amino acid.

the first limiting amino acid in all the three cases. Teotia and Ramakrishna[3] also reported that lysine was the first limiting amino acid of musk melon seeds. The data further indicate that the second limiting amino acid of wheat is threonine whereas sulphur amino acids (methionine and cystine) are the second limiting amino acids in musk melon seeds.

The protein intake by the wheat-melon chapati group was higher than wheat chapati group but differences were not significant (Table 5). The body weight gain of wheat-melon chapati group was also not significantly higher than the wheat chapati group. The protein efficiency ratio (PER) of skim milk group was 2.92 and that of the wheat chapati group was 1.97, the differences being significant ($P<0.01$). The PER of wheat chapati group was quite close to PER (2.04) of wheat chapatis reported by Shyamala and Kennedy[12]. The supplementation of wheat with melon seed flour resulted in an increase in PER from 1.97 for wheat chapati to 2.25 for wheat-melon chapati but it could not attain significance. The PER of skim milk group was however, significantly ($P<0.01$) higher compared to wheat-melon chapati.

The mean liver nitrogen and plasma protein of groups fed on skim milk, wheat-melon chapati and wheat chapati diets were not significantly different from one another. The results of plasma lysine, methionine, tryptophan and cystine contents of rats fed the three diets indicated that plasma of rats fed skim milk diet contained the highest amounts of amino acids followed by wheat-melon chapati and wheat chapati diets, respectively. The plasma lysine and methionine contents of rats fed casein diet have been reported to be 11.27 and 7.8 mg/100 ml, respectively by Guggenheim *et al*[13]. They have also reported values for plasma lysine and methionine of wheat gluten fed rats to be 5.9 and 1.2 mg per cent, respectively. The corresponding values for chapati diet fed group are higher in the present study because wheat flour and not gluten was fed to rats.

From the foregoing data, it is clear that musk melon seeds though rich in protein are not good as source of most of the essential amino acids. The supplementation of melon seeds to wheat flour could not improve the protein quality of chapatis significantly. But supplementation increased the protein quantity and energy content (fat content) of chapatis, which can provide increased amount of protein and energy. Thus, musk melon seeds which are usually thrown away could be used after dehusking to enrich the chapatis. This enrichment would be important especially for growing children in poor segments of the population.

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TABLE 5. PROTEIN QUALITY PARAMETERS OF VARIOUS DIETS FED TO ALBINO RATS

Parameter	Skim milk diet	Wheat-chapati diet	Wheat-melon chapati diet	F-ratio	CD at 1%
Protein intake (g)	23.03 ± 1.86	18.22 ± 2.09	19.54 ± 1.81	9.98**	3.31
Body wt gain, (g)	67.68 ± 11.20	36.45 ± 8.17	43.97 ± 2.44	22.12**	14.60
PER	2.92 ± 0.31	1.97 ± 0.31	2.25 ± 0.20	22.47**	0.43
Liver N (mg/g)	27.75 ± 1.85	25.75 ± 0.83	27.25 ± 4.93	0.80^{NS}	–
Plasma protein (g/100 ml)	8.00 ± 1.63	6.50 ± 0.14	7.00 ± 0.10	0.35^{NS}	–
Plasma lysine (mg/100 ml)	9.50 ± 0.28	6.80 ± 0.10	7.85 ± 0.14	–	–
Plasma methionine (mg/100 ml)	6.33 ± 0.31	3.20 ± 0.20	4.85 ± 0.14	–	–
Plasma tryptophan (mg/100 ml)	3.61 ± 0.48	1.40 ± 0.18	2.80 ± 0.30	–	–
Plasma cystine, (mg/100 ml)	1.62 ± 0.22	0.75 ± 0.13	1.04 ± 0.10	–	–

Values are means ± SD.
**Significantly different at 1% level from skim milk diet.
NS Not significant

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Influence of Soaking on Various Biochemical Changes and Dehusking Efficiency in Pigeon pea (*Cajanus cajan* L.) Seeds

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Effects of soaking seeds in different concentrations of sodium bicarbonate on leaching of various compounds and nutritional quality of seeds were studied in four varieties of pigeon pea. The effects were similar. Soaking in solutions of sodium bicarbonate caused leaching of sugars, proteins and electrolytes from seeds to the soaking medium, decrease in starch and protein contents in seeds and increase in amylase activity. The decrease in pectin and gum, and the increase in polygalacturonase and pectin methylesterase were more when seeds were soaked in sodium bicarbonate solutions than in water. A negative correlation was observed between dehusking efficiency and pectin and gum contents of seeds.

One of the important pulse crops of India used as dhal (dehusked split) is pigeon pea. Dhal is obtained by milling the seeds. In the traditional milling process, the recovery of dhal is 66 to 75 per cent[1]. The yield of dehusked split dhal and pearled grain depends upon splitting and scouring losses in the dehusking machine[2]. Undesirable hardness of seed is one of the most important factors responsible for poor splitting of grain[3]. The hardening factors have been found to be pectin, gums, mucilages and various complex polysaccharides present in the cotyledons and husk of grain[4]. Soaking the seeds in water and salt solutions is one of the treatments to reduce hardness of seeds and to maximize the yield of dhal[3]. Soaking has been found to be effective in decreasing oligosaccharides of legume seeds.[5]

Keeping the above facts in view, the effect of soaking in water and in different concentrations of sodium bicarbonate have been studied on leachate of grains, and on the nutritional quality of seeds in four varieties of pigeon pea. Effects of the soaking treatments on gums and pectins have also been investigated to correlate them with dehusking efficiency. Polygalacturonase and pectin methyl esterase activity were determined to explain hydrolysis of pectin due to soaking treatment.

Materials and Methods

Seeds of four varieties of pigeon pea (*Cajanus cajan* L). viz., 'UPAS-120', 'T-21' 'Pant A-3' and 'Pant-10' were obtained from the Plant Breeding Department of the University. Ten gram seeds were placed in 10 ml each of 4, 6 and 8 per cent $NaHCO_3$ solution and in distilled water as control. After 1 hr of soaking, the soaking medium (leachate) was decanted into test tubes.

Except for enzymatic activity determinations, the seedes were dried in an oven for 150 min at 65°C to attain 10 per cent moisture. The dry seeds were powdered (40 mesh sieve), and then used for various biochemical analyses. Absorption of sodium by seeds from sodium bicarbonate solutions was estimated using the procedure described in A.O.A.C[6].

Estimations in leachate: Total electrolytes in the leachate were measured in terms of specific conductance using a conductivity meter (Naina Electronics, India). Total sugars and soluble proteins in the leachate were measured according to the procedure of Morris[7] and Bradford[8], respectively.

Estimation in seeds: Sugars and starch in seeds were estimated using the procedure of Clegg[9]. Microkjeldahl method[10] was followed to estimate total proteins in seeds. Total amylase activity was measured using the method of Bernfeld[11]. The pectin content in seeds was estimated as per cent calcium pectate by the procedure of Ranganna[12]. Method described by Lineweaver and Ballon[13] was followed to assay polygalacturonase activity. One unit of enzyme was

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expressed as one millimole of uronic acid liberated per min per ml of enzyme. Pectin methyl esterase activity was measured according to Kertesz[14]. Isolation of total gums was made according to the procedure of Ryugo and Labavitch[15]. Sugars in the hydrolysed gum were estimated by the anthrone method[7].

*Dehusking efficiency of seeds:*Dehusking efficiency of seeds was measured according to the procedure of Saxena[3]. Seventy five grams of the soaked and unsoaked seeds of each variety were taken. The grains were dried to a moisture content of about 10 per cent. The seeds were milled in an abrasive mill (Satake Engineering Co. Ltd., Tokyo) for 16 sec, and denusking efficiency (E) was calculated by the following formula[3].

$$E = \left[\frac{(n_2 - n_1)}{n_2} - \frac{(fp)}{fp + p + b} \right] \times 100$$

where,

n_1 = weight of unhusked grains

n_2 = Total weight of grains used for milling

fp = Finish product weight (dehusked grains + dhal obtained after milling)

P = Weight of broken seeds

b = Weight of powder

Results and Discussion

In all the varieties, sodium uptake and leaching of electrolytes, sugars and proteins increased with increasing sodium bicarbonate concentration (Table 1). Sugars, proteins and electrolytes are common solutes which leach out of seeds[16,17]. Leakage phenomenon has been explained in terms of change in the integrity of plasma membrane[18]; it could be associated with the hexagonal configuration of membrane phospholipids[17]. Greater leaching in sodium bicarbonate solutions than in water (Table 1) could be due to increase in membrane permeability in sodium bicarbonate solutions.

Maximum leaching of sugars was found in 'T-21'; the other three varieties revealed nearly the same extent of sugars. Leaching of proteins was found to be lowest in 'Pant A-3', whereas other three varieties revealed higher amount of proteins in their leachate. Leaching of electrolytes was greater in 'UPAS-120', followed by 'Pant-10', 'Pant A-3' and 'T-21' (Table 1).

Conductivity of leachate in sodium bicarbonate solutions was lesser than the standard sodium bicarbonate solutions (Table 1). This fact is probably associated with the absorption of sodium ions by seeds. Further studies are required to find out distribution of salt between the husk and the cotyledons. The cotyledons is a human food while the husk is a cattle feed. All the varieties revealed similar changes in protein, sugar, starch and amylase activity in soaked-seeds (Table 2). Protein content of seeds decreased more on soaking seeds in sodium bicarbonate solutions than in water. Similar observations have

TABLE 1. SUGAR AND PROTEIN IN THE LEACHATE AND SODIUM UPTAKE BY PIGEON PEA SEEDS SOAKED IN SODIUM BICARBONATE SOLUTION.

Varieties	$NaHCO_3$ (%)	Sugar (μg/ml)	Protein (μg/ml)	Specific conductance: Standard (υ/cm/10ml)	Specific conductance: Leachate (υ/cm/10ml)	Sodium uptake (mg/g dry seeds)
UPAS-120	Water	163 ± 7.0	62 ± 4.9	0 ± 0	3.7 ± 0.6	ND
	4	175 ± 8.0	91 ± 6.9	29.9 ± 2.6	26.9 ± 1.6	0.6 ± 0.10
	6	205 ± 9.6	120 ± 5.8	43.7 ± 1.6	34.3 ± 3.2	1.6 ± 0.20
	8	305 ± 11.3	129 ± 7.8	54.8 ± 3.2	46.6 ± 3.6	2.8 ± 0.11
T-21	Water	301 ± 9.8	51 ± 3.9	0 ± 0	4.7 ± 1.2	ND
	4	340 ± 11.5	65 ± 6.8	29.9 ± 3.2	25.1 ± 2.6	1.1 ± 0.02
	6	365 ± 12.2	102 ± 5.9	43.7 ± 4.1	32.5 ± 4.8	2.0 ± 0.02
	8	420 ± 13.9	110 ± 4.8	54.8 ± 3.2	40.0 ± 2.6	3.0 ± 0.02
Pant A-3	Water	136 ± 4.2	20 ± 2.3	0 ± 0	5.6 ± 1.8	ND
	4	181 ± 6.9	25 ± 3.9	29.9 ± 2.1	23.2 ± 2.5	0.4 ± 0.05
	6	200 ± 7.6	45 ± 4.8	43.7 ± 3.8	38.1 ± 2.2	2.0 ± 0.12
	8	220 ± 11.2	52 ± 5.2	54.8 ± 2.6	41.9 ± 2.8	2.9 ± 0.11
Pant-10	Water	162 ± 5.9	56 ± 6.2	0 ± 0	4.4 ± 0.6	ND
	4	174 ± 6.8	93 ± 9.5	29.9 ± 3.6	26.0 ± 1.2	1.0 ± 0.01
	6	220 ± 10.6	114 ± 6.8	43.7 ± 2.8	37.2 ± 2.8	2.0 ± 0.02
	8	240 ± 13.2	140 ± 8.2	54.8 ± 4.6	44.6 ± 2.6	3.2 ± 0.02

Each value is mean of three replications ± S.E., N.D. = Not detected: One ml leachate corresponds to 1 g of seeds.

TABLE 2. CHANGES IN SOLUBLE SUGARS, STARCH, PROTEIN AND TOTAL AMYLASE ACTIVITY IN PIGEON PEA SEEDS ON SOAKING IN SODIUM BICARBONATE SOLUTION

Variety	$NaHCO_3$ (%)	Soluble sugars (mg/g)	Starch (mg/g)	Protein (mg/g)	Amylase (Sp. act. $\times 10^2$)
UPAS-120	Control	25.3±0.90	440.8±0.0	208.8±8.0	5.9±0.05
	Water	24.0±0.40	440.0±18.4	183.9±6.0	5.8±0.08
	4	21.8±0.72	351.6±12.2	147.0±23.0	5.7±0.36
	6	23.2±0.10	333.3±11.0	135.8±7.8	6.0±0.09
	8	23.3±0.16	297.0±20.8	128.5±9.0	6.3±0.03
T-21	Control	49.3±0.60	540.5±11.0	211.7±6.0	2.3±0.02
	Water	47.0±0.80	530.0±9.2	192.7±12.0	2.5±0.04
	4	43.3±0.50	466.6±13.0	169.3±11.0	2.7±0.05
	6	45.3±0.46	442.4±11.2	159.1±12.0	2.6±0.07
	8	47.7±0.39	420.3±8.8	155.7±8.3	2.9±0.06
Pant A-3	Control	31.1±1.00	531.9±10.5	208.7±8.2	2.9±0.01
	Water	30.0±0.40	510.0±6.2	194.1±5.1	3.0±0.02
	4	26.4±0.00	474.4±4.2	159.1±7.2	3.1±0.04
	6	28.5±0.34	450.0±12.4	153.3±13.0	4.3±0.03
	8	29.3±0.20	435.7±5.6	151.8±3.2	4.7±0.02
Pant-10	Control	41.8±1.30	641.0±8.4	236.5±3.1	4.4±0.05
	Water	39.0±0.20	620.0±4.6	220.4±10.0	5.0±0.06
	4	34.8±1.50	561.0±20.4	195.6±6.0	4.6±0.05
	6	39.6±1.20	482.0±13.5	186.8±11.0	5.3±0.03
	8	37.4±0.30	450.0±14.0	186.8±8.2	5.1±0.10

Values are means of three replications with ± S.E. Control represents the values for raw-grain.

been reported by Kadam *et al*[19]. Decrease in protein in soaked seeds is mainly associated with leaching. The amylolytic activity was higher on soaking seeds in the sodium bicarbonate solution than in water. This would lead to increased hydrolysis of starch to sugar. In the present study, starch content decreased with soaking treatments; sugar content was slightly decreased. This fact could probably be associated with the leaching of sugar into the soaking medium and with the conversion of sugar into organic acids as a result of activation of enzymes involved in energy cycles[20].

All the varieties showed similar decreases of pectins and gums, and increases of polygalacturonase (PG) and pectinmethyl esterase (PME) activities on soaking seeds in the different concentrations of sodium bicarbonate solutions (Table 3). The per cent calcium pectate of pigeon pea decreased on uptake of water[21]. Ganesh Kumar *et al*[4]. have explained the decrease in pectin content as a result of demethylation activity induced by the PME enzyme. The pectin enzyme PG acts simultaneously and it has favourable effect on PME action[22]. Thus, PG is mainly responsible for disintegrating pectin substances with the result that their content in seed decreases. The decrease in gum on soaking is probably associated with the activation

TABLE 3. CHANGES IN PECTINS, GUMS, PECTIN METHYL ESTERASE AND POLYGALACTURONASE IN PIGEON PEA SEEDS ON SOAKING IN SODIUM BICARBONATE SOLUTION

Variety	$NaHCO_3$ (%)	Pectin (mg/g)	Gum (mg/g)	Pectin methyl-esterase (Sp.act. $\times 10^2$)	Polygala-cturonase (Sp.act. $\times 10^4$)
UPAS-120	Control	71.0 ± 1.20	4.2 ± 0.12	0.6	0.22 ± 0.02
	Water	69.0 ± 1.10	3.5 ± 0.09	1.7	0.55 ± 0.03
	4	64.0 ± 1.30	2.8 ± 0.06	5.5	0.88 ± 0.04
	6	58.0 ± 0.60	3.0 ± 0.04	6.1	1.29 ± 1.00
	8	55.0 ± 0.30	3.3 ± 0.13	7.2	1.85 ± 1.20
T-21	Control	65.0 ± 0.16	3.1 ± 0.16	0.7	0.04 ± 0.01
	Water	61.0 ± 0.13	2.3 ± 0.12	1.2	0.45 ± 0.02
	4	57.0 ± 0.09	1.8 ± 0.14	5.3	1.03 ± 0.12
	6	53.0 ± 0.12	2.0 ± 0.09	5.9	1.24 ± 0.16
	8	51.0 ± 0.13	2.2 ± 0.06	6.7	1.62 ± 0.14
Pant A-3	Control	66.0 ± 0.13	3.4 ± 0.19	0.7	0.03 ± 0.01
	Water	62.0 ± 0.19	2.9 ± 0.13	1.8	0.19 ± 0.03
	4	59.0 ± 0.09	2.5 ± 0.16	6.0	0.77 ± 0.00
	6	53.0 ± 0.06	2.8 ± 0.18	6.3	1.51 ± 0.06
	8	52.0 ± 0.09	2.9 ± 0.06	7.9	1.81 ± 0.06
Pant-10	Control	54.0 ± 0.13	3.0 ± 0.13	0.6	0.33 ± 0.01
	Water	52.0 ± 0.08	2.6 ± 0.18	1.7	0.54 ± 0.08
	4	50.0 ± 0.08	1.9 ± 0.13	5.3	1.04 ± 0.06
	6	45.0 ± 0.13	1.9 ± 0.18	5.8	1.43 ± 0.08
	8	44.0 ± 0.12	2.0 ± 0.06	7.1	1.59 ± 0.06

Each value is the mean of three replications with ± S.E. Control represents the value for raw-grain. Replicate samples were not used in case of pectin methyl esterase.

TABLE 4. DEHUSKING EFFICIENCY OF PIGEON PEA SEEDS ON SOAKING IN SODIUM BICARBONATE SOLUTION

$NaHCO_3$ (%)	UPAS-120	T-21	Pant A-3	Pant-10
Control	65.4 ± 3.2	70.4 ± 2.0	69.1 ± 5.5	74.6 ± 4.8
Water	66.3 ± 4.3	71.2 ± 4.8	72.3 ± 3.7	77.8 ± 4.1
4	71.1 ± 4.9	87.2 ± 5.1	80.8 ± 4.3	87.2 ± 5.6
6	81.3 ± 5.4	80.5 ± 6.0	74.2 ± 5.1	88.1 ± 6.2
8	77.2 ± 6.5	80.3 ± 4.2	72.5 ± 5.9	85.3 ± 4.7

Each value is the mean of three replications with ±S.E.
Control represents the values for raw-grain.

TABLE 5. SIMPLE CORRELATIONS BETWEEN DEHUSKING EFFICIENCY AND PECTIN AND GUM CONTENTS OF PIGEON PEA SEEDS

Compound	UPAS-120	T-21	Pant A-3	Pant-10
Pectin	–0.93	–0.75	–0.30	–0.81
Gum	–0.64	–0.81	–0.92	–0.99

of gum hydrolysing enzyme. Further studies are required to investigate gum hydrolyzing enzymes in the seeds after various soaking treatments.

In all the varieties, the dehusking efficiency of seeds was much higher on soaking seeds in sodium bicarbonate solution than in water (Tabe 4). In 'T-21' and Pant 'A-3', the highest value of dehusking efficiency was found in seeds soaked in 4 per cent sodium bicarbonate solution; but in 'UPAS-120' and 'Pant-10', it was observed at 6 per cent. The dehusking efficiency of seeds decreased on soaking seeds in 8 per cent sodium bicarbonate solution. The increase in dehusking efficiency after soaking in sodium bicarbonate solutions could probably be due to reduction of attachment between dhal and seed coat, which is caused by hydrolysis of pectin and gum in the seed. The husk of grain is strongly attached to the cotyledons due to the presence of gums, pectins and mucilages. A negative correlation has been observed between dehusking efficiency and pectin and gum contents of seeds (Table 5).

Although milling quality of seeds has been found to be improved on soaking in sodium bicarbonate solution, the nutritional value decreased. Therefore, it can be concluded that sodium bicarbonate concentrations as high as 4, 6 and 8 per cent cannot be recommended for commercial use. However, its academic aspect may be interesting. Further studies are required to investigate the effect of soaking period and concentration of sodium bicarbonate solution to optimize the milling and nutirtional quality.

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Nitrate and Nitrite Contents in Vegetables

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The nitrate and nitrite contents of 23 fresh vegetables, as available at retail markets, are reported. The samples included 6 leafy, 10 tubers/roots/bulbs and 7 fruit vegetables. The nitrate values on fresh weight basis ranged from 310 to 3086, 65 to 2920 and 70 to 512 ppm. in leafy, root and fruit vegetables, respectively. Some samples of mustard Raya *(Brassica campestris)* green leaves contained as much as 3750 ppm. nitrate. The nitrite contents in the respective groups of vegetables were generally low ranging between 2.5 and 4.7, 0.7 and 5.1 and 2.1 and 3.7 ppm, the highest of 5.8 ppm occurring in some samples of mustard Raya green leaves and black carrots.

Our diet, particularly vegetarian diet comprises a variety of plant materials to derive the necessary nutrients. The plant materials are known to contain many anti-nutritional or toxic components of which nitrate occurs in abundance.[1]. Nitrate is also found in drinking water and its presence in the soil is vital for the normal growth of plants. The leafy and root vegetables are generally rich in nitrate. The most important agro-climatic factors that favour accumulation of nitrate in vegetables have been summarised[2-6]. The nitrite content of vegetables is usually very low; however, plants that accumulate very high concentrations of nitrate may also contain significant amounts of nitrite[7-8]. Post-harvest storage of vegetables may also lead to the accumulation of nitrite arising from the reduction of nitrate caused by plant enzymes or microbial activity[9,10]. The toxic effects of nitrate and nitrite have been reviewed[11].

The preponderance of nitrates in foodstuffs and their possible conversion to toxic nitrite has prompted the International Agency for Research on Cancer[12] and WHO[13] to recommend reducing the concentration of nitrate and nitrite in foods and vegetables in particular. In view of this, it was felt desirable to assess the levels of nitrate and nitrite in vegetables commonly available in the local retail markets since the level of nitrate in vegetables varies with the agricultural practices and other environmental factors. The present paper reports the nitrate and nitrite contents in 23 fresh vegetables procured from different retail markets.

Materials and Methods

Fresh samples of different vegetables as listed in Tables 1, 2 and 3 were procured during the period January '86 to March '87 from retail vegetable markets located in different parts of Ludhiana. All the reagents used were of analytical grade. Absorbance was measured in an ELICO Spectrocol spectrophotometer.

Preparation of vegetable extract: The vegetables received from the market were washed well under running tap water, excess water removed and cut into small pieces. One hundred grams of the cut vegetables were taken, blended with 100 ml distilled water in a blender and made to volume (250 ml) with two washings. The pH of this blend was measured. It was filtered and the residue washed twice with 100 ml distilled water. The filtrate with washings was made to volume (500 ml). This extract was used for the determination of nitrate and nitrite.

Determination of nitrate: The nitroxylenol method of Lip and Dolberg[14] as given by Heisler *et al*[15], was used with some modifications. Suitable aliquots (0.5, 1.0 and 2.0 ml) of the vegetable extract were pipetted into 3 conical flasks (100 ml) wrapped with aluminium foil instead of iodine flask. To each flask was added 0.1g, 3-4-dimethyl phenol followed by careful addition of 10 ml concentrated H_2SO_4. The flask was then placed in an ice bath instead of cold tap water and 25 ml distilled water was added slowly. The reaction was allowed to proceed for 30 min. The entire reaction mixture with the washing was then transferred into a previously cleaned micro-Kjeldahl steam distillation apparatus. The nitroxylenols were distilled off slowly and the distillate collected in a 50 ml volumetric flask containing 6 ml of 5 per cent NaOH instead of 25 ml flask containing 3 ml of 5 per cent NaOH. This modification was necessary to ensure complete distillation of the chromogen. The distillate was made

TABLE 1. NITRATE AND NITRITE CONTENTS IN LEAFY VEGETABLES

Vegetables	No. of samples analysed	Moisture (%)	Nitrate (ppm)	Nitrite (ppm)	pH
Spinach *(Spinicia oleracea* Linn)	5	92.2	1703 (1075–2300)	4.7 (4.0–5.3)	5.86
Fenugreek leaves *(Trigonelia foenum graecum* Linn.)	5	89.8	310 (200–500)	4.1 (1.1–5.3)	5.69
Mustard – Raya *(Brassica campestris)*	5	90.1	3086 (2750–3750)	3.9 (2.8–5.8)	5.58
Mustard – Gobi (B. campestris)	4	89.0	1368 (875–2075)	2.6 (1.9–3.4)	5.97
Bathua leaves *(Chinopodium album)*	5	89.2	1854 (1175–2313)	4.2 (3.4–5.3)	5.81
Cabbage *(Brassica oleracea* var. capitata)	5	90.1	931 (800–1063)	2.5 (1.6–4.3)	5.62

Figures in parenthesis indicate range

TABLE 2. NITRATE AND NITRITE CONTENTS IN VEGETABLES – TUBERS/ROOTS/BULBS

Vegetables	No. of samples analysed	moisture (%)	Nitrate (ppm)	Nitrite (ppm)	pH
Turnip *(Brassica rapa)*	3	91.5	576 (550–602)	2.3 (1.4–3.5)	5.30
Radish – white *(Raphanus sativus* var. hortensis)	4	93.6	2466 (1719–3125)	2.8 (1.6–3.8)	5.45
Radish – pink *(R. sativus)*	4	92.3	2920 (2635–3125)	0.7 (0.4–1.0)	6.05
Carrot – Red *(Daucus carota* var. sativa)	6	91.0	81 (67–95)	2.4 (1.1–3.4)	5.23
Carrot – Black *(Daucus carota* var. sativa)	4	86.0	65 (33–98)	5.1 (4.7–5.8)	5.50
Beetroot *(Beta vulgaris)*	4	88.1	1685 (1263–2400)	2.2 (1.9–2.6)	5.46
*Ginger (fresh) *(Zingiber officinale* Rosc.)	5	73.7	652 (488–1062)	2.5 (1.2–3.3)	5.85
Colocasia *(Colocasia antiquorum)*	5	79.0	164 (43–300)	2.1 (1.0–3.0)	5.77
Onion-greenbulb *(Allium cepa)*	4	89.4	157 (144–172)	2.2 (1.8–2.7)	5.18
Onion-dry bulb *(Allium cepa)*	3	90.7	266 (163–425)	1.3 (1.0–1.8)	5.30

Figures in parenthesis indicate range
*Condiment

to volume with distilled water and the absorbance was measured at 430 nm. A blank determination was also carried out.

A standard curve was similarly prepared using standard solutions containing 0-10 μg nitrate/ml. The results were expressed as ppm.

Determination of nitrite: The method employed was that used by Fudge and Truman[16] with some modification. The vegetable extract (25 ml or appropriate volume) was pipetted into a 250 ml volumetric flask to which 10 ml $Al(OH)_3$ suspension was added, and then made to volume. The flask was shaken several times and allowed to stand for 30 min. The extract was filtered through Whatman no.1 filter paper into a 250 ml volumetric flask. This modification in the procedure was necessary to decolorize the extract. Aliquots (5, 10 and 20 ml) of the filtrate were pipetted into 50 ml volumetric flasks. Distilled water was added to each flask to make the volume to approximately 40 ml; then 5 ml freshly prepared

TABLE 3. NITRATE AND NITRITE CONTENTS IN FRUIT VEGETABLES

Common/Botanical name	No. of samples analysed	Moisture %	Nitrate (ppm)	Nitrite (ppm)	pH
+Cauliflower (*Brassica oleracea* var. botrytis)	4	91.1	512 (281–863)	2.1 (0.8–2.9)	5.73
Peas green (*Pisum sativum*)	4	74.5	118 (25–207)	3.8 (2.2–4.8)	5.72
Tomato (*Lycopersicon esculentum*)	6	93.9	201 (100–279)	2.5 (1.9–2.7)	4.13
Brinjal (*Solanum melongena* Linn)	5	92.8	163 (43–375)	3.7 (2.2–4.5)	5.16
Bittergourd (*Momordica charantia*)	5	92.3	122 (94–219)	3.5 (1.9–4.8)	5.42
Lady's finger (*Abelmoschus esculentus*)	5	89.4	70 (27–94)	3.4 (2.9–4.0)	5.68
Roundgourd ("Tinda") (*Citrullus vulgaris* var. fistulosus)	3	92.7	148 (59–238)	2.3 (1.8–3.3)	6.30

Figures in parenthesis indicate range
+A composite flower

sulphanilamide solution (0.5 per cent in dilute HCl, 1:1) was added to each flask, mixed and allowed to stand for 3 min. Finally, 2 ml of a freshly prepared solution of N-(1-napthyl)-ethylenediamine hydrochloride (0.5 per cent in distilled water) was added and the volume made to the mark with distilled water. The contents were mixed well and after standing for 20 min. absorbance was read at 540 nm. A blank determination was carried out at the same time.

A calibration graph was prepared by treating aliquots of the standard sodium nitrite solution containing 0.50 μg nitrite/ml as described above. The results were expressed as ppm.

Results and Discussion

The leafy vegetables, as shown in Table 1, were found to contain high amounts of nitrate. Mustard 'Raya' leaves, which are frequently consumed as 'saag', contained 2750 to 3750 ppm of nitrate, close to the values reported for Malaysian mustard leaves[17]. The nitrate values reported here in the case of other leafy vegetables viz., spinach and cabbage are similar to those reported by other workers.[6,8,18,19] Fenugreek leaves, mustard 'Gobi' and 'Bathua' have, however, not previously been analysed for their nitrate content. The nitrite content of the leafy vegetables, on the other hand, ranged from 2.5 to 4.7 ppm, slightly higher than the values reported in the literature[8].

Among the tubers and root vegetables analysed, radish contained the highest amount of nitrate-as much as 3125 ppm, and carrots the lowest amount ranging from 33 to 98 ppm. (Table 2). These nitrate data are well within the range of reported values [8,17] Onions were found to accumulate more of nitrate towards the end of maturity as is evident from Table 2. The values for nitrite in root/tubers/bulb vegetables ranged from 0.7 to 5.1 ppm, the highest amount occurring in black carrots.

The fruit vegetables, in general, contained lower proportion of nitrate when compared with some of the leafy and root vegetables, the highest amount of 201 ppm nitrate was present in tomatoes. Cauliflower contained 281 to 863 ppm nitrate which is close to the values reported in the literature.[8,20] The nitrate data for other fruit vegetables viz., peas, brinjal, bittergourd and lady's finger were similar to the values previously reported.[8,17,20] The nitrite content in these vegetables rangeu from 2.1 to 3.8 ppm the highest amount occurring in peas and bittergourds.

The nitrate and nitrite values for vegetables as reported in this study do not necessarily pose any health hazard to the consumer under normal conditions. However, frequent consumption of large quantities of leafy and some root vegetables high in nitrate contents may prove hazardous, particularly to infants. Besides its direct toxicity, nitrate has the tendency to get reduced to nitrite when vegetables are stored for long periods under abnormal conditions. Conversion of nitrate to nitrite also takes place in our saliva when food is chewed[21]. Infants are particularly put to risk as nitrate gets reduced to nitrite under suitable conditions in their upper gastrointestinal tract.[11] The nitrite consumption not only brings about methaemoglobinaemia in infants but also increases the risk of formation of nitrosamines which possess cancer causing properties.[11] It is, therefore, highly advisable

to restrict the consumption of such food materials that are high in nitrate and nitrite contents. Moreover, the processing of vegetables high in nitrate is difficult because of can corrosion. Hence, developing suitable vegetable cultivars that accumulate less of nitrate would be advantageous to obviate the health risks to the consumers.

Acknowldegement

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Effect of Incorporation of Cheese Slurry and Supplementation with *Lactobacillus* casei on the Quality of Buffalo Milk Cheddar Cheese

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Cheddar cheese was prepared from buffalo milk by adding upto 3% cheese slurry and viable cells of *L. casei* to the milled curd and ripening at 8±1°C upto 12 months. Both flavour and body and texture scores were higher in cheese samples containing both 2% slurry and cells of *L. casei* at the end of 9 months. Biochemical characteristics such as pH, free fatty acids and soluble nitrogen were higher but titratable acidity was lower in cheese samples containing slurries than control samples.

Cheese is one of the most important dairy products in the world market. Good quality cheddar cheese is made from cow's milk only. Buffalo milk is not considered suitable. In India, it is the buffalo milk which contributes to the major share of total milk production. FAO[1] reported that 66 per cent of the total milk production was from buffaloes in India. The cheddar cheese made from buffalo milk is always criticized for its lack of full cheese flavour even after a prolonged ripening period of 1-12 months. The ripening period is of great economic importance to the cheese industry. An attempt was made to accelerate the ripening of cheddar cheese from buffalo milk by adding ripened cheddar curd slurries to the milled curd. Ripening was further quickened by adding *L. casei* to the cheese system.

Materials and Methods

Manufacture of Cheddar cheese: Buffalo milk was procured from the Experimental Dairy of this Institute. Cheddar cheese was manufactured from 90 l of buffalo milk by Presalting method[2,3]. The milk was standardized to casein/fat ratio of 0.70, pasteurized at 63°C for 30 min and cooled to 28°C. To this milk, 1 per cent salt (sodium chloride) was added before culturing and renneting. Calf rennet (@ 2.5 g/100 l milk) was used for setting curd within 45-50 min. The curd was cut and cooked to 37°C in 50 min and cheddaring was done for about 3 hr to attain acidity of 0.5 per cent lactic acid. The cheese slurry and *L. casei* cells were incorporated into the milled curd before pressing. The cheddar cheese was ripened at 8±1°C. Each treatment was repeated 3 times.

Preparation of cheese slurry: The well ripened cow's milk cheddar cheese of 9 months age was selected for preparation of slurry. Cheddar cheese was macerated and ground using cheese grinder with the addition of sterile water. The moisture content of slurry was 40 per cent.

Lactobacillus casei 300: Mother culture of *L. casei* was obtained from the Dairy Bacteriology Division of the Institute. Bulk culture was prepared using buffalo skim milk.

Sensory characteristics evaluation: The sensory characteristics of cheddar cheese in terms of flavour, body and texture and colour were evaluated by a panel of 5 judges using cheddar cheese score card[4], at 3 months interval upto 12 months of ripening.

Chemical analysis: Cheddar cheese was analysed for chemical qualities and subsequent biochemical changes, at 3 months interval upto 12 months.

The fat in milk was determined by Gerber method[5] and in cheese by Mojonnier method[6]. The pH of the cheese was measured by using Digital pH meter, (Elico Pvt. Ltd. Hyderabad). Titratable acidity was determined by the A.O.A.C. method[7]. The soluble protein content in cheese was analysed by the method described by Kosikowski[8]. The total free fatty acids were determined by the method recommended by Ramamurthy and Narayanan[9].

Results and Discussion

Sensory characteristics: The effect of incorporating different levels of cheese slurries and viable cells of *L. casei* on the sensory characteristics of buffalo milk cheddar cheese during ripening is shown in Table 1. Incorporation of cheese slurry upto 2 per cent greatly improved the flavour scores of cheese samples ripened upto 9 months. Addition of viable cells of *L. casei* to cheese samples containing cheese slurry (2 per cent) further improved the flavour scores indicating the combined effect of cheese slurry and cells of *L. casei*.

TABLE 1. MEAN TASTE PANEL SCORES FOR CHEESE PREPARED USING CHEESE SLURRIES AND *L. CASEI* CELLS

Additives (%)	Flavour at indicated periods (months)				Body and texture at indicated periods (months)				Colour at indicated periods (months)			
	3	6	9	12	3	6	9	12	3	6	9	12
Control	35.0 Fl	36.1 Fl	36.8	36.0	25.0 Cu	25.4 Cu	26.0	26.0 H	9.0	9.0	9.0	8.5
Slurry – 1%	35.5 Fl	36.8	37.0	36.8	25.5	26.0	27.0	27.2	9.0	9.0	8.5	8.5
Slurry – 2%	36.2	37.6	38.5	37.2	26.4	28.0	28.2	27.5	9.0	9.0	8.5	8.5
Slurry – 3%	35.8	35.5 Fr Un	34.2 Un R.B	33.0 Un R.B	26.0 We	26.4 We	26.2 We	25.5 We P	8.0 M	7.2 M	7.0 M	7.0 M
Slurry – 2% + *L. casei* 0.2%	36.8	38.8	39.2	37.6	26.8	28.5	28.8	27.6	8.5	8.5	8.0 M	8.0 M

Flavour : Fl – Flat; Fr – Fruity; Un – Unclean; R – Rancid; B – Bitter

Body & Texture : Cu – Curdy; We – Weak; P – Pasty; H – Hard

Colour : M – Mottled

Average of 3 replicates

It may be seen that the flavour scores which were 35.0 in control and 36.8 in cheese containing slurry and cells at the end of 3 months, increased to 36.8 and 39.2 respectively after 9 months of ripening. The flavour enhancing ability of cheese slurry has been attributed to faster proteolysis and lipolysis in cheese during ripening and it was also observed by various workers[10-12]. The role of *L. casei* in enhancing the flavour development has also been reported by John and Cole[13], Jha[3], Singh and Kanawjia[11] and Kristoffersen and Nelson[14] and Franklin and Sharpe[15]. The results of this study also indicated that increasing the slurry concentration to 3 per cent drastically reduced the flavour scores and this effect has been attributed to uncontrolled excessive proteolysis and lipolysis during ripening. These samples showed flavour defects such as fruity, unclean, bitter and rancidity.

Incorporation of cheese slurry upto 2 per cent markedly improved the body and texture scores of cheese samples ripened upto 9 months. Addition of viable cells of *L. casei* to cheese containing slurry further enhanced the body and texture development. It is apparent that the body and texture scores which were 25 in control and 26.8 in cheese containing slurry and cells at the end of 3 months increased to 26 and 28.8, respectively, after 9 months of ripening. Increasing the concentration of slurry to 3 per cent drastically reduced the body and texture scores and this effect has been attributed to weak and pasty defects in cheese.

The colour of all cheeses was normal except in the cheese made with 3 per cent slurry which was criticized for being mottled. The colour score ranged from 8.0 to 9.0 at 3 months of storage. The scores decreased after 9 months regardless of treatments. At the end of ripening, the scores ranged from 7.0 to 8.5. The cheese made with 3 per cent slurry was criticized for mottled defect throughout the ripening. The cheese made with 2 per cent slurry and 0.2 per cent *L. casei* was also commented for mottled defect towards the end of ripening.

Biochemical changes: The effect of addition of cheese slurry and *L. casei* on various attributes such as pH, titratable acidity, soluble protein and free fatty acids, of cheddar cheese is presented in Fig. 1. Initially the pH of cheeses ranged from 5.30 to 5.42, being maximum in cheese with 3 per cent slurry and minimum in controls. The pH values were relatively higher in all the experimental cheeses as compared to the control from the very beginning. The pH increased in all the cheese samples as ripening progressed. At the end of ripening, these values ranged from 5.62 to 5.92, being the highest in case of cheese made with 3 per cent slurry. The higher pH in the experimental cheeses may be attributed to the release of ammonia and ammonium compounds after deamination of amino acids.

The 0-day titratable acidity ranged from 0.68 to 0.78 per cent being the lowest for cheese made with 3 per cent slurry. These values increased throughout the ripening. The changes in experimental cheeses were lower than control. This may be due to faster utilization of breakdown products of glycolysis. The

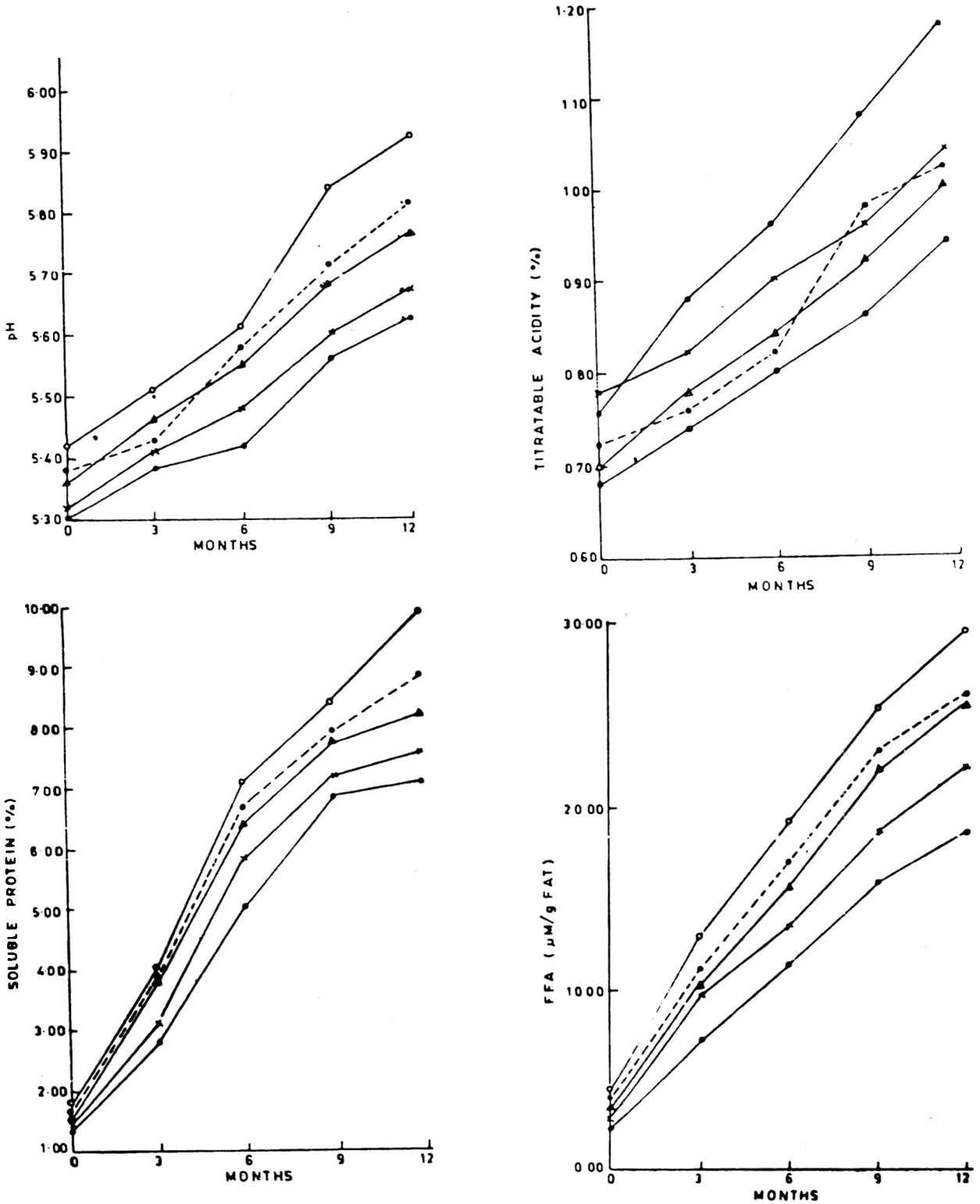

Fig. 1. Effect of addition of different concentrations of cheese slurry and viable cells of *L. casei* on various attributes of cheddar cheese during ripening. (Average of 3 replications)

● : Control; O: 1% slurry; △: 2% slurry; ▲ : 3% slurry
□ : 2% slurry + 0.2% *L. casei*.

final titratable acidity ranged from 0.94 to 1.18 per cent, being the highest in the control.

The concentration of soluble protein on 0-day values ranged from 1.35 to 1.78 per cent being the highest for the cheese made with 3 per cent slurry and the lowest for control. These values increased throughout the ripening period in all the cheese samples. The addition of slurry had tremendous effect on proteolysis of cheese. The supplementation of slurry with *L. casei* further enhanced proteolysis. Kristoffersen and Nelson[14], Bassette *et al*[16]. and Jha[3] also reported that *L. casei* contributed to the flavour due to its proteolytic activity. The cheese made with 3 per cent slurry showed excessive proteolysis which had adverse effect on the flavour and body texture of cheese. The final values of soluble protein ranged from 7.10 to 9.98 per cent, being the lowest in control. The addition of slurry and *L. casei* had a marked effect on proteolysis of cheese.

The free fatty acids (FFA) in cheese initially ranged from 2.36 to 4.04 μM/g fat, being maximum in cheese made with 3 per cent slurry and minimum in control. These values increased as the ripening progressed. The FFA content was higher in all the experimental cheeses as compared to control. The addition of *L. casei* further enhanced the lipolytic changes. It is apparent that higher the amount of slurry added higher was the FFA content. The final values ranged from 18.82 to 29.66, being the maximum in cheese made with 3 per cent slurry and the minimum in control. The stimulatory effect of *L. casei* on lipolysis was also observed by many workers[3,10,13,14].

The foregoing studies revealed that an acceptable quality cheddar cheese could be obtained by incorporating 2 per cent cheese slurry and 0.2 per cent cells of *L. casei*. The incorporation of slurry and cells resulted in balanced proteolytic and lipolytic changes in cheese during ripening.

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Studies on the Electrophoretic and Immuno Diffusion Methods in the Differentiation of Mutton and Beef Subjected to Severe Thermal Processing

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Thermostable muscle proteins (TMP) were prepared by ethanol precipitation from saline extracts of sterilized muscle homogenates of goat, sheep, ox and buffalo. On electrophoresis, all of them exhibited four major bands of molecular weights 16000, 26000, 28000 and 35000-37000. It was found that the thermostable protein component was derived from the myofibrillar fraction of the muscle. The TMP of all the four species were antigenic in rabbits, but showed considerable cross reactions. To eliminate this, sheep antiox and antibuffalo sera were first prepared and then all sera were made species specific by absorption techniques. Both antibeef and antisheep TMP sera were able to identify their respective TMP when extractives from raw beef as well as those from canned spiced mutton curry were tested. However, ox and buffalo muscle TMP could not be distinguished by the use of either or both antiox and anti-buffalo sera.

Species identification of meat in meat products is possible by electrophoresis of the soluble meat proteins[1-4]. This method is particularly useful for the identification of fish species[5]. Electrophoretic data obtained for beef, pork, horse, chicken, turkey and whale meat in the raw[1,2] and heated[4] state (dry heat upto 120°C for 6 min) were found to be useful in species identification. The electrophoretic patterns of raw, minced, heated or sausage type products are not comparable to those of canned meat where sterilization temperatures cause extensive alteration and denaturation of the constituent proteins. Immuno diffusion tests[6,7] and enzyme linked immunosorbent assays[8,9] have also been used for species identification but there are problems in preparing antisera for sterilized meat using totally heat denatured constituents specific to the species. However, boiled ethanol insoluble protein extractives (BE) from adrenal glands of different animals have been shown to be antigenic[10].

In India, the presence of beef in meat products is objectionable to a section of population on religious grounds. To distinguish mutton (meat of goat and sheep) from beef (meat of ox and the Indian buffalo) when the latter may have been used in the manufacture of canned spiced mutton curry, investigations were carried out on finding both electrophoretic differences and likely serological differences in the meat animals. The electrophoretic patterns of the whole muscle and muscle fractions have been compared and examined for features that might prove useful in differentiating meats of different origins. Further, the origin of the thermostable protein moiety was traced to a muscle fraction and its antigenicity established in experimental animals. Antisera prepared in different animals were used to test for the presence of a particular species of meat in cooked canned meat curry.

Materials and Methods

The chemicals used – sodium chloride, urea, sodium dodecyl sulphate (SDS), sodium dihydrogen orthophosphate, disodium hydrogen phosphate, bromophenol blue, mercaptoethanol, methanol, ethanol and acetic acid – were of the highest purity commercially available. Acrylamide, bisacrylamide, merthiolate were products of Sigma Chemical Co,, USA. Agar (purified), Freund's complete and incomplete adjuvants were obtained from Difco and Detroit, USA respectively.

Thermostable meat proteins (TMP) from muscle: The method employed was adopted from Hayden[11] which was used with adrenal glands. A flow diagram for the preparation of thermostable fraction from muscle is shown in Fig. 1.

TMP from canned curry: The curry (100 g) was blended in a waring blender for 2 min in 300 ml chloroform-methanol (2:1) mixture. The slurry was passed and squeezed through muslin cloth. The

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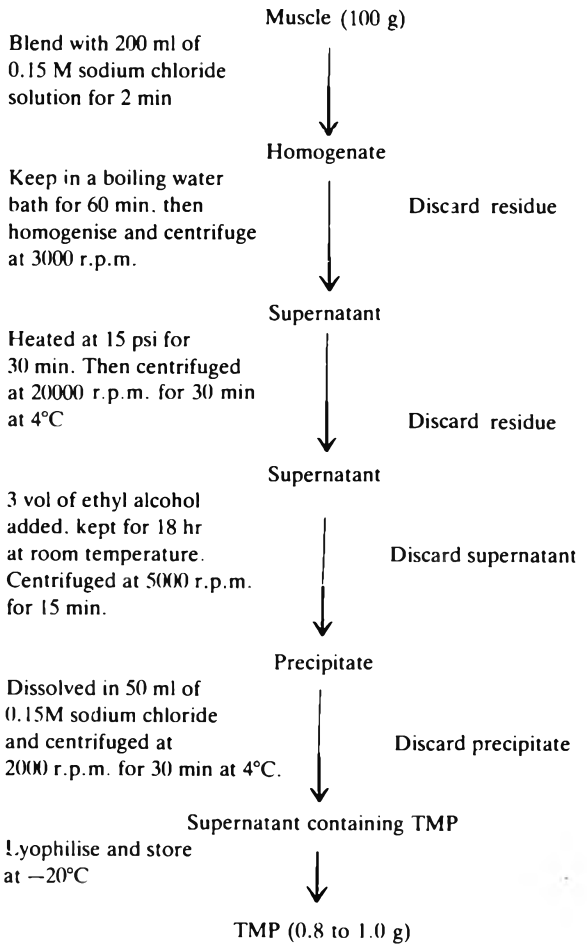

Fig. 1. Flow diagram for preparing TMP.

defatted residue was ground in a mortar with 0.15 M saline (1:4 w/v) for 2 min, filtered and centrifuged and the clear supernatant collected and concentrated by freeze drying.

Fractionation of muscle proteins: Raw sheep muscle was fractionated into sarcoplasmic, myofibrillar and stromal proteins by the method of Cole and Smithies[12]. The individual fractions were used after subjecting them to cooking, autoclaving and ethanol extraction procedure described for obtaining TMP.

Protein content was estimated by the biuret method[13].

SDS gel electrophoresis: Electrophoresis was carried out by the method of Weber and Osborn[14]. Molecular weight determination was done using Sigma molecular weight markers (Sigma Kits MWSDS 70 and 200). The protein preparations from muscle as well as molecular weight standards (1 mg/ml) were dissolved in 0.01 M sodium phosphate buffer (pH 7.0) containing 1 per cent SDS, 1 per cent β-mercapto-ethanol and 8M urea and heated at 97°C for 2 min or kept at 37°C for 3 hr. Fifty μl of the sample was applied on top of the gel (10 cm long, 5 mm dia) and run under a current of 8mA per gel for 4 and 6 hr for 5 and 10 per cent gel respectively. The gels were fixed in a fixing solution (methanol-acetic acid-water; 40:7:53) for 16 hr and stained with Coomassie blue (0.25 per cent in fixing solution) for 16 hr. The gels were then destained using several changes of destaining solution (methanol : acetic acid : water – 5:7.5:85).

The molecular weights of the unknown proteins were calculated from their mobility by standard procedures.

Preparation of antisera: Antibodies for sheep and goat TMP were raised in rabbits by injecting intramuscularly at two different places, a mixture of 1 ml antigen (5 mg/ml in 0.15M NaCl) and 1 ml Freund's complete adjuvant. Three booster shots were given every 15 days using antigens mixed with Freund's incomplete adjuvant. The animals were bled (cardiac) 10 days after the last dose and again after 15 days. Merthiolate (1:100000) was added to the pooled serum before refrigeration. Sheep antiox and antibuffalo sera were similarly raised in yearling sheep using 2 ml (10 mg/ml) intramuscular injections of antigen preparation mixed with 3 ml Freund's complete adjuvant. Three booster doses were given, once in every 20 days. Blood was collected from the jugular vein 10 days after the last dose and then twice again with an interval of seven days. The pooled sera were used in the tests.

Absorption of antisera: Absorption of the ox and buffalo antisera was done according to the method described by Milgrom *et al*[15]. Different concentrations of TMP of sheep and goat were mixed with ox or buffalo antisera and held at 28°C for 2 hr. The mixture was centrifuged at 3000 rpm for 10 min and the supernatant used in the immuno diffusion experiments.

Ouchterlony's test: Double dimmuno diffusion tests were done using 10 ml of 1 per cent agar (Noble) in 0.15M phosphate buffered saline (pH 7.2) poured into 100 × 15 mm glass petri dishes[16]. Wells of 5 mm diameter were made 10 mm apart in the set agar. Twenty five μl of antiserum was added to the centre well and the test antigens were kept in the surrounding wells. Precipitin lines were recorded after a 24 hr reaction in a humid chamber kept at room temperature.

Results and Discussion

Electrophoretic studies: Separation of the thermostable muscle proteins was not satisfactory in the 5 per cent gel system as some of the components were found to be moving ahead of the dye front; therefore all further experiments were done using 10 per cent gel system. The electrophoretic patterns of

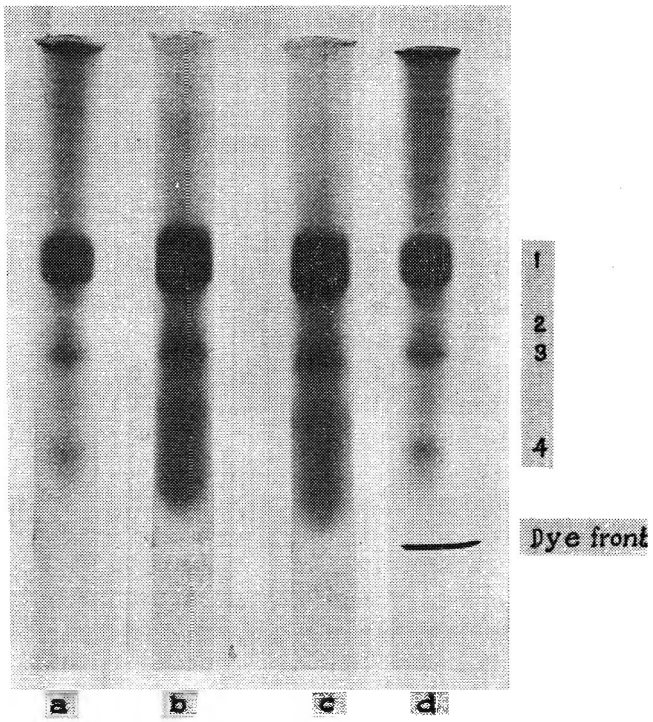

Fig. 2. Electrophoretic patterns of TMP from (a) Goat (b) Ox (c) Buffalo & (d) Sheep

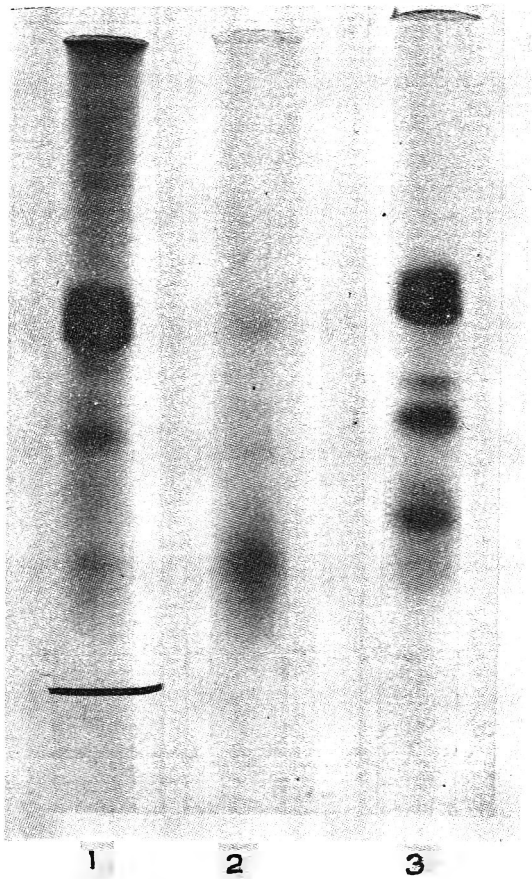

Fig. 3. Electrophoretic patterns of (1) Sheep TMP (Whole muscle), (2) Sarcoplasmic fraction and (3) Myofibrillar fraction.

TMP from goat, sheep, ox and buffalo are shown in Fig.2. Four distinct and major bands appeared along with some diffuse lines which were common to all the four species. By their relative intensity and width, these appeared to be in the low molecular weight region. From the mobility (Rm) relative to dye front, the molecular weights of the bands are as follows: Band 1, mol. wt. 35000-37000; Band 2, mol. wt. 28000; Band 3, mol. wt. 26000 and Band 4, mol. wt. 16000. Between bands 3 and 4, additional faint and diffuse bands are also found. Above band 1, a few very faint lines could also be observed in the high molecular weight range but these constituents are present in very minute quantities and were found in all 4 species. The diffuse lines could not be clearly identified.

A comparison of the electrophoretic pattern of sheep TMP precipitated from whole muscle, sarcoplasmic fraction and myofibrillar fraction (Fig.3) showed that the four major bands detected in the whole muscle TMP corresponded to the pattern and mobilities exhibited by the myofibrillar fraction. The diffuseness observed in the whole muscle electrophoretogram was absent in the myofibrillar fraction derived from all the four species (Fig.4).

To differentiate meat of food animals, Ramdass and Misra[3] used polyacrylamide gel electrophoresis of raw meat extractives and reported that some distinctive bands were apparent which could be used to identify the meat source. Studies on the electrophoretic patterns of heat treated muscles have been restricted to lower temperatures such as heating between 65° and

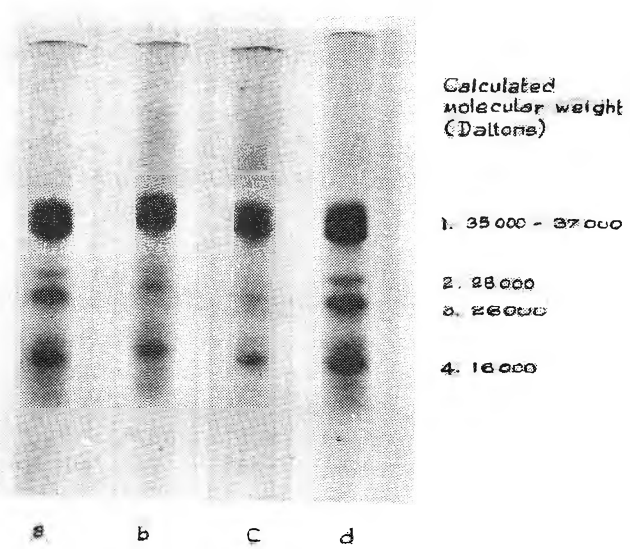

Fig. 4. Electrophoregram of myofibrillar fractions of (a) Goat (b) Ox (c) Buffalo & (d) Sheep muscles.

90°C by Lee *et al.*[17] whose aim was to determine if the bovine muscle had been cooked at 69°C for the required period to destroy foot and mouth disease virus. They reported that whereas there were six bands in extractives of muscle heated to 65°C, only one band was observed in samples heated at 90°C. Mattey *et al.*[4] and Babiker *et al.*[18] who conducted experiments with muscles of various animals exposed to hot air at 120°C for 6-20 min, found that several bands remained and reported that differentiating and qualitatively identifiable patterns were discernible between beef and rabbit meat and between horse meat and beef. Our studies show that after cooking and sterilizing in moist heat at 120°C for 30 min, only four major bands were detected in the electrophoretograms of all the four species and that there were no distinguishing features that can be found by using either the whole muscle extracts or their myofibrillar fractions.

Although it has been reported that electrophoretic patterns of myoglobin from various meat species were distinctive, the work pertained again to studies on raw meat[2]. Similarly, sarcoplasmic protein patterns reported to be useful for the identification of beef, pork, chicken and turkey, were obtained from raw meat. Our studies on cooked meat showed that sarcoplasmic proteins did not contribute to the electrophoretic patterns formed by the whole muscle extract and the only persistent components were detected in the TMP derived from the myofibrillar fraction. Kaiser and Krause[19] in a recent review on the subject stated that even closely related animal and plant foods could be distinguished by the uniqueness of their gel patterns but our observations so far have shown that neither the TMP from the whole muscle of goat, sheep, ox and buffalo nor their myofibrillar fractions could in themselves be sufficient for this purpose. In our studies to characterise the TMP, there is evidence (unpublished) to suggest that either it is troponin/tropomyosin or is directly derived from them. Experiments to purify and characterise the TMP are continuing.

Immuno diffusion test: Anti sheep, goat, ox and buffalo sera prepared in rabbits exhibited considerable cross reactions in the double immuno diffusion tests making it difficult to distinguish any one species clearly. However, ox and buffalo antisera raised in sheep also cross reacted with sheep and goat antigens (Fig.5) but absorption with these antigens rendered them capable of distinguishing between goat and sheep on the one hand and ox and buffalo on the other.

Even after the absorption step, ox and buffalo antigens continued to cross react with antisera of each other. Kangethe *et al.*[8] used rabbits for raising antisera against bovine serum albumin and sheep serum albumin and even after absorption by affinity chromatography with cyanogen bromide activated sepharose, it was not possible for them to eliminate cross reactions between the species antigens and antisera. According to Johnston *et al.*[20] sheep antibeef serum should completely abrogate any cross reactions with sheep antigen but our efforts to prepare antibeef sera in sheep did not produce beef specific sera necessitating further absorption with the cross reacting antigens. Kangethe *et al.*[21] also recently reported successful identification of the different animal species by preparing their antisera in sheep and goat and

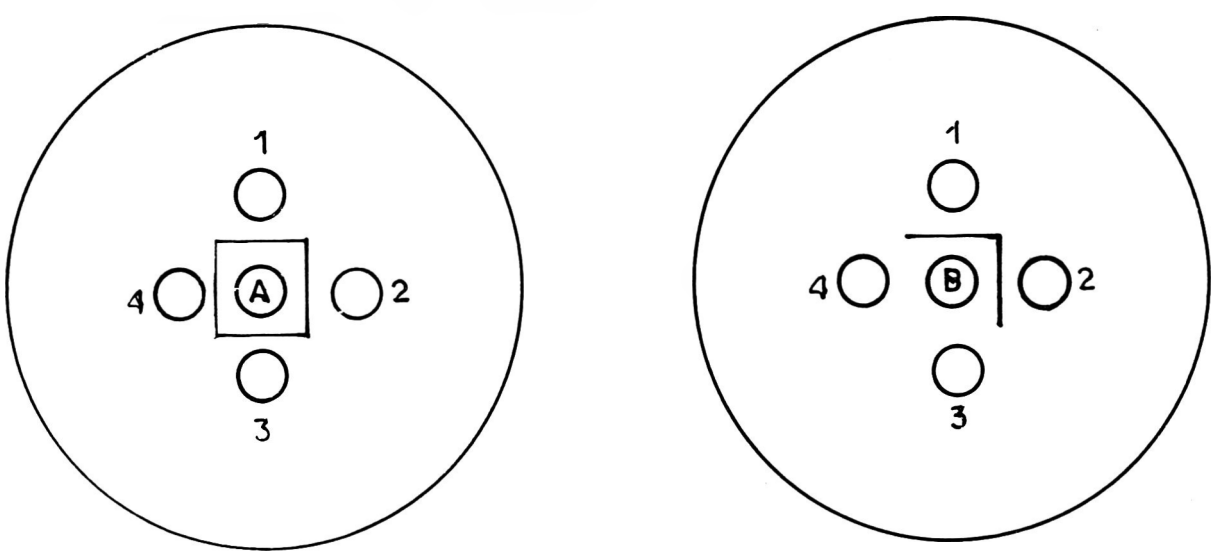

Fig. 5. Double diffusion results: (A) Unabsorbed sheep antiserum to ox TMP (B) Absorbed sheep antiserum to ox TMP against cooked meat extracts from (1) Buffalo (2) Ox (3) Sheep and (4) Goat muscles.

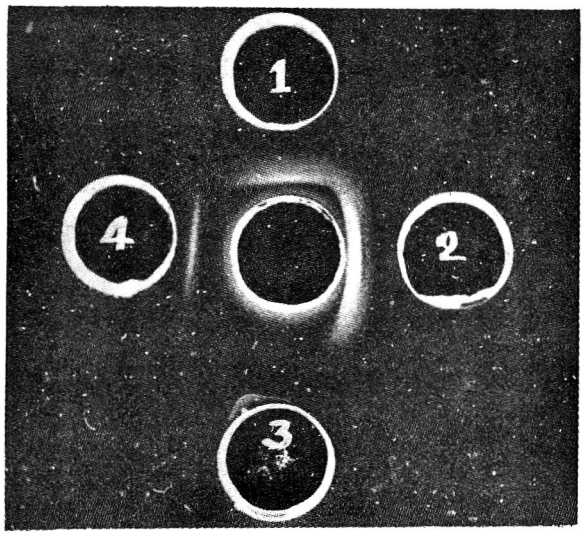

Fig. 6. Double difussion results: Absorbed sheep antiserum to ox TMP in the central well against cooked meat extracts of (1) Buffalo (2) Ox (3) Sheep and (4) Fresh meat extract of ox muscles.

removing cross reactions by absorbing with individual species antigens.

The anti beef and sheep TMP sera were also tested against fresh ox and buffalo muscle extractives as well as proteins from canned mutton curry. The appearance of precipitation lines for fresh as well as canned meat indicated (Fig.6) that the TMP was present in the native raw state also and was not the breakdown product derived from heat denatured muscle proteins. While the antibeef serum did not cross react with antigens from mutton curry, antisheep serum clearly formed a precipitin line. This showed that even though the mutton was cooked in spices and then canned (at 120°C for 35 min), antigens could be successfully extracted from the defatted meat directly and tested by the Ouchterlony procedure.

By raising antibodies in sheep against ox and buffalo and after cross absorption, the antisera were made specific to beef but the preparations were not able to distinguish between ox and buffalo. More refined absorption techniques are required to achieve this. But for the purpose of detecting adulteration of mutton preparation with beef irrespective of whether it was ox or buffalo meat, this level of specificity in the serum has been adequate.

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Evaluation of Synergistic Effects Obtained in Emulsion Systems for the Production of Wieners

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A 2.5:3.0 (per cent of meat block) combination of hard wheat flour and extenders (Promax 70 and sodium caseinate) was used to examine the results from emulsion systems for laboratory preparation of wieners. Texture profile analysis and sensory evaluation revealed that the wieners containing sodium caseinate had the highest scores for hardness, texture, flavour and overall acceptability. The emulsions produced were stable. The performance of fillers and extenders in a meat emulsion product could be predicted based on the data obtained in the emulsion system.

Bawa[1] reported on the interactions upon combining 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 systems in combination with extenders and fillers were found to be the best when emulsifying capacity (EC), emulsion stability (ES) and water holding capacity (WHC) were considered. Of the extender sources evaluated, hard wheat flour is most widely used, while skim milk powder is not a common extender. Therefore, the medium meat system as well as 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. A 2.5:3.0 (per cent of meat block) combination of hard wheat flour and non-meat proteins produced the optimal overall response of emulsifying capacity, emulsion stability and water holding capacity[2]. Very little has been reported in the literature on the extension of the results from a model system to a commercial emulsion system. Chattoraj *et al.*[3] reported that sausages prepared from sheep meat, utilizing the information on model systems, were found to be acceptable by a taste panel. With this background, the present study was aimed to evaluate the synergistic effects obtained in emulsion systems for the production of wieners under laboratory conditions.

Materials and Methods

The lean pork trim, pork hearts and pork back fat were obtained from the regular inventory of the Meat Science Laboratory at University of Guelph. The beef tripe was procured from J.M. Schnieder Inc. of Kitchner, Ontario. The lean pork trim, pork hearts and beef tripe were ground twice through a 4.8 mm plate and pork back fat was ground once through a 12.7 mm plate. The hard wheat flour, sodium caseinate and Promax 70 (a soy concentrate produced using acid wash technique) were obtained from Griffith Laboratories United, Toronto. The medium meat system was formulated following the system of Comer and Dempster[4] with slight modifications and used in combination with hard wheat flour, Promax 70 or sodium caseinate for wiener formulation as shown in Table 1.

Proximate analysis was carried out using standard AOAC procedures[5]. The meat ingredients, except the back fat, were removed from the freezer and tempered overnight in a cooler at 4°C and required quantities were weighed. The salt, sodium nitrite, and sodium erythorbate were weighed and mixed together in a polythylene bag. The meat ingredients except fat were transferred into a previously cooled small Hobart silent cutter (Model 84142). The mixed dry ingredients, filler, extender and one-half of the chilled (10°C) water were uniformly distributed over the meat ingredients and then chopped for 1 min. The fat and remaining water were then added and chopping was continued for 2 min. The temperature at the end of chopping was 13-15°C.

Emulsion stability of the Wiener batter: The

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TABLE 1. WIENER FORMULATION FOR LABORATORY TRIAL USING THE MEDIUM MEAT SYSTEM

Ingredients	Control	Treatments I	II
Lean pork trim (g)	900	900	900
Pork hearts (g)	300	300	300
Beef tripe (g)	300	300	300
Pork back fat (g)	500	500	500
Sodium chloride (g)	68	68	68
Sodium nitrite (g)	0.62	0.62	0.62
Sodium erythorbate (g)	1.55	1.55	1.55
Hard wheat flour* (g)	–	57.4	57.4
Promax 70* (g)	–	64.2	–
Sodium caseinate* (g)	–	–	64.6
Added water (g)	908	908	908
Total fresh emulsion (g) (rounded off)	2978	3100	3100

*Non-fat dry wt basis

stability of the raw emulsion (ES) was determined using the method of Saffle *et al.*[6]

Stuffing of the Wiener batter and linking: Approximately 3 kg of each homogenate was stuffed into 22 mm diameter cellulose casings using a hand stuffer (Model Super R.L.6, 5 Officina Meccanica, Bologna, Italy) to make 15 cm long wieners. The raw wieners were weighed and left overnight in a refrigerator at 4°C. A portion of the homogenates was transferred to plastic bags, sealed and kept in a refrigerator until required for proximate analysis.

Cooking: The next day, raw wieners were removed from the refrigerator and weighed. The loss from the initial weight (mainly water) was expressed as percentage of original weight and identified as overnight cooling loss. The cooled raw wieners were immersed instantaneously in liquid smoke and cooked in domestic type cooking ovens to an internal temperature of 75°C. The ovens were preheated to a temperature of 93°C and then the temperature was raised slowly at the rate of 14°C every 15 min until the required internal temperature was reached; this was recorded by 7.5 cm long and 2 mm thick thermocouples at the tip placed in the centre of the product. Following cooking, the wieners were cooled to room temperature and weighed. The loss of weight during the cooking process was expressed as percentage of the cooled and original raw wiener weights.

The cooled wieners were peeled manually and the separated gel and fat were washed from them. The wieners were then drained and weighed. The weight lost in the process of peeling was expressed as a percentage of the weights of cooked wieners and original raw wieners. The total loss in original weight of the raw wieners through cooling, cooking and peeling was expressed as percentage of the weight of original raw wieners and characterized as per cent yield after substracting from hundred. The cooked wieners were vacuum packed in plastic bags and stored in a cooler at 4°C until required for analysis.

Consumer cook test: A consumer cook test was performed on the wieners using the procedure described by Tauber and Lloyd.[7]

Sensory panel evaluation: A semi-trained panel was used for the sensory evaluation of the wieners. A semi-structured scale[8] was used for the evaluation of samples for colour, texture, flavour and overall acceptability. It consisted of 150 mm long horizontal lines with verbal anchors of the most undesirable description on the left and the most desirable one on the right. The panelists were asked to taste the samples in a given order and place a vertical mark across the line at the point at which it best reflected the magnitude of his or her perceived intensity of that attribute. The distance from the left hand end of the scale to the panelist's rating was measured in millimeters in order to obtain a numerical value for each attribute tested. These values were used in the statistical analysis.

Shear force: Shear force was measured using a Warner Bratzler shear press[9] set at 5 per cent sensitivity and standardized to give a reading of 4 with a 1000 g weight. The peak force was measured in kg and converted into Newtons for use in the statistical analysis.

Texture profile analysis (TPA): An Instron (model 1122) universal testing machine set at a maximum limit of 7.5 mm, return limit of 15.0 mm, minimum limit of 2.0 mm, chart speed of 50 mm/min and crosshead speed of 200 mm/min was used to determine the texture profile[10] of the wieners.

Preparation of samples for scanning electron microscope (SEM): An Etech Scanning Electron Microscope was used to examine the microstructural differences among the meat emulsions, the wieners before and after the consumer cook test and wieners prepared from different extender sources. Two wieners were randomly selected from each treatment for SEM evaluation. Two samples were prepared by cutting a thin slice (2 mm) from the centre of each wiener with a razor blade. Each centre slice was then cut into squares approximately 3 mm × 3 mm. The procedure of Theno and Schmidt[11] was then used with slight modifications. Immediately after cutting, specimens from each treatment were immersed in 0.1 M phosphate buffer (pH 7.2) in glass vials. All the specimens were then fixed by replacing buffer with 2 per cent glutaraldehyde and 0.5 per cent acrolein and storing the vials overnight in a refrigerator. The following day, each specimen was given five washings

with the phosphate buffer over a period of 1 hr and the buffer was replaced with 1.0 per cent osmium tetroxide solution (phosphate buffered) to post-fix the specimens for 2 hr. This was followed by five washings in 0.1 M phosphate buffer. The specimens were then dehydrated in graded ethanols of 10, 20, 30, 50, 70, 80, 95 per cent concentration and three changes of 100 per cent ethanol for 15 min each. All the specimens were then washed three times with chloroform followed by two 15 min changes of absolute alcohol. The samples were then dried to the critical point using liquid carbon dioxide as the transitional medium. The wiener specimens were mounted on 13 mm aluminium stubs with silver paint for increased conductivity. The stubs were then coated *in vacuo* with gold/palladium using a sputter coater (Technics Hummer V) for 5 min and examined on an Etech Automatic Scanning Electron Microscope operating at 10 KV accelerating potential. Photographs were taken at various magnifications on a polaroid film (Polaroid Corp., Cambridge, Mass.).

The completely randomized block design consisting of three blocks with two replications were used for the wieners produced in the laboratory. Within each replicate, two determinations were made for each parameter. An average of the two determinations was used in the statistical analysis. The general linear model (GLM) programme of the Statistical Analysis System (SAS) was used for analysis of variance. All tests of statistical significance were made at the probability level of $\alpha = 0.05$. The LSD and HSD procedures[12] were used to test for significant differences among the means when main effects were significant.

Results and Discussion

The drip loss due to overnight cooling of the stuffed wieners as well as the cooking loss calculated on the basis of either fresh or cooled weight (Table 2) were not significantly ($P>0.05$) affected by the treatments. This can be explained by the non-significant effect of extenders on water holding capacity (WHC) shown by Bawa[1]. There were significant differences among the treatments for peeling losses when calculated on either fresh or cooked weight basis. The loss was greatest for the control (20.8 per cent) and lowest for the sodium caseinate (9.6 per cent) treatment. The peeling loss can be an indication of ES and the results are in accordance with the superior ES shown for caseinate as compared to Promax 70[1]. The LSD test revealed that the peeling loss was significantly different among the treatments when expressed on a fresh weight basis, while on a cooked weight basis, the control was significantly different from Promax 70 and sodium caseinate treatments but the latter two were not significantly different from each other. The yield values for the control were significantly different from Promax 70 and sodium caseinate treatments but the difference between the latter two was not significant ($P>0.05$). This may be due to the non-significant effect of the addition of non-meat protein sources on the cooling loss which is one way to evaluate WHC. Thus, it was found that the addition of extender sources significantly improved the ES over the control without significantly affecting WHC.

TABLE 2. MEAN PER CENT LOSSES DURING THE PREPARATION AND COOKING OF STUFFED WIENER BATTERS MADE FROM MEDIUM MEAT SYSTEM AND IN COMBINATION WITH HARD WHEAT FLOUR AND PROMAX 70 OR SODIUM CASEINATE. n= 2.

Treatment	CL_1	CL_2	CL_3	CL_4	CL_5	Yield (%)
Control	5.2	29.4	25.5	20.8^a	29.4^a	49.8^a
Promax 70	4.5	28.4	24.1	11.8^b	16.1^b	60.7^b
Sodium caseinate	4.0	26.5	24.7	9.6^c	13.2^b	63.0^b
S.D.*	±0.60	±1.47	±0.74	±6.28	±9.03	±7.05

Means superscripted with different letters in the same column are significantly different from each other ($P<0.05$)
CL_1 – overnight cooling loss; CL_2 – cooking loss on fresh wt basis; CL_3 – cooking loss on cooled wt basis; CL_4 – peeling loss on fresh wt basis; CL_5 – peeling loss on cooked wt basis;
* degree of freedom = 5.

The stability of the raw emulsion was significantly different among the treatments with a maximum fat separation in the control (5.13) and a minimum for the sodium caseinate (2.88) treatment. This result is consistent with peeling losses expressed on a fresh weight basis (Table 2). The loss in weight of wieners during the consumer cook test was not significantly affected by the treatments but the value was considerably higher for the control (5.84 per cent) as compared to the Promax 70 (1.19 per cent) and sodium caseinate (0.72 per cent) treatments. Thus, the amount of fat released during cooking was higher for the control as compared to the Promax 70 and sodium caseinate treatments which were essentially the same, indicating the improved stability of wieners.

The analysis of variance revealed that the moisture and protein contents of the raw emulsions were significantly ($P<0.05$) different among the treatments which can be attributed to the differences in protein content of the extenders added. The control had a higher moisture content (67.7 per cent) and a lower protein content (7.5 per cent) when compared to the Promax 70 (moisture 65.4 per cent and protein 8.3 per cent) and sodium caseinate treatments (moisture 65.6 per cent and protein 8.8 per cent). There were no significant differences in moisture and protein contents of wieners among the treatments. Fat content was not

TABLE 3. MEAN COLOUR, FLAVOUR, TEXTURE AND OVERALL ACCEPTABILITY SCORES (mm) FOR WIENERS PREPARED FROM THE MEDIUM MEAT SYSTEM AND IN COMBINATION WITH HARD WHEAT FLOUR AND PROMAX 70 OR SODIUM CASEINATE, n = 40

Treatment	Colour	Flavour	Texture	Overall acceptability
Control	80.0[a]	76.8[a]	56.5[a]	55.9[a]
Promax 70	88.8[a]	79.8[a]	89.5[b]	78.1[b]
Sodium caseinate	88.9[a]	93.4[b]	96.2[b]	99.0[c]
S.D.*	±26.5	±20.0	±19.9	±21.2

Means superscripted with different letters in the same column are significantly different (P<0.05).

* degrees of freedom = 119

significantly (P>0.05) affected by the treatments with respect to both raw emulsions and wieners.

Colour was not significantly (P>0.05) affected by the treatments, but flavour, texture and overall acceptability scores (Table 3) were (P<0.05) affected. The LSD test for comparison of individual treatment means revealed that the flavour scores for the control and Promax 70 were not significantly (P>0.05) different. The sodium caseinate treatment received flavour scores which were significantly higher than the other two treatments. The sensory panel scores for texture of the control were significantly lower than those for Promax 70 and sodium caseinate but the latter two were not significantly (P>0.05) different from each other. The overall acceptability scores for the treatments were significantly (P<0.05) different with sodium caseinate receiving the highest score. The lower flavour and overall acceptability scores for the Promax 70 treatment can be partially explained by the suppressing effect of the soy proteins on the characteristic flavour and tastes of meat emulsion products, also reported by Schut and Veghal[13].

The texture profile analysis data (Table 4) reveal that none of the parameters were significantly affected by the treatments except for hardness. Sodium caseinate significantly improved the hardness in comparison with the control wieners and those made with Promax 70. The Warner Bratzler shear values were not significantly (P>0.05) affected by the treatments. The comparison of the hardness scores with the texture and overall acceptability scores from the sensory panel evaluation revealed that the harder product (sodium caseinate) was preferable to the others in this experiment.

Microstructure of wieners: The scanning electron micrographs of wieners containing all meat and those with hard wheat flour in combination with Promax 70 or sodium caseinate are shown in Fig. 1. The cavities represent fat globules because fat was extracted during the preparation of samples for SEM. All three samples showed structures consisting of a protein matrix and fat globules which were coarser in the case of sodium caseinate (Fig. 1). The fat globules were larger in the case of the control as compared to the other two treatments. These observations indicated the suspension or mechanical fixing of the fat with a protein matrix as described by Hamm[14] and Van den Oord and Visser[15]. The fat globule distribution in the case of sodium caseinate samples was not found to be as uniform as for the control and Promax 70. Cooking of wieners in boiled water for the consumer cook test adversely affected their structure. It resulted in disrupting the protein matrix (Fig. 2) causing loss in weight; the effect was greater in Promax 70 samples than controls but the sodium caseinate samples were not affected very much. This may be explained by the indirect contribution of sodium caseinate to water retention and texture of meat emulsion reported by Schut and Brouwer[16] and the non-coagulation of sodium caseinate at normal pasteurization and sterilization temperatures[13]. It also supports the minimum loss in weight for the consumer cook test for sodium caseinate containing samples as reported earlier in this study.

The synergistic effect of the incorporation of extenders and fillers into the medium meat system, observed in the emulsion system could be reproduced when wieners were made under laboratory conditions. Sodium caseinate was superior to Promax 70 which had an adverse effect on flavour.

TABLE 4. MEAN HARDNESS SPRINGINESS, COHESIVENESS, GUMMINESS, CHEWINESS AND WARNER BRATZLER SHEAR FORCE FOR WIENERS PREPARED FROM MEDIUM MEAT SYSTEM AND IN COMBINATION WITH HARD WHEAT FLOUR AND PROMAX 70 OR SODIUM CASEINATE, n = 4.

Treatment	Hardness (Newton)	Springiness (meter)	Cohesiveness	Gumminess (Newton)	Chewiness (Newton meter)	W.B. Shear (Newton)
Control	15.51[a]	0.0125	0.338	5.40	0.066	6.89
Promax 70	16.92[b]	0.0125	0.280	4.73	0.059	5.17
Sodium caseinate	18.39[c]	0.0125	0.385	6.99	0.088	6.52
Standard deviation*	0.85	0.00	0.0197	0.21	0.0022	0.17

Means superscripted with different letters in the same column are significantly different (P<0.05).

* degrees of freedom = 5

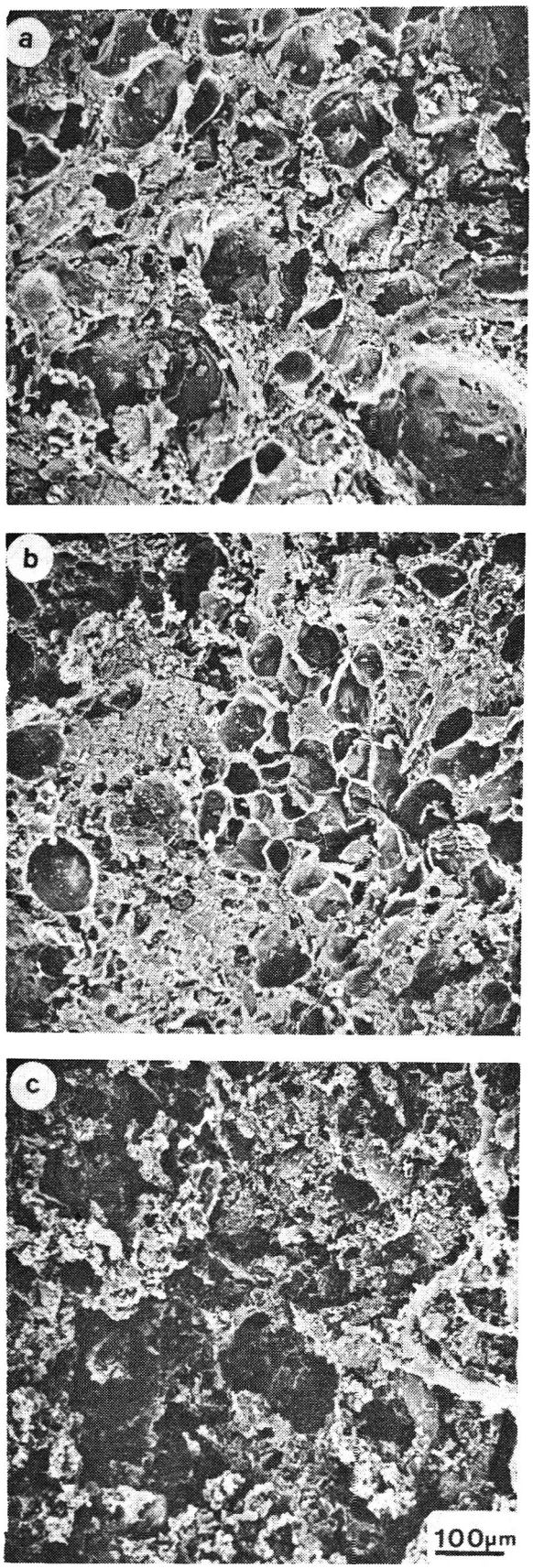

Fig. 1. SEM micrographs of wieners made from medium meat system (a) and in combination with hard wheat flour and Promax 70 (b) or sodium caseinate (c).

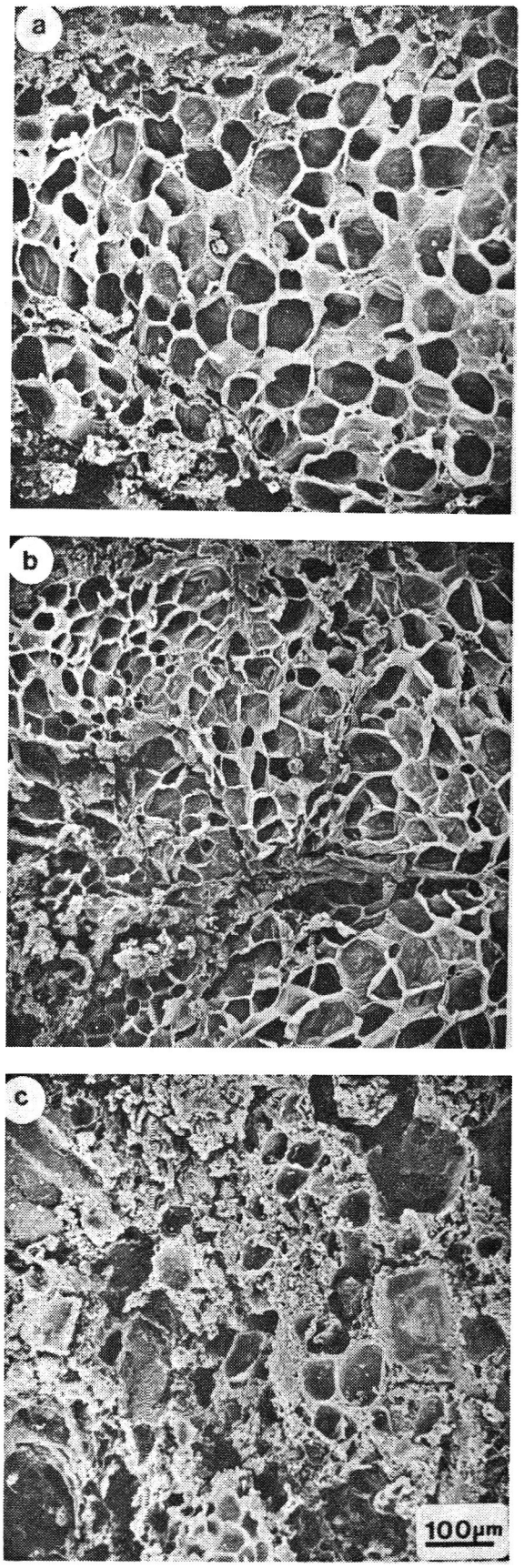

Fig. 2. SEM micrographs of cooked wieners made from medium meat system (a) and in combination with hard wheat flour and Promax 70 (b) or sodium caseinate (c).

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The authors thank The Griffith Laboratories, Scarborough, Ontario for supplying the non-meat ingredients. Financial support for this research was from the Ontario Ministry of Agriculture and Food. A.S. Bawa was supported by a Canadian Commonwealth Scholarship.

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Evaluation of Antagonistic Effects Obtained in Emulsion Systems for the Production of Wieners

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Wieners were made from the strong meat systems in combination with hard wheat flour, mustard flour and Promax 70 which were shown to give an antagonistic effect in the emulsion system studies. Mustard flour resulted in the lowest emulsion stability and a soft product with an undesirable flavour. The control wieners were preferred to those containing hard wheat flour in combination with Promax 70 or mustard flour. The performance of fillers and extenders in a meat emulsion product could be predicted based on the data obtained in a model system.

Bawa *et al.*[1] explored the interactions upon combining strong, medium and weak meat systems with corn starch, hard wheat flour and skim milk powder as fillers and mustard flour, sodium caseinate and Promax 70 as extenders in emulsion systems. The extenders and fillers were found to act synegistically or antagonistically depending on the levels and types of meats used. The functional properties of a strong meat system were antagonistically affected by a combination with filler (Hard wheat flour) and extenders (mustard flour and Promax 70). The synergistic effects obtained in emulsion systems were evaluated in the production of wieners under laboratory conditions[2]. This study was conducted to evaluate the antagonistic effects obtained in emulsion systems using the strong meat system alone and in combination with hard wheat flour and mustard flour or Promax 70 for the production of wieners under laboratory conditions.

Materials and Methods

The lean beef chuck, lean pork trim and pork back fat were obtained from the regular inventory of the Meat Science Laboratory at the University of Guelph. The lean beef chuck and lean pork trim were ground twice through a 4.8 mm plate and pork back fat was ground once through a 12.7 mm plate. The hard wheat flour, mustard flour and Promax 70 (a soy concentrate produced using acid wash technique) were obtained from Griffith Laboratories Limited, Toronto. The strong meat system was formulated following the system of Comer and Dempster[3] with slight modifications and used in combination with filler (Hard wheat flour) and extenders (sodium caseinate, Promax 70) for wiener formulations as shown in Table 1. All methods were the same as reported by Bawa *et al.*[2]

Results and Discussion

The loss due to overnight cooling of raw wieners and the cooking loss calculated on the basis of either fresh or cooled weight basis (Table 2) were significantly ($P<0.05$) affected by the treatments. However, cooking loss on the basis of cooled weight was not

TABLE 1. WIENER FORMULATION FOR LABORATORY TRIAL USING THE STRONG MEAT SYSTEM

		Treatments	
Ingredients	Control	I	II
Lean beaf chuck (g)	1000	1000	1000
Lean pork trim (g)	400	400	400
Pork back fat (g)	600	600	600
Sodium chloride (g)	68	68	68
Sodium nitrite (g)	0.62	0.62	0.62
Sodium erythorbate (g)	1.55	1.55	1.55
Hard wheat flour* (g)	–	69	69
Mustard flour* (g)	–	105.6	–
Promax 70* (g)	–	–	74.6
Added water (g)	906	906	906
Total fresh homogenate (rounded off)	2976	3144	3120

*Non-fat dry weight basis

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TABLE 2. MEAN PERCENT LOSSES DURING THE PREPARATION AND COOKING OF STUFFED WIENER BATTERS MADE FROM STRONG MEAT SYSTEM AND IN COMBINATION WITH HARD WHEAT FLOUR AND PROMAX 70 OR MUSTARD FLOUR. n = 2.

Treatment	CL_1	CL_2	CL_3	CL_4	CL_5	% yield
Control	6.7[a]	26.6[a]	21.3[a]	13.6	18.6	59.4[a]
Promax 70	4.9[b]	19.7[b]	15.6[b]	5.1	6.6	75.1[b]
Mustard flour	3.9[c]	23.2[c]	20.1[a]	13.6	17.6	63.3[a]
S.D.*	±1.42	±3.45	±3.0	±4.91	±6.66	±8.12

Means with different superscripts in the same column are significantly different ($P< 0.05$)
CL_1 – overnight cooling loss
CL_2 – cooking loss on fresh wt basis
CL_3 – cooking loss on cooled wt basis
CL_4 – peeling loss on fresh wt basis
CL_5 – peeling loss on cooked wt basis
*degree of freedom = 5

significantly different for the control and mustard flour treatments, but Promax 70 resulted in a significantly ($P<0.05$) smaller loss. Peeling loss expressed on the basis of either fresh or cooked weight was not significantly affected by the treatments; however, the values were almost the same for the control and mustard flour but lower for the Promax 70 treatment. This indicated that the addition of mustard flour to the strong meat system did not improve the emulsion stability (ES) as measured by peeling loss over that of the all-meat control. The yield values were significantly ($P<0.05$) affected by the treatments. Promax 70 resulted in a significantly ($P<0.05$) higher yield than the all-meat control. The yield values were significantly incorporation of mustard flour into the strong meat system resulted in a slightly higher yield than the control but these were not significantly different.

Promax 70 incorporated samples (2.65) gave a significantly lower value for the consumer cook test as compared to the control (5.17) and the mustard flour incorporated ones (3.01). The ES was not significantly affected by the treatments; however, the separation of fat was the highest for mustard flour (7.25) followed by Promax 70 (4.25) and the all-meat control (4.00). These results are similar to those observed by Bawa *et al.*[1] where ES was the lowest for mustard flour treatments while Promax 70 had little effect on the emulsification properties of the strong meat system. It was observed that neither the results from the ES test nor for peeling loss, which can be an indicator of ES during cooking, were significantly different among the treatments. The differences in yield appeared to be due to both ES and water holding capacity (WHC). The higher yields in this experiment as compared to the one with medium meat system[2] point out the importance of higher ES and WHC values for the strong meat systems containing lean beef[1]. Similar views have been expressed by Comer[4] indicating the importance of gelling and water binding properties of meat proteins in meat emulsion products.

The extenders did not have a significant ($P>0.05$) effect on moisture, fat or protein contents of Wieners. However, the moisture and protein contents of raw emulsions (Table 3) which were significantly ($P<0.05$) different among the treatments.

The mean sensory panel scores for colour, flavour, texture and overall acceptability are shown in Table 4. The colour scores were the highest for the mustard flour treatments probably because most of its colour was extracted into the gel which separated out during the cooking of the wieners. Promax 70 received the lowest colour scores. The flavour scores in the case of mustard flour were found to be significantly different from the control and Promax 70 treatments. Wieners containing mustard flour had the lowest flavour and overall acceptability values. The texture scores were significantly lower for the control than the Promax 70 and mustard flour treatments. There were no significant differences with regard to the overall acceptability scores among the three treatments. However, the overall acceptability was adversely affected by the extenders and fillers, resulting in lower scores for Promax 70 and mustard flour containing wieners when compared to the control. Similar results have been reported by Patel *et al.*[5] while working with navy bean protein extender and non-fat dry milk in combination with lean beef.

TABLE 3. PROXIMATE COMPOSITION OF RAW EMULSION AND WIENERS

	Raw emulsion			Wieners		
	Moisture (%)	Fat (%)	Protein* (%)	Moisture (%)	Fat (%)	Protein (%)
Control	65.3[a]	20.9	8.9[a]	48.7	28.8	14.9
Promax 70	63.6[b]	20.7	10.6[b]	50.3	26.8	15.5
Mustard flour	62.3[b]	19.9	9.6[c]	52.3	25.0	16.4
Standard deviation[1]	± 0.89	± 0.73	± 0.28	± 0.18	± 0.84	± 0.47

*Means with different superscripts in the same column are significantly different ($P < 0.05$)
[1]degrees of freedom = 5

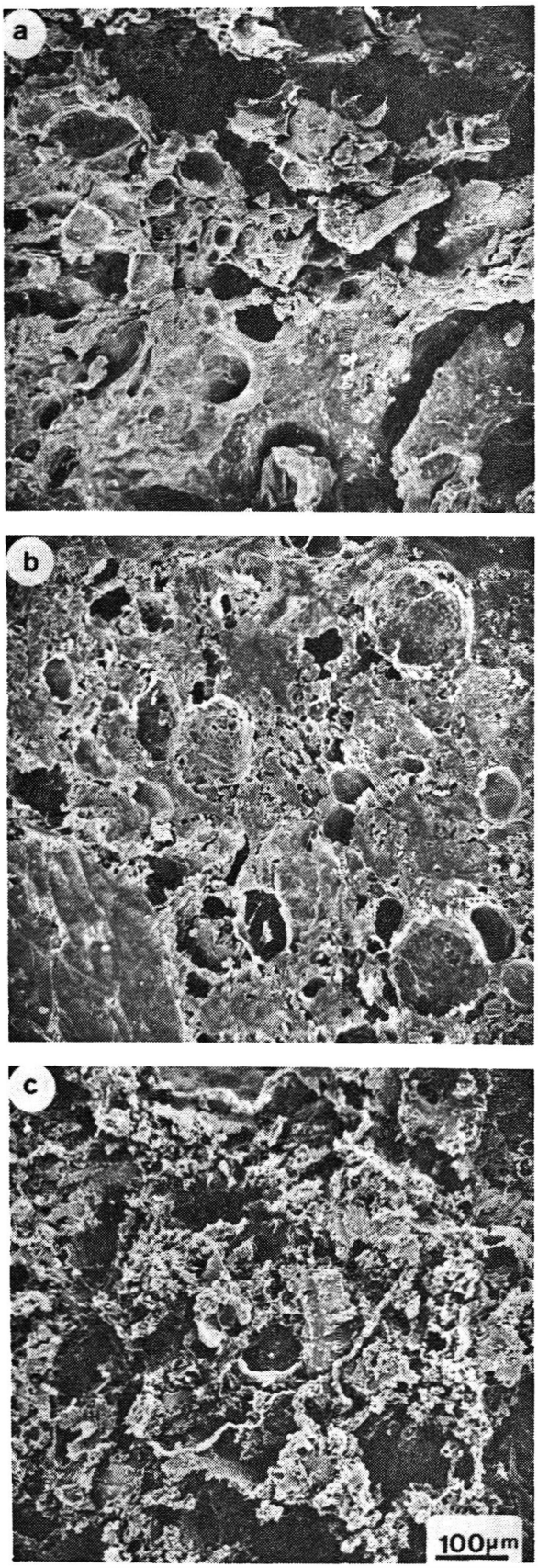

Fig. 1. SEM micrographs of wieners made from strong meat system (a) and in combination with hard wheat flour and Promax 70 (b) or mustard flour (c)

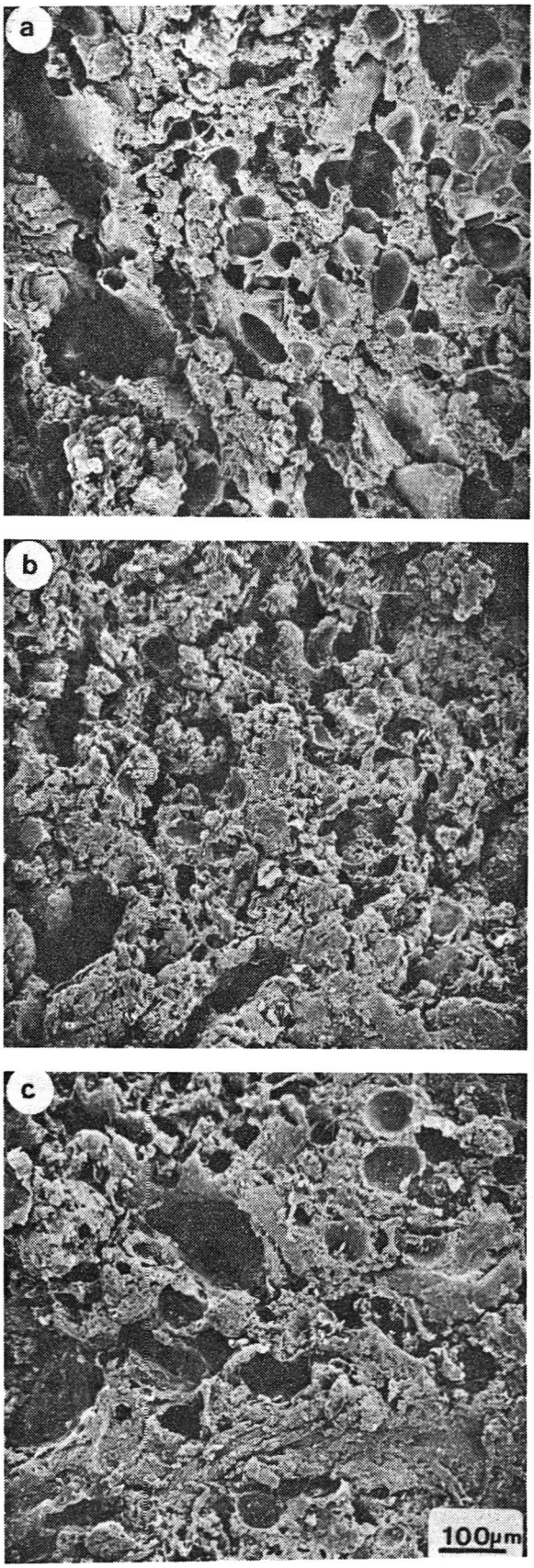

Fig. 2. SEM micrographs of cooked wieners made from strong meat system (a) and in combination with hard wheat flour and Promax 70 (b) or mustard flour (c).

TABLE 4. MEAN COLOUR, FLAVOUR, TEXTURE AND OVERALL ACCEPTABILITY SCORES (mm) FOR WIENERS PREPARED FROM THE STRONG MEAT SYSTEM AND IN COMBINATION WITH HARD WHEAT FLOUR AND PROMAX 70 OR MUSTARD FLOUR, n = 32

Treatment	Colour	Flavour	Texture	Overall acceptability
Control	74.3[a]	77.4[a]	61.0[a]	71.1[a]
Promax 70	56.3[a]	73.5[a]	76.7[b]	69.8[a]
Mustard flour	91.2[c]	60.3[b]	82.0[b]	59.0[a]
S.D.*	±26.9	±32.3	±24.9	±31.7

Means with different superscripts in the same coloumn are significantly different ($P < 0.05$)
*degrees of freedom = 95

The values for all the texture profile parameters (Table 5) were somewhat lower for the mustard flour treatment as compared to control and Promax 70 treatments which were similar. The extenders significantly affected the hardness of the wieners. The presence of Promax 70 increased the hardness and mustard lowered it when compared to the all meat control. Mustard flour resulted in a softer product probably because of the poor ES and formation of emulsions with low consistency observed in the model system[1]. A similar effect has been reported for potato starch and baked cereals by Comer[4]. This may be due to the dilution of the muscle proteins combined with the poor gelation characteristics of mustard flour in the strong meat system, as indicated by gel separation during cooking of wieners and greater peeling losses.

Microstructure of Wieners: The scanning electron micrographs taken for the all meat control and wieners containing hard wheat flour in combination with Promax 70 or mustard flour are shown in Fig 1. Since the samples were defatted during their preparation, the fat globules show up as cavities. The samples containing mustard flour did not show a definite protein matrix (Fig 1c). This may explain the poor ES and greater separation of gel during cooking of wieners. The control and Promax 70 containing samples (Fig 1a and 1b) showed a coarse protein matrix but the cavities due to fat globules were slightly larger in the case of the controls. These observations indicate suspension or mechanical fixing of the fat within a protein matrix as described by Hamm[6] and Van den Oord and Visser[7]. The SEM micrographs of cooked wiener samples revealed disruption of the protein matrix to a greater extent (Fig 2) especially in the control and Promax 70 samples but there was almost no change in the structure of wieners containing mustard flour. This may be due to the denaturation of the protein matrix. The absence of structure in samples containing mustard flour may be responsible for the softer texture.

The addition of hard wheat flour in combination with either Promax 70 or mustard flour did not improve the ES of the strong meat systems confirming the negative effect observed by Bawa *et al.*[1] in model systems. The analysis of cooking and preparation parameters showed that the WHC is as equally important as the emulsification properties of the comminuted system in obtaining higher yields.

Acknowledgement

The authors thank The Griffith Laboratories, Scarborough, Ontario for supplying the non-meat ingredients. Financial support for this research was from the Ontario Ministry of Agriculture and Food. A.S. Bawa was supported by a Canadian Commonwealth scholarship.

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TABLE 5. MEAN HARDNESS SPRINGINESS, COHESIVENESS, GUMMINESS, CHEWINESS AND WARNER BRATZLER SHEAR FORCE FOR WIENERS PREPARED FROM THE STRONG MEAT SYSTEM AND IN COMBINATION WITH HARD WHEAT FLOUR AND PROMAX 70 OR MUSTARD FLOUR, (n = 4)

Treatment	Hardness (Newton)	Springiness (Meter)	Cohesiveness	Gumminess (Newton)	Chewiness (Newton meter)	W.B. shear (Newton)
Control	26.36[a]	0.0138	0.41	10.92	0.150	4.57
Promax 70	35.06[a]	0.0138	0.42	15.05	0.205	4.39
Mustard flour	18.76[a]	0.0128	0.35	6.56	0.085	3.65
Standard deviation*	± 0.93	± 0.0004	± 0.0299	± 0.79	± 0.0108	± 0.23

[1]Means with different superscripts in the same column are significantly different ($P < 0.05$)
*degrees of freedom = 5

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Natural Plant Enzyme Inhibitors: Effect of Tuber Protease Inhibitors on Bovine Enzymes with Protein and Synthetic Substrates

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Inhibitory effect of plant tuber extracts on bovine trypsin, chymotrypsin and the crude pancreatic preparation was assessed using protein and synthetic substrates. Potato alone suppressed the caseinolytic activity of the pancreatic extract more than those of trypsin and chymotrypsin. Arrow root or colocasia tuber inhibitor decreased the activity of trypsin or chymotrypsin to comparable degrees with different substrates. Ratios of trypsin/chymotrypsin inhibition for these two tubers were comparable whether crude pancreatic preparations or purified enzymes were used for the assay. While alocasia inhibitor showed comparable inhibiton of chymotrypsin with different substrates, its trypsin inhibition was more with casein than with albumin and benzoyl arginine p-nitroanilide. Potato extract showed lesser inhibition of trypsin and chymotrypsin with casein than with albumin. Sweet potato showed significantly higher inhibition of chymotrypsin with albumin as substrate than with casein.

Considerable amount of work has been done in recent years on the *in vitro* action of plant protease inhibitors on different pancreatic enzymes to evaluate the toxicological effects of these factors[1–4]. A major difficulty encountered in comparative studies is the differential behaviour of inhibitors in the presence of casein and synthetic substrates. The data of Mallory and Travis[5] indicate that human trypsin is inhibited by soya bean inhibitor and lima bean inhibitor to comparable levels with casein as substrate whereas in the presence of ester substrate, the latter inhibitor is more powerful. Kumar and coworkers[6] observed higher inhibition of trypsin and chymotrypsin by the sorghum inhibitor when synthetic substrates were used. Similar differences in the inhibition of bovine trypsin by several seed extracts were reported recently[7]. Extension of data obtained with synthetic substrates to evaluate the action of plant inhibitors will therefore not be correct in all cases. Further, extrapolation of information obtained with crystalline enzymes to assess the action of the inhibitors on pancreatic digestive system can also lead to erroneous conclusions.

A comparative study on the action of plant inhibitors on purified enzymes and the corresponding crude pancreatic extract with natural and synthetic substrates for the proteases concerned, will help in overcoming these technical difficulties. In this communication, we report the studies on the inhibition of the hydrolytic activities of bovine pancreatic preparation, bovine crystalline trypsin and chymotrypsin by plant tuber inhibitors using casein, albumin, benzoyl arginine p-nitroanilide and acetyltyrosine ethyl ester as substrates.

Materials and Methods

The tubers, alocasia *(Alocasia macrorhiza)*, arrow root *(Maranta arundinaceae)*, coleus *(Coleus parviformis)*, colocasia *(Colocasia antiquorum)*, potato *(Solanum tuberosum)*, sweet potato *(Ipomoea batatas)*, tapioca *(Manihot utilissima)* and yam *(Amorphophallus companulatus)* were procured locally. Bovine pancreas was collected from a freshly killed animal. Preparation of the pancreatic acetone powder and its activation by bovine enterokinase was described earlier[2]. Bovine trypsin (twice crystallized), bovine α-chymotrypsin (thrice crystallized), α-N-benzoyl DL-arginine p-nitroanilide (BAPNA) and α-N-acetyl L-tyrosine ethyl ester (ATEE) were procured from Sigma Chemical Company, St. Louis, MO, U.S.A. Other reagents were analytical grade commercial chemicals.

Measurement of proteolytic activity with casein (10 mg) and its inhibition by plant extracts were performed as described earlier[8]. Under the assay conditions (ph 7.6, 37°C, 10 min incubation), 6 μg of active trypsin, 6.3 μg of active chymotrypsin and 110 μg protein of the pancreatic preparation, yielded trichloroacetic acid soluble fragments equivalent to an absorbance of 0.6 (λ 540) when analyzed by the method of Lowry *et al*[9]. With bovine serum albumin as

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substrate under the same conditions, 18 μg of trypsin was used to get an absorbance of 0.5 and 36.5 μg of chymotrypsin yielded an absorbance of 0.3. Beyond these points, enzyme activities were not linear.

Tryptic activity and its inhibition were assayed using BAPNA as substrate[10]. Under the assay conditions (pH 7.6, 37°C, 30 min incubation), 6 μg of trypsin and 75 μg protein of pancreatic extract released p-nitroaniline equivalent to an absorbance of 0.6 (λ 405). Chymotryptic activity and its suppression were determined using ATEE as substrate[11]. Under the assay conditions (pH 7.6, 37°C, 15 min incubation), 0.63 μg of chymotrypsin and 10 μg protein of pancreatic extract released acetyltyrosine equivalent to an absorbance of 0.6 (λ 540).

Fleshy portion (25 g) of the fresh tuber was homogenized with 25 ml of 0.02 M sodium phosphate buffer, pH 7.6 for 2 min. After stirring for 15 min, the homogenate was centrifuged at 10 000 × g for 20 min. All operations were carried out at 4°C. The supernatant was tested for inhibitory activity by including a wide range of aliquots in duplicate in the assay system described above.

Protein in tuber extracts was determined by the method of Lowry *et al*[9]. using bovine serum albumin as standard. Active site titrations of trypsin and chymotrypsin were performed according to the method of Kezdy and Kaiser.[12]

Results

The data on the inhibition of caseinolysis catalyzed by bovine pancreatic preparation, trypsin and chymotrypsin by the different tuber extracts are shown in Table 1. Suppression of the action of pancreatic extract by potato extract was more than those of the purified enzymes. In other cases, decrease in caseinolytic activity of the pancreatic extract was intermediate between the values obtained for trypsin and chymotrypsin. With sweet potato and colocasia especially the magnitudes of inhibition of the activity of pancreatic extract were significantly lower than the inhibition of tryptic activity. Coleus and yam were found to be relatively poor inhibitors and tapioca extract did not exhibit any measurable activity.

Table 1. Relative inhibitory activities against bovine pancreatic preparation, trypsin and chymotrypsin with casein as substrate

Tuber extract	Pancreatic extract	Trypsin A	Chymotrypsin B	Ratios A/B
Potato	12.50	9.30	7.00	1.33
Arrow root	2.75	4.50	2.30	1.96
Alocasia	2.50	3.33	2.08	1.60
Sweet potato	2.22	7.33	0.17	43.10
Colocasia	1.40	3.33	0.14	23.80
Yam	0.50	0.67	0.33	2.03
Coleus	0.19	0.22	0.09	2.44
Tapioca	Nil	Nil	Nil	–

Values are expressed as absorbance units/mg tuber protein. Inhibitory values were calculated based on the linear range of inhibition and adjusting enzyme concentrations to give the same proteolytic activity in the controls.

Inhibitory patterns of the activities of trypsin, chymotrypsin and the pancreatic preparation with casein as substrate, by potato, alocasia and arrow root extracts, as functions of inhibitor concentration were somewhat similar. The decrease in activity was directly proportional to protein concentration in plant extracts over a wide range (upto 50-70 per cent) and complete inhibition of trypsin and chymotrypsin (> 95 per cent) could be observed at high inhibitor concentrations. With pancreatic extract, maximal inhibition observed was around 80-90 per cent. The profiles of inhibition by arrow root as a representative example, are shown in Fig 1. Sweet potato and colocasia that formed another group, showed similar patterns of inhibition of trypsin like the other three tubers mentioned above. Both were poor inhibitors of chymotrypsin. Maximal inhibition of this enzyme was around 25 per cent by sweet potato and colocasia at inhibitor (protein) concentrations of 2.0 mg. The pancreatic caseinolytic activity was suppressed to 50 and 35 per cent respectively by sweet potato and colocasia at this protein concentration. The patterns with colocasia are shown in Fig 1. Coleus and yam were found to be poor inhibitors of all the three enzyme systems. Coleus inhibited trypsin, chymotrypsin and the pancreatic extract to the maximal extents of 25, 15 and 30 per cent respectively at a protein concentration of 2.4 mg. Corresponding values for yam were 35, 25 and 30 per cent. The inhibitory patterns with yam are shown in Fig. 1. In further studies, coleus, yam and tapioca were excluded. The suppression of trypsin action and of the tryptic activity (adjusted to be equal to that of trypsin action on BAPNA) in the pancreatic extract by the tuber inhibitors were compared. Similarly, inhibition of chymotrypsin and chymotryptic activity in pancreatic preparation was studied with ATEE as substrate. The results are shown in Table 2. Arrow root and colocasia showed comparable action on the pure enzymes and the pancreatic extract. Potato, which showed comparable action on chymotryptic activity, was two times more effective on the tryptic activity on pancreatic extract compared to action on pure trypsin. Alocasia inhibited the tryptic activity of pancreatic extract by an order of magnitude higher than that of trypsin. Both alocasia and sweet potato showed lesser

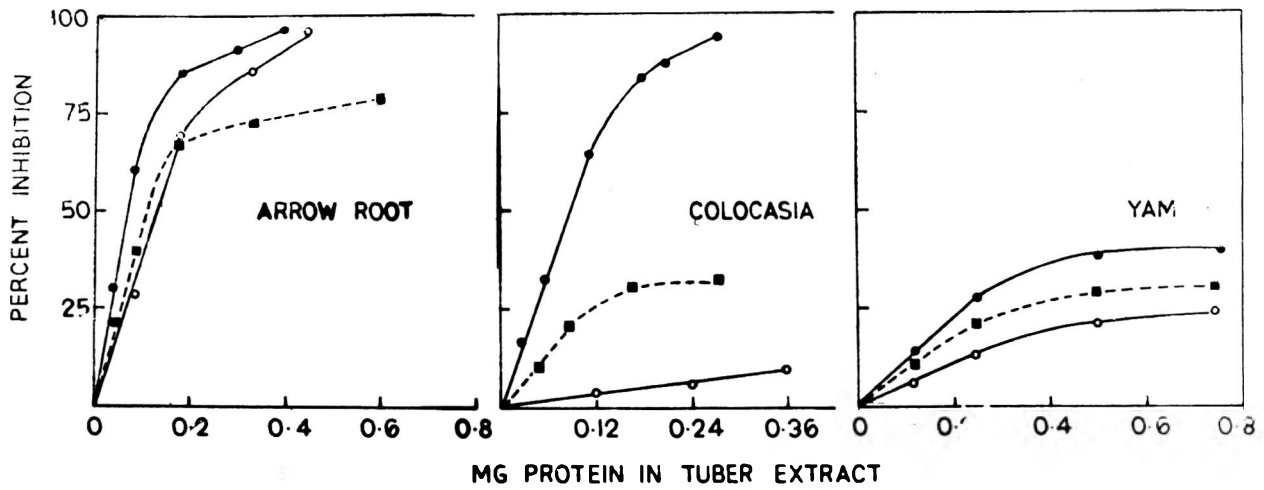

Fig. 1. Inhibition of the caseinolytic activities of trypsin (●——●), chymotrypsin (o——o) and the pancreatic extract (■-----■) by tuber extracts.

inhibition of ATEE hydrolysis by the pancreatic extract than by chymotrypsin itself.

Correlation of the data in Tables 1 and 2 shows that potato and sweet potato showed higher inhibition of crystalline trypsin and chymotrypsin with synthetic substrates than with casein. While alocasia did not show significant difference of chymotrypsin inhibition depending on the nature of substrate, its action on trypsin was less with BAPNA than with casein. The ratios of inhibition of tryptic activity and chymotryptic activity in the pancreatic preparation (Table 2) and the ratios with pure enzymes (Table 2) for arrow root or colocasia were comparable suggesting that inhibitory data obtained with pure enzymes are valid when extended to crude pancreatic system. Even though potato showed comparable ratios for the pure enzymes with casein and synthetic substrates (Table 1) the values are lower than that obtained for the pancreatic system due to greater inhibition of trypsin than chymotrypsin with synthetic substrates. The data with sweet potato and alocasia also lead to similar conclusion.

The inhibition of pure trypsin and chymotrypsin by arrow root and colocasia extracts was not significantly altered with different substrates. While alocasia showed a similar behaviour with chymotrypin, it showed reduced inhibition of trypsin in the presence of albumin (29.4 per cent) compared to casein. On the other hand, potato and sweet potato showed much higher inhibition of the two enzymes with albumin as substrate compared to casein, the effect being very much pronounced in regard to chymotryptic inhibition by sweet potato (58.5 times more with albumin).

TABLE 2. RELATIVE INHIBITION OF TRYPTIC ACTIVITY AND CHYMOTRYPTIC ACTIVITY USING PANCREATIC EXTRACT AND PURE ENZYMES

Tuber extract	Inhibition of BAPNA hydrolysis		Inhibition of ATEE hydrolysis		Ratios	
	Pancreatic extract A	Trypsin B	Pancreatic extract C	Chymotrypsin D	A/C	B/D
Potato	70.00	32.50	20.90	21.20	3.37	1.53
Arrow root	5.00	4.42	2.50	2.16	2.00	2.05
Alocasia	12.50	1.46	1.25	2.38	10.00	0.61
Sweet potato	16.00	25.00	0.14	0.90	114.00	27.70
Colocasia	3.46	3.50	0.12	0.14	28.10	25.00

Values are expressed as absorbance units/mg protein based on the linear range of inhibition. One unit of antitryptic activity is the amount that suppressed BAPNA hydrolysis by an O.D. of 1 and one unit of antichymotryptic activity equals the amount that suppressed ATEE hydrolysis by an O.D. of 10.

Discussion

Potato tuber contains inhibitors specific for trypsin or chymotrypsin and polyvalent inhibitors for serine proteases[13]. The higher degree of inhibition of the caseinolytic activity of the pancreatic extract than those of trypsin and chymotrypsin individually, by potato extract can be due to the concerted action of several inhibitors. Alocasia[14] and arrow root[15] tubers are reported to contain inhibitors acting on both trypsin and chymotrypsin, with common sites for enzyme binding. The intermediate inhibitory values of caseinolytic inhibition of the pancreatic extract in relation to the values with the pure enzymes can be explained as due to competition between the enzymes. The distinctly low inhibition of the activity of pancreatic extract by colocasia and sweet potato compared to tryptic inhibition can be due to infructuous binding of

the inhibitors to chymotrypsin. These two tubers contain factors which are essentially trypsin inhibitors with feeble action on chymotrypsin[8,16].

Arrow root or colocasia showed comparable amount of inhibition of trypsin or chymotrypsin in the presence of different substrates. With these two inhibitors, information obtained with crystalline enzymes are reasonably close to the inhibition data provided by studies using crude bovine pancreatic preparation. Even though potato extract inhibited ATEE hydrolysis by chymotrypsin and the pancreatic extract to comparable levels, it was distinctly more potent on the hydrolysis of BAPNA in the crude system. Alocasia showed a similar behaviour with respect to inhibition of tryptic activity. The possibility of the presence of esterases acting on BAPNA being susceptible to some of these inhibitors in crude pancreatic preparation needs to be investigated. On the other hand, alocasia and sweet potato were less effective in blocking ATEE hydrolysis by pancreatic extract than by the pure enzyme. The exact reason for this is not clear. The data suggest that information obtained on inhibition with synthetic substrates may not be valid in all cases to evaluate the suppression of proteolysis by pancreatic enzymes. While emphasizing the evaluation of assays for measurement of protease inhibitors in relevance to nutrition, Holm and Krogdahl[17] concluded that effects on caseinolytic activity will be most relevant. The present studies also show that magnitude of inhibition of crystalline enzymes vary depending on whether casein or albumin is used as substrate. Further studies using different protein substrates of common dietary origin will be more meaningful in evaluating protease inhibitor levels in plant systems.

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INCIDENCE OF ENTEROTOXIGENIC *KLEBSIELLA PNEUMONIAE* IN VARIOUS FOOD GROUPS

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Various food samples were screened for the presence of *Klebsiella pneumoniae*. Maximum isolations were from sweets (60%) and least from meat and meat products (40%). Large number of these isolates were found to be enterotoxigenic (61%) as detected by anti LT-coated staphylococcal co-agglutination test. All the isolates from chat, curd and snacks were enterotoxigenic. Incidence of multiple drug resistance (MDR) was 37% and resistance to individual antibiotics was in the range of 8 to 92%.

Diarrhoeal infections are the major cause of mortality especially in the developing countries. Besides non-invasive enterotoxin producing strains of *Escherichia coli*, strains of *Klebsiella pneumoniae* have also been isolated from cases of acute diarrhoea, chronic diarrhoea and tropical sprue.[1-3] Transient colonization of small intestine by strains of enterotoxigenic *K. pneumoniae* produces a heat stable (ST) or heat labile (LT) or both LT and ST enterotoxins[4-7] which are commonly responsible for episodes of diarrhoea.

Prevalence of *K. pneumoniae* strains in various food samples has been reported from India and abroad.[8-12] Results of these studies indicate that *K. pneumoniae* is a frequent isolate among *Enterobacteriaceae* indicating that this organism might signal unhygienic handling of the food products as it has a faecal orgin like typical *E. coli* and hence is of considerable importance from the public health point of view. In the present study, various food samples in our community were screened for the incidence of *K. pneumoniae* and enterotoxigenicity.

Collection of samples and processing: Samples were collected from different shops in the city in sterile containers or polythene bags and brought to the laboratory immediately. For enrichment, nutrient broth was used and finally the samples were streaked on MacConkeys agar. Lactose fermenting, mucoid colonies were picked up and identified biochemically as suggested by Orskov.[13] One hundred strains identified as *Klebsiella pneumoniae* were stored at 4°C in nutrient agar stabs.

Co-agglutination test for enterotoxigenicity: The enterotoxin producing ability of all the strains of *Klebsiella pneumoniae* was determined by the co-agglutination test as described by Ronnberg and Wadstrom.[14] Each strain was grown on blood agar at 37°C for 16-18 hr. A loopful of bacteria from blood agar plates was suspended in 100 µl of saline containing 2mg/ml of polymyxin B. The tubes were incubated in a water bath at 37°C for 1hr. Later, Triton X-100 was added to these tubes to achieve a final concentration of 0.1 per cent and the mixture was further incubated for 10 min. The lysate was separated by centrifugation (3000 g, 20 min). Equal volumes of cell lysates and co-agglutination reagent (25 µl each) were mixed on phadebact cellulose paper cards. The results were read within 2 min. A positive result (co-agglutination) was indicated by the aggregation of anti LT-coated staphylococci. Lysates of *E. coli* (1628-15) and normal saline were used as positive and negative controls.

Antibiotic sensitivity: All the isolates were subjected to antibiotic sensitivity test by the method of Bauer *et al.*[15]. Discs of various antibiotics (Hindustan Dehydrated Media, Bombay, India), and the concentrations used were: ampicillin (10µg), streptomycin (10µg), chloramphenicol (30µg), tetracycline (30µg), kanamycin (30µg), gentamycin (10µg) nalidixic acid (30µg).

TABLE 1. FREQUENCY OF OCCURRENCE OF *K. PNEUMONIAE* AMONG DIFFERENT FOODS.

Food groups	Screened (No.)	+ve for *K. pneumoniae* (No.)	+ve for LT toxin (No.)
Raw milk	20	9	6
Curd	5	3	3
Ice cream	60	28	18
Cheese	5	1	NIL
Vegetables	45	27	17
Sweets	20	13	8
Snacks	15	5	5
Meat and meat products	35	14	4
Total	205	100	61

TABLE 2. DISTRIBUTION OF ENTEROTOXIGENIC *K. PNEUMONIAE* ISOLATED FROM VARIOUS FOODS

Samples	Tested (No.)	+ve for LT enterotoxin (No.)
Raw milk	9	6
Curd	3	3
Ice cream	28	18
Cheese	1	–
Roots and tubers	15	10
Green vegetables	12	7
Sweets	13	8
Snacks	5	5
Sausages	3	1
Salami	2	1
Keema	4	2
Total	100	61

One hundred out of the 205 samples of different foods showed the presence of *K. pneumoniae* (Table 1). Maximum isolations were from sweets (60 per cent) and the least from meat and meat products (40 per cent). More than 45 per cent of ice cream samples were positive for *K. pneumoniae*. A large proportion of the isolates were enterotoxigenic (61 per cent). Isolations of enterotoxigenic strains were maximum from sweets and snacks (72.2 per cent) followed by milk and milk products (65.8 per cent). The incidence was very low in the meat and meat products (25 per cent). Maximum number of LT enterotoxin producing *Klebsiellae* were isolated from chat, curd and snacks (100 per cent respectively). Isolations from milk, ice-creams, roots, tubers and sweets were in the range of 60 to 66 per cent (Table 2).

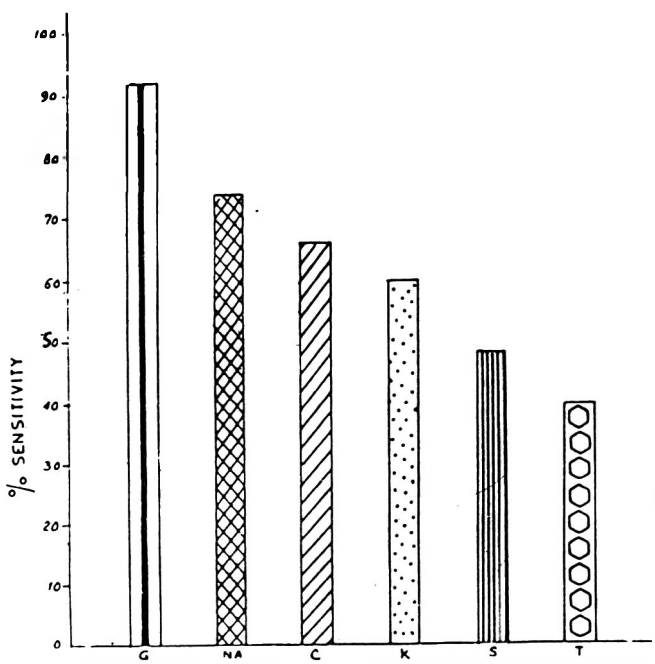

Fig. 1. Sensitivity of isolates towards antibiotics Gentamycin (G), Nalidixic acid (NR), Chloramphenicol (C), Kanamycin (K), Streptomycin (S), Tetracycline (T).

Out of these strains; about 37 per cent of the strains showed resistance towards more than three antibiotics and were labelled as multiple drug resistant. However, no correlation betwen enterotoxin production and multiple drug resistance could be established ($P<0.01$). Gentamycin and nalidixic acid were the most effective drugs. Resistance to individual antibiotics was in the range of 8 to 92 per cent (Fig.1).

The high percentage (48.7) of incidence of these organisms is in agreement with earlier findings[8-12] and can be attributed to the ignorance and insanitary conditions employed by the vendors and shopkeepers during storage and distribution of foods. If the source of these organisms gets established to be faecal in origin, then this organism can be used as an indicator for judging the hygienic quality of foods.

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QUALITY CHARACTERISTICS OF SEEDS OF FIVE OKRA *(ABELMOSCHUS ESCULENTUS* L.) CULTIVARS

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The seeds of five okra cultivars viz., *'Punjab-7', 'Pusa Sawani', 'Punjab Padmini', 'L-Sel-1'* and *'Crimson Red'*, developed by Punjab Agricultural University, Ludhiana were analysed for proximate composition. The seeds were found to contain oil and protein in the range of 18.83 to 20.18 and 20.2 to 23.2% respectively. The seeds of cultivar *Punjab-7'* were highest in oil content followed by *'Pusa Sawani'*. Fractionation of the crude protein from okra seeds of cultivar *'Pusa Sawani'* showed that they are rich in globulin and prolamine. The seed oils of the three cultivars were examined for their characteristics and fatty acid composition. A positive Halphen colour test indicated the presence of cyclopropene fatty acids in the oil. The oil was composed of about 66 to 73% unsaturated fatty acids.

Among the seed bearing plants, only soybean and cotton seeds have been utilized to an appreciable extent for the production of protein isolates or concentrates and edible oil.[1,2] Okra seeds are reported to be rich in protein and oil[3,4] and have often been used for various purposes. Roasted and ground seeds have been used as a coffee substitute.[5] Martin, *et al.*[3] have suggested the preparation of vegetable curd from okra seed meal. Crossley and Hilditch[6] reported that okra seeds could be substituted for cotton seeds in the extraction of oil for edible purposes or for other industrial uses.

The yield of okra seeds of different cultivars is estimated to be 9 to 12 quintals/hectare. However, no reports are available on the commercial production of okra seeds for industrial use. At present, okra seeds are produced for seed purposes only. This paper reports on the physico-chemical characteristics of the new cultivars of okra seeds developed by PAU.

Representative samples of the seeds from five okra cultivars namely 'L-Sel-1', 'Pusa Sawani', 'Punjab-7', 'Punjab Padmini' and 'Crimson Red' grown under identical agro-climatic conditions were obtained from Punjab Agricultural University, Ludhiana.

Standard AOAC[7] methods were followed for the determination of moisture, total sugar, protein (N × 6.25), crude fibre and total ash in whole seeds of the five okra cultivars. Fractionation of crude protein of okra seed meal of cultivar 'Pusa Sawani' was carried out by the solubility method of Mitchel.[8] The seeds were ground in a Waring blender and passed through a 30 mesh sieve. A known weight (10g) of defatted powdered sample was successively extracted four times with (a) distilled water, (b) 3 per cent sodium chloride, (c) 70 per cent ethanol and (d) 0.2 per cent sodium hydroxide for two hr each by shaking them in a rotary shaker followed by centrifugation. The volume was made upto 250 ml in each case. Aliquots of 50 ml were then taken in duplicate for the estimation of protein by microkjeldahl method.[7]

In order to assess the potential of okra seeds for oil production, the oil from seeds of three okra cultivars namely 'Punjab-7', 'Pusa Sawani' and 'Punjab Padmini' was extracted with hexane using a Soxhlet apparatus for 16 hr. Solvent and moisture free oil were filtered and analysed for colour, refractive index at room temperature (16°C), specific gravity (16°C), iodine value, free fatty acids and saponification value according to the AOCS[9] official methods. The oil was subjected to Halphen colour test according to the method of Coleman and Firestone.[10] The fatty acid composition of the oil was determined by gas chromatography of the methyl esters using a Neucon series 5700 gas chromatograph equipped with a flame ionization detector as described by Batta, *et al.*[11]

The proximate composition of the seeds is presented in Table 1. The husk was removed by coarse grinding of seeds in a Waring blender and passing through a 30 mesh sieve. The husk and seed meal contents of the

TABLE 1. PROXIMATE COMPOSITION OF OKRA SEED CULTIVARS

Parameters	'Punjab-7'	Punjab Padmini'	'L-Sel-1'	'Pusa Sawani'	'Crimson' Red
Moisture (%)	9.24	9.17	10.41	10.73	10.34
Fat (%)	20.18	19.13	18.98	19.20	18.83
Protein (%) N × 6.25)	21.60	20.20	23.20	22.80	21.48
Crude fibre (%)	23.24	24.11	21.54	23.30	24.15
Total sugars (%)	2.26	1.99	2.44	1.88	2.10
Total ash (%)	6.65	5.60	7.15	6.52	5.44
Carbohydrates (%) (by diff.)	16.83	19.80	16.28	15.57	17.66

Average of three replicates; results are on moisture free basis.

whole seed ranged from 42.6 to 44.5 per cent and 55.5 to 57.4 per cent, respectively, indicating that there was no marked difference in the recovery of seed meal among the cultivars examined. The okra seeds of cultivar 'Punjab-7' contained the highest amount of oil (20.18 per cent) followed by 'Pusa Sawani' (19.2 per cent). On the other hand, the seeds of okra cultivar 'L-Sel-1' contained the highest amount of protein (23.2 per cent), closely followed by 'Pusa Sawani' (22.8 per cent).

The okra seed meal from cultivar 'Pusa Sawani' contained 34.2 per cent protein and 26.7 per cent oil (Table 2) and may be exploited for protein and oil production. The okra seeds are lower in protein than soybean. The seed husk contained 10 per cent protein, 5.8 per cent oil, 54.3 per cent crude fibre and hence may be used in cattle feed.

The crude protein in okra seed-meal of cultivar 'Pusa Sawani' upon fractionation was found to be composed of 29.2 per cent globulin, 26.1 per cent albumin, 16.4 per cent glutelin, 13.3 per cent prolamine and 5.4 per cent non-protein nitrogen.

Characteristics of the oil: The oil recovered from the seeds of the three okra cultivars was examined for its physical constants and other characteristics (Table 3). The oil was bright yellow in colour. There was no significant variation in the refractive index of the oil among the cultivars. However, specific gravity of the oil varied slightly among the cultivars. The iodine value of oil was found to be maximum for the seeds of cultivar 'Punjab-7' and minimum for 'Pusa Sawani'. Free fatty acid content of the oil was found to be slightly higher in 'Punjab-7' and minimum in 'Punjab Padmini'. The oil from the three cultivars gave positive Halphen test indicating the presence of cyclopropene fatty acids. More than 3g of oil was needed to produce a light orange-red colour suggesting the occurrence of only traces of these fatty acids.

The fatty acid composition of the three okra cultivars namely 'Punjab-7', 'Punjab Padmini' and 'Pusa Sawani' is presented in Table 4. The oil from cultivar 'Punjab-7' contained the highest proportion of unsaturated fatty acids, an advantageous trait from the nutritional point of view. Okra seed oil may thus be used advantageously as edible oil in place of cotton seed oil since it closely resembles the latter in fatty acid composition and other oil characteristics.

It may be concluded that the okra seed cultivar 'Punjab-7' is a potential source of protein and oil.

TABLE 2. PROXIMATE COMPOSITION OF OKRA SEED, SEED MEAL AND SEED HUSK (cv. *PUSA SAWANI*)

Parameter	Okra seed		
	Whole	Meal	Husk
Moisture (%)	10.7	8.0	11.6
Fat (%)	19.2	26.7	5.8
Protein (%) (N × 6.25)	22.8	34.2	10.0
Crude fibre (%)	22.3	4.6	54.3
Total ash (%)	6.5	6.5	6.3
Carbohydrates (%) (By diff.)	18.5	20.0	12.0

Average of three replicates
Results expressed on moisture free basis

TABLE 3. CHARACTERISTICS OF OKRA SEED OIL

Property	Okra seed cultivar		
	Punjab-7	Pusa Sawani	Punjab Padmini
Refr. index at 16°C	1.47122	1.47121	1.47122
Sp. gr. at 16°C	0.9199	0.9191	0.9196
Iodine value (Wij's method)	88.78	77.60	79.41
Sapon. value	166.91	162.49	163.60
FFA (as oleic acid %)	1.10	0.86	0.76

Means of three determinations

TABLE 4. FATTY ACID COMPOSITION OF OKRA SEED OIL

Fatty acid	Per cent of total in		
	Punjab-7	Punjab Padmini	Pusa Sawani
16:0	21.92	25.96	29.04
16:1	0.24	0.15	0.15
16:2	0.53	0.33	0.31
18:0	4.53	4.90	4.03
18:1	31.20	29.76	23.63
18:2	36.15	34.42	39.26
18:3	3.48	1.57	1.54
20:0	Trace	0.81	Trace
20:1	1.00	1.07	0.46
22:0	0.20	Trace	0.45
22:1	0.46	0.46	0.51
24:0	Trace	0.21	0.23
Total sat.	26.65	31.88	33.75
Total unsat.	73.06	67.76	65.96

Trace < 0.1%.
Means of two determinations

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DEACIDIFICATION ACTIVITY OF *SCHIZOSACCHAROMYCES POMBE* IN PLUM MUSTS

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Deacidification behaviour of *Schizosaccharomyces pombe* was studied in plum musts of varying sugar contents. It was found that the deacidification activity decreased as the percentage of T.S.S. is increased in the plum musts. Alcohol content and volatile acidity of the fermented musts increased with the T.S.S. For effecting deacidification of plum musts, extra addition of sugar is not required.

The yeast, *Schizosaccharomyces pombe,* has been reported to metabolize malic acid[1,2] and its use has been suggested for reducing the acid contents of grape musts for producing wines of acceptable quality[3,4]. Plum fruits also contain malic acid[5] and fermentation with this yeast has been found to be effective in reducing the total titrable acidity in the plum musts[6]. But whether the metabolised acid is converted into ethyl alcohol or not is not clear[2]. The activity of yeast like other micro-organisms could be influenced by various factors like types of substrates, nutrient concentration, temperature, pH, etc. In the absence of any published information on these aspects in plum musts, the present study was undertaken to look into the deacidification behaviour of yeast in musts containing varying degrees of total soluble solids and the results obtained are reported here.

Fully ripe and sound plum fruits of 'Santa Rosa' variety procured locally, were made into pulp by boiling the destoned fruits with sufficient water. The resulting pulp had total soluble solids (T.S.S.) of 6.8°B and 0.92 per cent acidity as malic acid. The pulps were ameliorated to varying sugar contents i.e. 12°(P_2), 15° (P_3), 20°(P_4) and 25°(P_5) except the control 6.8°B(P_1). Analysis of musts for various characteristics like T.S.S., acidity and pH were carried out as per standard methods[7]. These musts were then sterilized and inoculated with five per cent active culture of *Schizo. pombe* obtained from the Indian Institute of Horticultural Research, Bangalore. The fermentations were carried out in duplicate at a temperature of 25 ± 1°C. During fermentation, changes in T.S.S., titrable acidity and pH were recorded. After the completion of fermentation as evidenced by stable °Brix and acidity, the musts were siphoned, filtered, bottled and processed in boiling water. They were analysed for T.S.S (°B), alcohol (%), titrable acidity, volatile acidity and pH. Microscopic examination of the musts at random confirmed the presence of the yeast.

The results (Table 1) show that the deacidification activity of the yeast decreases as the T.S.S. of the musts increases. The highest percentage of acid reduced was 88 when the T.S.S. was only 6.8° B (P_1) and then there was a continuous decrease in per cent acid and it was 63 per cent when the T.S.S. was raised to 25°B(P_5). Corroborating with the acid used, the pH increased in all the musts (Table 1). The reduction in titrable acidity is obviously due to the metabolisation

TABLE 1. CHARACTERISTICS OF PLUM MUSTS FERMENTED WITH *SCHIZOSACCHAROMYCES POMBE*

Musts	Initial				Final			
	T.S.S. (°Brix)	Titratable acidity (% MA)	pH	Alcohol (% V/V)	T.S.S. (°Brix)	Titratable acidity	Volatile acidity (% A.A.)	pH
P_1	6.8	0.92	3.27	2.7	2.8	0.11	0.04	4.42
P_2	12.0	0.92	3.28	5.5	4.0	0.22	0.09	4.02
P_3	15.0	0.91	3.28	8.2	5.0	0.30	0.09	3.93
P_4	20.0	0.91	3.28	11.6	6.0	0.32	0.13	4.15
P_5	25.0	0.92	3.27	12.6	8.6	0.34	0.20	4.16

MA = malic acid.

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of malic acid in plum musts but the percentage of acid metabolised is not the same in all the musts. The highest percentage of acid used (88 percent) in P_1 must show that almost complete utilization of the acid has taken place since it is reported that about 90 per cent of total acidity of plum fruit is contributed by malic acid[5]. Similar results about the per cent malic acid used by this yeast in grape must have been reported[4].

As is evident from the data, the deacidification of the yeast decreased as the percentage of sugar (T.S.S.) increased. From this, it appears that both the acid and sugar are metabolised simultaneously by the yeast but in the presence of high concentration of sugar lesser quantity of acid is utilized. However, in case of grape musts fermented with *Schizo. pombe* the yeast utilized malic acid before fermenting glucose to completion[8].

The data about the behaviour of sugar utilization in different musts (Table 1) show that with the increase of the T.S.S. from P_1 to P_5 there was an increase in sugar utilization. But the optimum utilization of sugar occurred when T.S.S. was about 20°B (70 per cent) and decreased to 58.8 and 64 per cent in P_1 and P_5 musts, respectively. The alcohol contents of the fermented musts increased from 2.7 to 12 per cent as the T.S.S. of the musts were increased from 6.8 to 25°B. Almost similar percentage of alcohol content from the grape musts with comparable sugar levels (°Brix) and fermented with the yeast have been reported[3]. Volatile acids of the fermented musts showed a general increasing trend with the increase in the T.S.S. of the musts. This characteristic has been found to be associated with *Schizo pombe* in the studies carried out on grape musts[1,4]

From the findings, it can be concluded that in plum musts addition of sugar does not enhance deacidification and the yeast can be employed to decrease the acidity to a desirable level for preparation of wine of acceptable quality.

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PROXIMATE COMPOSITION OF JACK FRUIT SEEDS

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The proximate composition of the seeds of 'Kathari' and 'Bharat Baramasi' varieties of jack fruit suggests that they are good sources of carbohydrates (26.83-28.01%), protein (6.25-6.75%) and minerals (1.16-1.27%). Fractionation of nitrogen revealed that non-protein nitrogen forms 5.56% and 7.00% of the total nitrogen in 'Kathari' and 'Bharat Baramasi' seeds, respectively. Globulin – nitrogen forms the major portion of total nitrogen in both the varieties.

Tender jack fruit comes to the market in spring and continues until summer and it is used as a popular vegetable. Since common vegetables are scarce and costly at that time of the year, jack fruit enjoys a high demand and premium price[1]. The ripe fruit is not liked by many people due to its characteristic flavour; however, its seed is cooked and used in many culinary preparations[2]. Little work is done on the biochemical composition of jack fruit seed[3,4]. The present communication reports on the chemical composition and nutritive value of jack fruit seed.

Seeds of two local varieties of jack fruit i.e. 'Kathari' and 'Bharat Baramasi' were used in the present study. They were cut into small pieces, dried in an oven at 60°C for about 24 hr (till constant weight), powdered and passed through a 60 mesh sieve. Moisture content was calculated by subtracting dry weight from fresh weight and expressed as per cent of fresh weight. The methods as described in A.O.A.C.[5] were used to analyse protein, fat and total mineral contents. Total carbohydrate content was determined by difference i.e. subtracting from 100 the sum of values for moisture, protein, fat and total mineral contents[6]. Reducing, non-reducing and total sugars were estimated by Shaffer-Somogyi's micro-method and starch content by the method of McReady *et al*[7]. For fractionation of nitrogen, the method of Nagy *et al*[8]. was adopted; accordingly known amounts of seeds were extracted twice with water, 1 M NaCl, 90 per cent ethanol and 1 per cent NaOH for 1 hr with each. After centrifugation, the supernatant was made up to a known volume and N content in each fraction was estimated by Kjeldahl method[5]. Non-protein nitrogen was determined in the filtrate after precipitating the protein with TCA.

TABLE 1. PROXIMATE COMPOSITION OF JACK FRUIT SEEDS*

Parameters	'Kathari'	'Bharat Baramasi'
Moisture (%)	63.19	64.87
Protein (%)	6.75	6.25
Fat (%)	0.78	0.89
Total carbohydrates (%)	28.01	6.83
Starch (%)	15.50	14.35
Reducing sugars (%)	2.00	2.50
Non-reducing sugars (%)	4.14	4.00
Total sugars (%)	6.36	6.71
Total minerals (%)	1.27	1.16
Energy (k.cal/100g)	146.06	140.33

*Average of three replicates.

A perusal of data presented in Table 1 shows that there were considerable differences in the levels of all the biochemical constituents between both the varieties. Higher values for protein (6.75 per cent), total carbohydrates (28.01 per cent) and starch content (15.50 per cent) were observed in 'Kathari' seeds whereas seeds of 'Bharat Baramasi' gave higher values for fat (0.89 per cent). Reducing, non-reducing and total sugars ranged from 2.00-2.50, 4.00-4.14 and 6.36-6.71 per cent respectively. 'Kathari' variety had the higher non-reducing sugar content whereas 'Bharat Baramasi' gave the higher values for reducing and total sugar contents. Higher total mineral content (1.27 per cent) was observed in 'Kathari'. Energy value was higher in 'Kathari' (146.00 K.Cal.) than in 'Bharat Baramasi' (140.33 K.Cal.).

Data regarding the fractionation of nitrogen are presented in Table 2. The seeds of 'Kathari' variety contained more nitrogen than the seeds of 'Bharat Baramasi'. Of the total nitrogen make up, the seeds of 'Kathari' variety contained more of globulin and glutelin nitrogen, while the seeds of 'Bharat Baramasi'

TABLE 2. FRACTIONATION OF NITROGEN IN JACK FRUIT SEEDS*

Nitrogen fraction	'Kathari'		'Bharat Baramasi'	
	N (%)	% of total N	N (%)	% of total N
Total nitrogen	1.08	–	1.00	–
Non protein nitrogen	0.06	5.56	0.07	7.00
Albumin	0.08	7.41	0.09	9.00
Globulin	0.35	32.41	0.31	31.00
Prolamin	0.16	14.81	0.18	18.00
Glutelin	0.18	16.67	0.16	16.00

*Average of three replicates

scored over the 'Kathari' in respect of non-protein nitrogen, albumin and prolamin. Globulin nitrogen formed the major portion of total nitrogen in both the varieties whereas albumin formed the least. The overall variability in quality components may be due to the varieties chosen for analysis and agroclimatic factors under which they were grown. 'Kathari' variety was observed to be nutritionally excelling the 'Bharat Baramasi' on the basis of most of the constituents analysed.

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OCCURRENCE OF *SALMONELLA* IN MEATS

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The study assesses the prevalence of *Salmonella* in buffalo meat and meat products. About 5% samples each of fresh and frozen buffalo meats, 6.6% minced meat, 10% *kabab* and 5% each of prescapular and popliteal lymph nodes were positive for *Salmonella*. These included *S. anatum* (63.2%), *S. weltevreden* (20.4%), *S. typhimurium* (10.2%), *S. poona* and *S. newport* (2.04% each). Of these about 91% were found to be enteropathogenic when tested in ligated ileal loops of rabbit by whole cell cultures. Dilation Index for strains giving positive response varied from 0.85-1.08.

Role of *Salmonella* as an agent of food-borne disease has received increasing attention all over the world and foods of animal origin are still the major source of human salmonellosis[1]. The present investigation was undertaken to explore the magnitude of *Salmonella* incidence in meats and to suggest preventive measures. It reveals the prevalence of these organisms in buffalo meats and the lymph nodes along with the frequency of their occurrence and entero-pathogenicity.

Salmonella have been reported to occur in abattoir drains and fresh carcass meats viz. bovines, pigs and poultry[2,3]. Das Gupta[4-5] and Manickam and Victor[6] isolated number of *Salmonella* serotypes from beef (2.5-5 per cent), pork (3 per cent) and mutton (2-5 per cent) samples. Similarly, many *Salmonella* serotypes were isolated from meat cuts (8 per cent) and sheep carcasses[7] (9 per cent). Mandokhot *et al*[8]. and Randhawa and Kalra[9] demonstrated the presence of many *Salmonella* serotypes from sheep and goat meats and prescapular, inguinal and mesenteric lymph nodes of goat carcasses. Sherikar *et al*[10]. reported high incidence rates of *Salmonella* (2.0×10^5/g) in porkies and in *kabab* (3.5×10^5/g). Meat and meat products have been reported to harbour large number of *Salmonella* serotypes like *S. anatum, S. weltevreden, S. dublin, S. typhimurium, S. newport, S. infantis, S. london, S. paratyphi B, S. bareilly* and *S. enteritidis*.[1-10]

Fresh meat samples from thigh region and the prescapular and popliteal lymph nodes of buffalo carcasses were collected from the slaughter house. Minced meat and cooked meats (curry and *kabab)* were collected from road side restaurants and meat shops. Fresh meat samples were aseptically divided into two portions. One portion was subjected to bacteriological examination immediately and the other was stored for subsequent analysis after one month of storage at –10°C. Frozen samples were thawed in a refrigerator at 4-5°C for 7 ± 1 hr before analysis.

Lymph nodes were subjected to bacteriological examination after sterilizing their surfaces[11]. Indian standards method[12] was followed to isolate and identify *Salmonella* from meats and the lymph nodes. Enteropathogenicity of the cultures was determined by ligated ileal loop technique (RLIL) in rabbits by whole cell cultures (WCC)[13-15]. Ratio of fluid volume to loop length was calculated and expressed as dilation index. An index of 0.4 and above was considered a positive reaction.

In all, 246 meat samples were analysed. Prevalence rates of salmonellae are presented in Table 1. Fresh buffalo meats harboured highly infectious types of salmonellae like *S. anatum, S. weltevreden* and *S. typhimurium*. These could be recovered from as many samples in frozen meats as in fresh i.e. these organisms survived subthermal stress during frozen storage at –10°C for one month. Thus, fresh and frozen buffalo meats would not only act as an

TABLE 1. OCCURRENCE OF *SALMONELLA* IN BUFFALO MEAT, LYMPH NODES AND MEAT PRODUCTS

Product	Number of samples		% positive	Serotypes isolated
	Tested	Positive		
1. Fresh meat	78	4	5.13	*S. anatum, S. weltevreden, S. typhimurium* and rough strains
2. Frozen meat	78	4	5.13	*S. anatum, S. weltevreden* and *S. typhimurium*
3. Raw minced meat	30	2	6.66	*S. newport, S. poona*
4. Cooked meats				
Kabab	10	1	10.00	*S. anatum*
Curry	10	Nil	Nil	Nil
5. Lymph nodes				
Prescapular	20	1	5.00	*S. anatum*
Popliteal	20	1	5.00	*S. anatum*
Total	246	13	5.28	Vide supra

important source of human salmonellosis, but would cross-contaminate other products during processing, transportation, distribution and storage. Infected lymph nodes would act as an important source of endogenous contamination in meats particularly ground or minced meats.

Table 2 shows the order of occurrence of *Salmonella* in buffalo meats and the lymph nodes. *Salmonella anatum* was found to be the commonest species and was isolated from almost all the types of meat tested. This was followed by *S. weltevreden*, *S. typhimurium*, *S. poona*, *S. newport* and rough strains. Infected lymph nodes constitute an important source of endogenous contamination in meats, particularly the ground and minced meats. These have been reported to be important reservoirs of *Salmonella* in buffaloes. Isolation of *S. anatum* has been reported not only from prescapular and popliteal lymph nodes but also from a large number of other lymph nodes from different animals[9,16-18]. These may transfer salmonellae to other parts during carcass cutting and inspection. These should be inspected and discarded carefully.

Table 1 shows that prevalence rates of *Salmonella* were higher in raw minced meat and *kabab* as compared to fresh meats. Sherikar *et al*[10]. reported high *Salmonella* incidence in *kababs*. Presence of *Salmonella* in ready-to-eat meat products is a direct health hazard. Inadequate cooking and/or recontamination are the factors that contribute *Salmonella* to such products. Al Rajab and Hussain[19] who isolated salmonellae from 6.6 per cent samples of *kabab* prepared from beef had made a similar observation. Presence of *Salmonella* in *kabab* could be attributed to post-cooking contamination, poor personal hygiene, poor sanitary practices, or inadequate heating. Proteinaceous materials have poor heat penetration rate. Miller and Ramsdon[20] reported that baking meat pies at 204.4°C oven temperature produced browning of surface crust. When the browning of crust occurred, the central internal temperature was 47.2°C. This was not sufficient to kill *Salmonella*. Minced meats are used to prepare a number of meat products. Prevalence rates of *Salmonella* in such meats vary widely depending upon the production practices and the degree of sanitation[20-22].

Most of the *Salmonella* species were found to be enteropathogenic when tested in RLIL by WCC. The volume of fluid accumulated in ligated loops varied from 1.8 to 10 ml with a corresponding dilation index of 0.22-1.25. On the whole, about 91 per cent of the eleven strains tested gave positive reaction except *S. poona*.

In view of the complex epidemiological distribution, *Salmonella* contamination may arise at any step in the food chain viz. animals – foods – human. It is generally accepted that any serotype of *Salmonella* is potentially dangerous to man[23]. Number of *Salmonella* cells/g required to produce illness depend upon the virulence of the strain. However, less than 1.0 to large numbers of *Salmonella*/g of foods have been implicated in outbreaks[23]. Hence, presence of *Salmonella* at any level in meats is objectionable. For these reasons, meat should be shown to be free from *Salmonella* employing presence/absence test in "the two class attribute plan"[23,24]. Keeping in view the

TABLE 2. SPECIES AND SOURCE-WISE DISTRIBUTION OF *SALMONELLA* IN BUFFALO MEATS AND MEAT PRODUCTS

Serotype	Antigenic structure	Source of isolation	No. of strains	% of total isolates	Number of samples		% positive
					Tested	Positive	
S. anatum	3,10:eh:1,6	Fresh meat	18	36.73	78	1	1.28
		Frozen meat	4	8.16	78	1	1.28
		Kabab	4	8.16	10	1	10.00
		Lymph nodes	5	10.20	40	2	5.00
S. weltevreden	3,10:r:Z6	Fresh meat	1	2.04	78	1	1.28
		Frozen meat	9	18.36	78	2	2.56
S. typhimurium	4,5,12:i:1,2	Fresh meat	2	4.08	78	1	1.28
		Frozen meat	3	6.12	78	1	1.28
S. poona	13,22:Z:1,6	Raw minced meat	1	2.04	30	1	3.33
S. newport	6,8:eh:1,2	Raw minced meat	1	2.04	30	1	3.33
Rough strain	–	Fresh meat	1	2.04	78	1	1.28

Total number of strains tested = 49.

prevalence rates, a minimum of 5 per cent sample needs to be examined in a lot.

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EFFECT OF COLD STORAGE ON THE PROTEOLYTIC ENZYMES OF FISH MUSCLE

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Occurrence of proteolytic enzymes, aminopeptidase (Arg-NNap hydrolase) and cathepsin B was demonstrated in the muscle extracts of seven marine fish species viz. doma *(Johnius dissumieri)*, seer *(Scomberomorous guttatus)*, mackerel *(Rastrelliger kanagurta)*, pomfret *(Stromateus cinereus)*, Indian salmon *(Eleutheronesma tetradactylum)*, shark *(Caracharhinus spp.)* and sardine *(Sardinella longiceps)*. The activity of aminopeptidase showed a progressive decline during storage at 0°C and frozen condition in doma and sardine. Cathepsin B activity, on the other hand, decreased in mackerel, shark and pomfret muscle during cold storage. In Indian salmon, activities of both enzymes were reduced as a consequence of storage. The possibility of the decline in the enzyme activity during storage serving as an index of the freshness of fish is indicated.

Fish muscle contains several lysosomal enzymes including proteinases[1]. Some of the endopeptidases such as cathepsin D[2], alkaline proteinase[3] and cathepsin B[4] of fish muscle have been purified and characterized in our laboratory with a view to understanding the mechanisms underlying protein catabolism and spoilage pattern during post mortem storage. It is apparent that both endo – and exopeptidases are involved in the total proteolysis[5]. Therefore, the present investigation was aimed at analysing the activities of a major endopeptidase, cathepsin B and an aminopeptidase in seven tropical fish varieties. The activities of these enzymes were assessed at different periods of storage both at 0°C and under frozen condition in order to examine whether the enzyme activity could be used to differentiate fresh fish from the stored one.

Freshly caught marine fish varieties were purchased from the local market. The fish were beheaded, eviscerated, cleaned and filleted. The muscle freed from skin and bones was minced with scissors and homogenized in distilled water to get a 10 per cent (W/V) homogenate. The homogenate was centrifuged at 12,000 × G for 20 min and the clear supernatant was collected. This supernatant formed the source of the enzyme. Endopeptidase activity of cathepsin B was determined using N-CBZ-α-arginyl-arginine β-naphthylamide (Z-Arg-Arg-NNap) as a substrate at pH 6.0 in the presence of 2mM cysteine and 1mM EDTA[5]. The reaction mixture (1 ml) was incubated at 40°C for 30 min and mersalyl reagent was used to terminate the reaction and to couple the liberated β-naphthylamine with Fast Garnet GBC. The intensity of the colour was measured at 520 nm. Arginine amino peptidase (Arg-NNap-hydrolase) activity was determined similarly at pH 6.8 using L-arginine β-naphthylamide (Arg-NNap) as substrate. The unit of activity equals n moles β-naphthylamine released in one hour. Protein content was measured according to Miller[6] employing bovine serum albumin as standard.

Presence of a number of proteolytic enzymes active at acid, near neutral and alkaline pH was reported in four fish species[5]. Concerted action of these proteolytic enzymes seemed to accomplish total proteolysis in fish muscle. In the present study, the occurrence of two proteolytic enzymes, an aminopeptidase and an endopeptidase possessing carboxypeptidase activity which could presumably hydrolyse fish muscle protein completely by their synergistic action is reported. All the fish species screened exhibited both aminopeptidase and cathepsin B activities which are included in Table 1. However, there were significant differences between the specific activities of the enzymes in different species of fish. Aminopeptidase activity was highest in shark muscle (118.05 units/mg) and lowest in pomfret (60.60 units/mg). In contrast, maximum activity of cathepsin B was observed in mackerel muscle (100.87 units/mg) and least in shark muscle (16.34 units/mg). Activities of both aminopeptidase and cathepsin B declined during storage at 0°C. Decrease in aminopeptidase activity was more pronounced in the case of doma and Indian salmon. It can be seen from Table 1 that aminopeptidase activity was reduced from 90.4 to 59.8 and from 90.6 to 60.8 in doma and Indian salmon respectively during 5 days of storage at 0°C. Cathepsin B activity, however, showed a reduction of 50-70 per cent in mackerel, pomfret, Indian salmon and shark.

Alteration in the levels of aminopeptidase in the muscle of frozen stored fish species is depicted in Fig. 1. A progressive decrease in enzyme activity could be observed in all cases. However, the extent of decrease varied with respect to different fish species. For example, Indian salmon showed maximum loss of activity (55 per cent) whereas mackerel showed minimum reduction during 4 months of frozen storage. Since bacterial growth is retarded during cold storage including frozen storage, low molecular weight compounds produced by the enzymic action continue

TABLE 1. AMINOPEPTIDASE AND CATHEPSIN B ACTIVITIES IN FISH STORED AT 0°C

Fish	Enzyme	Enzyme activity (units/mg protein) at indicated days of storage		
		0 – day	2 days	5 days
Doma	Aminopeptidase	90.40 ± 10.72	65.60 ± 7.33	59.80 ± 12.64
	Cathepsin B	23.79 ± 0.92	11.95 ± 1.23	19.28 ± 1.36
Seer	Aminopeptidase	99.30 ± 12.30	98.90 ± 15.98	90.60 ± 9.51
	Cathepsin B	48.33 ± 1.46	45.50 ± 1.12	35.51 ± 3.41
Shark	Aminopeptidase	118.05 ± 8.55	94.80 ± 10.80	93.30 ± 8.10
	Cathepsin B	16.34 ± 1.53	10.23 ± 1.81	5.19 ± 1.23
Sardine	Aminopeptidase	94.80 ± 4.40	74.40 ± 2.50	63.13 ± 9.34
	Cathepsin B	47.52 ± 1.85	25.00 ± 2.95	34.34 ± 5.41
Mackerel	Aminopeptidase	107.00 ± 12.73	108.53 ± 10.41	80.36 ± 5.50
	Cathepsin B	100.87 ± 5.01	49.64 ± 1.07	39.24 ± 2.75
Pomfret	Aminopeptidase	60.60 ± 2.54	55.80 ± 2.40	57.20 ± 0.69
	Cathepsin B	81.69 ± 7.86	44.29 ± 2.93	31.98 ± 3.52
Indian Salmon	Aminopeptidase	90.60 ± 10.80	81.20 ± 11.73	60.80 ± 12.00
	Cathepsin B	32.00 ± 2.39	21.51 ± 2.82	15.33 ± 1.46

Values represent average of four estimations ± SD.

to accumulate; these compounds can act as inhibitors of the enzymes. Alternatively, the fall in enzyme activity could be due to the release of endogenous inhibitors from the muscle which suppress the enzyme activities.

Since there is no appreciable change in the protein content, the enzyme data could apparently be used to differentiate fresh fish from the stored one. If the limits of specific activities in fresh fish were set, a fall in aminopeptidase activity would indicate the storage status of doma and sardine. Similarly, low cathepsin B activity could be taken as an index to differentiate fresh and stored mackerel, shark and pomfret. Fresh and stored Indian salmon could be identified either by the decrease in aminopeptidase or cathepsin B activity. However, in seer fish this cannot be taken as an index because there was only a marginal decrease in both aminopeptidase and cathepsin B on storage. This would mean that in seer fish, either the major component of proteolytic products is generated by other proteolytic enzymes such as cathepsin L or the fish lacks the endogenous inhibitors of proteases.

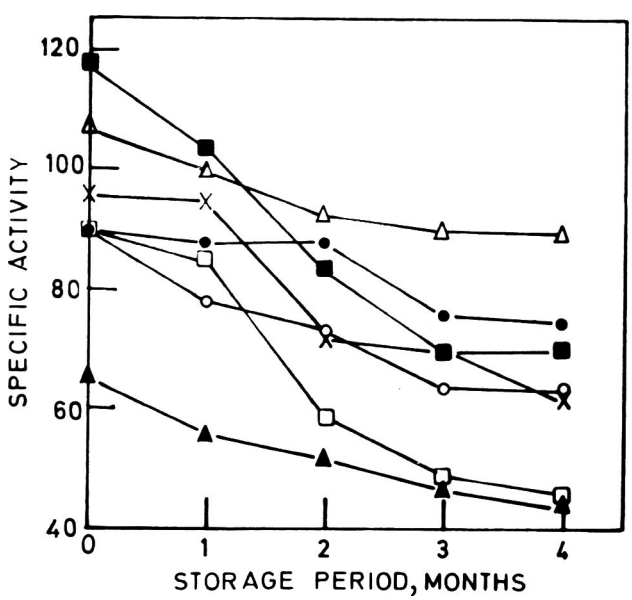

Fig. 1. Aminopeptidase activity in muscle of frozen stored fish species.

▲——▲ Pomfret, □——□ Indian salmon, ■——■ Shark, X——X Sardine.

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PREPARATION OF LIME JUICE FOR CARBONATED DRINK

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The effect of methods of lime juice extraction on the acceptability of lime juice based carbonated drink has been investigated. The combined juice of the cut fruits and grated whole limes in the ratio of 2:1 gave an acceptable carbonated lime drink with stable cloud.

Lime juice is used in several products and its quality is affected by the method of extraction and preservation[1]. The juice extracted mechanically contains more pulp than the juice extracted manually[2]. Most of the carbonated drinks are made by addition of synthetic colouring, and flavouring agents to sugar syrup and these are potentially allergenic[3]. Fruit juice based carbonated drinks can obviate the need for addition of synthetic colouring, flavouring and thickening agents. It has been reported that a temperature of 76.7°C was required to destroy pectinesterase in lime juice and ensure cloud stability in 1.7°C storage[4]. Verma and Sastry[5] have also reported the presence of natural clouds in comminuted orange and lime squashes. The present study was conducted to find out a suitable method to prepare lime juice for carbonated drink.

Fresh limes were obtained from local market (wholesale) (Azadpur-Delhi) for the experiments. Fruits were washed with water thoroughly and prepared as follows: In treatments (Table 1) 1 to 5, limes were cut into two halves with a stainless steel knife and the juice was extracted manually with a wooden lime squeezer and 10 to 40 per cent water was added to the peels on the fruit weight basis and the juice was again extracted by hand pressing through two fold of muslin cloth. In treatments 6 and 7, limes were cut into small pieces with a stainless steel knife and the juice was extracted through filter cloth in a hand operated wooden basket press. In treatemnts 8 and 9, whole limes were crushed in a grater (apple grater) and the juice was extracted through filter cloth in a hand operated wooden basket press. In treatments 7 and 9, 30 per cent water to the weight of original fruit was added to the peels and pomace respectively and the juice extracted as mentioned above. The pure juice and water extract were designated as juice I and juice II respectively and when both were combined together, it was termed "Juice".

TABLE 1. EFFECT OF LIME JUICE EXTRACTION METHOD ON THE YIELD OF JUICE AND PEEL.

Treatment*	Juice I (%)	Peel (%)	Water added to peel (%)	Juice II (%)	Peel (%)	Juice (%)
			Hand Pressed			
1	46.5	46.5	0	–	–	–
2	45.0	47.5	10	13.5	44.2	58.5
3	44.5	51.2	20	25.0	47.0	69.5
4	52.0	44.8	30	33.5	41.5	85.5
5	48.0	47.7	40	43.0	43.7	91.0
			Basket Pressed			
6	39.2	59.2	–	–	–	–
7	38.0	63.6	30	38.6	53.8	76.6
8	48.3	42.7	–	–	–	–
9	47.2	48.3	30	36.6	41.7	83.8

*For details of treatment see text.

The juices were heated to 80°C and filled at that temperature into clean, sterilized glass bottles (200 ml capacity), crown corked and air cooled. The bottles of lime juice were stored at 3°C until required.

The lime drink containing 3 to 5 per cent juice with 10° Brix and 0.2 per cent acidity was cooled to 4°C and then carbonated at 100 p.s.i. CO_2 gas pressure in a carbonating unit and sealed immediately with crown cork. The carbonated lime drink was kept at 5°C before sensory evaluation.

Physico-chemical characteristics: Soluble solids (°Brix) content was determined with a hand refractometer and the values corrected to 20°C. Acidity, pH and specific gravity were determined by the standard methods of A.O.A.C.[6]. Ascorbic acid was determined by the titrimetric method[7]. Sensory evaluation was carried out by a panel of seven judges using a 9-point Hedonic scale[8]. The cloudiness of the carbonated lime drink was evaluated by visual observation.

It is evident from the data in Table 1 that the method of juice extraction from limes greatly affected the juice yield. The yield of juice I varied from 44.5 to 52.0 per cent when hand pressed. This variation in the juice yield might be due to the non-uniform pressure of hand. The yield of juice I was 38.0 to 39.2 per cent when cut fruits were basket pressed and 47.2 to 48.3 per cent when grated limes were basket pressed. The grating of the whole limes gave higher yield of juice I in comparison to the cutting of limes into pieces and it

TABLE 2. PHYSICO-CHEMICAL COMPOSITION OF LIME JUICE

Treatment*	°Brix (20°C)	Acidity (%)	pH	Ascorbic acid (mg/100 ml)	Specific gravity
1	7.22	7.10	2.25	20.10	1.02875
2	6.72	6.20	2.35	18.04	1.02383
3	5.24	5.33	2.35	16.49	1.02247
4	4.74	4.60	2.38	14.43	1.01760
5	4.24	4.13	2.40	12.89	1.01474
6	5.74	5.22	2.43	14.95	1.02723
7	4.74	4.15	2.55	11.21	1.02380
8	6.24	4.27	2.70	20.21	1.03070
9	4.74	3.39	2.75	14.40	1.01780

*For details of treatment see text
Values are average of duplicate analysis

TABLE 3. EFFECT OF JUICE EXTRACTION METHOD ON PH, CLOUDINESS AND SENSORY QUALITY OF CARBONATED LIME DRINK

Treatment*	pH	Cloudiness	Quality score		
			Colour	Flavour	Overall
1	3.05	Slight cloudy	6.44	6.72	6.58
2	3.05	Clear	6.22	6.22	6.22
3	3.10	Slight cloudy	6.66	6.38	6.52
4	3.10	Clear	6.00	5.83	5.92
5	3.10	Clear	6.11	6.27	6.19
6	3.10	Clear	6.10	6.10	6.10
7	3.10	Slight cloudy	6.70	6.61	6.66
8	3.23	Slight cloudy	6.50	5.30	5.90
9	3.23	More cloudy	7.10	5.30	6.20

*For details of treatment see text

was similar to the average juice I yield (47.2 per cent) by hand pressing method. Hand pressing gave a higher yield of juice I than basket pressing of cut pieces. This may be due to the higher surface area of the fruit exposed to pressure in the hand pressing method.

Since it was felt that peels still contain the soluble solids including acid, water was added to recover them. The yield of juice II increased with increase in water added to the peels and varied from 13.5 to 43.0 per cent. The peel content varied from 41.5 to 53.8 per cent.

The pH of the juice was greatly influenced by the method of juice extraction and varied from 2.25 to 2.75. The grating and basket pressing method resulted in highest pH and lowest acidity in the juice (Table 2).

It is also evident that the juice of treatment 4 (mixture of juice I and juice II) was highest in ascorbic acid (12.34 mg/100g), total soluble solids (4.05 per cent), acid content (3.93 per cent) and total drink (1964 ml/100 g) when calculated on fruit weight basis. It was observed that the best drink for carbonation could be made with a Brix/acid ratio of 50, even though others were found to be acceptable to the taste panel.

It is to be noted that the method of juice extraction greatly influenced the sensory quality and cloudiness in the carbonated lime juice based drink (Table 3). The drinks made out of the juice extracted from grated limes (treatments 8 and 9) were rejected by the taste panel members in respect of flavour, due to bitterness. All other drinks were acceptable. The colour score of the carbonated drinks was affected by the cloudiness, and the drinks made from the juice of the grated limes obtained the highest colour score.

Since the cloudiness has a great influencing factor in the acceptability of the carbonated drink, the "juice" of treatments 7 and 9 were mixed and then carbonated drinks made from them in order to have some cloudiness with reduced bitterness. It was observed that the juices of treatments 7 and 9 could be combined in the ratio of 2:1, and the drink made out of this juice obtained highest flavour score with moderate colour score and acceptable cloudiness in the drink.

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PROTEIN CONTENT AND AMINO ACID PROFILE OF SELECTED COMMERCIAL INFANT AND WEANING FOODS

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Analysis of ten commercial infant and weaning foods indicated that the protein content of infant formulae ranged from 13 to 30%. While the protein content of weaning foods was similar to infant foods (16.7%), one of them had only 6.2% protein. The total essential amino acid content of the infant foods ranged from 421.1 to 468.2 mg/g protein and that of weaning foods from 416.5 to 446.8 mg/g protein. The sulphur amino acids were the first limiting amino acids in infant and weaning foods when human milk was taken as the reference.

Infant foods are products prepared primarily and specifically for use in bottle feeding from birth as complete or partial human milk substitutes. Therefore, the manufacturer tries to produce a reasonable likeness to the composition of human milk. Most of the commercial formulae available are based on cow's milk[1]. The quality of cow's milk protein as measured by biological tests is high and this would appear to make them ideal ingredients for infant foods. However, the cow's milk has three times the concentration of protein found in human milk and the major proteins of cow's milk and human milk are present in different proportions. Therefore, in this study the protein content and composition of selected infant foods was estimated to assess their similarity to human milk. A few weaning foods were also analyzed.

Samples of 7 different infant foods and 3 weaning foods were bought from different local markets. For each sample, different tins were pooled together and mixed thoroughly. An aliquot of the mixed sample was taken for analysis. The protein and the non-protein nitrogen (NPN) content was estimated by micro-kjeldahl method[2]. The amino acid composition was determined after acid hydrolysis[3] in a Beckman model auto analyser.

The quality of the protein was estimated from its amino acid composition as compared with the FAO/WHO pattern of amino acids[4] and human milk[5]. The amino acid score of the protein was calculated as follows:

$$\text{Amino acid score} = \frac{\text{mg of amino acid in 1 g of test protein}}{\text{mg of amino acid in reference pattern}} \times 100$$

TABLE 1. PROTEIN CONTENT OF INFANT AND WEANING FOODS

Sample	Crude protein as analysed	(g/100 g) as in label	NPN (% of total protein)	True protein (g/100 g)
		Infant Formula		
1	25.0	22.0	0.28	24.9
2	30.2	25.7	0.32	30.1
3	14.9	14.5	0.27	14.9
4	16.8	16.1	0.37	16.7
5	23.9	21.6	0.31	23.8
6	22.2	22.0	0.32	22.1
7	13.3	12.0	0.56	13.2
		Weaning Food		
1	16.7	15.5	0.29	16.7
2	16.8	15.5	0.34	16.7
3	6.2	6.0	0.35	6.2

It was observed that the protein content of the infant foods varied from 13.2 to 30.1 per cent (Table 1). Our results are consistent with those reported for similar infant foods[6-8]. The protein contents of two weaning foods were 16.7 and 16.8 per cent whereas that of a cereal based one was lower – 6.2 per cent.

When the analyzed protein value was compared with the labelled value on the tin, it was seen that most of the values observed by us were higher than those on the label. All the seven samples of infant food had protein content higher than the minimum recommended for infant formulae by the Committee on Nutrition of the American Academy of Pediatrics[9]. As there is a tendency of many mothers to use excessive amounts of powder in preparing the formula, there is a possiblity of overloading the neonatal kidney.

It has been reported that the most severe form of damage to the protein during processing is the transformation of protein nitrogen to other forms[10]. The low non protein nitrogen (NPN) content (0.27 to 0.56 per cent) of all the samples suggest that processing has not damaged their protein.

The quality of a protein depends on its ability to supply all essential amino acids in sufficient amounts to fulfil the requirements for maintenance and growth. The total essential amino acid content of the infant formulae and weaning foods analyzed did not differ significantly; it ranged from 416.5 to 468.2 mg/g protein (Table 2).

Milk powders have been reported to have sulphur amino acids as the limiting amino acids[11]. This was also true for four infant formulae and one weaning

TABLE 2. ESSENTIAL AMINO ACID (MG/G PROTEIN) PROFILE OF COMMERCIAL INFANT AND WEANING FOODS

Type of food	His.	Ile.	Leu.	Lys.	Met.	Cys.	Phe.	Tyr.	Thr.	Val.	Total	Amino acid score: Ref. human milk	Amino acid score: Ref. recommended levels
Infant formula													
1	29.7	53.0	83.5	68.7	25.7	11.4	46.4	49.8	42.6	57.4	468.2	88	97
2	27.4	45.8	59.6	52.8	21.3	13.8	51.7	55.5	41.3	51.9	421.1	64	75
3	29.6	52.8	78.2	58.8	22.4	10.0	46.7	49.9	44.1	56.3	448.8	77	98
4	29.1	53.5	74.5	60.7	22.4	8.0	44.3	50.9	41.3	55.3	440.0	72	93
5	29.7	52.6	83.3	63.8	22.0	12.0	46.3	49.4	42.8	54.1	456.0	81	97
6	29.9	52.1	81.1	65.3	24.1	11.0	46.0	58.7	40.4	55.2	453.8	87	92
7	26.1	50.1	79.6	54.5	24.9	12.4	44.8	47.8	40.1	51.9	432.2	83	91
Human Milk*	26	46	93	66	42		72		43	55	443		
FAO/WHO Requirements	14	35	80	52	29		63		44	47	364		
Weaning food													
1	27.6	48.9	76.5	55.0	21.0	10.0	45.1	44.5	35.6	52.3	416.5	74	81
2	25.9	49.2	84.6	53.3	21.5	13.4	42.5	46.3	38.5	51.2	426.4	81	88
2	29.7	54.7	84.6	40.8	23.1	11.2	50.7	51.3	45.6	55.1	446.8	62	79

*Composition reported by FAO

food when human milk was used as the reference standard. However, when FAO/WHO[4] recommended levels were used as a reference, threonine was the first limiting amino acid in five infant formulae and leucine in the other two.

Weaning foods are cereal-milk blends or purely cereal based; hence sulphur amino acids or lysine was the first limiting amino acid. Similar observations were reported for weaning foods analyzed in Pakistan[6].

The results of the study show that the protein content of commercial infant formulae is higher than the minimum recommended by the American Academy of Pediatrics and except for formula two, the amino acid composition is very close to FAO/WHO suggested requirements.

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FAT CONTENT AND FATTY ACID COMPOSITION OF INFANT AND WEANING FOODS

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Ten commercial infant and weaning foods and five home made weaning foods, *Khichadi*, *Sheera*, *Ravakheer*, Rice *Kheer* and *Dalia* used very frequently by mothers, were analyzed for their fat content and fatty acid composition. The fat content of commercial infant foods ranged from 6 to 24%, of commercial weaning foods from 1 to 9% and in home made weaning foods from 0.5 to 3%. Only 3 infant formulae and one weaning food had essential fatty acids sufficient to provide more than 3% of total calories.

The appropriate level of fat and a balance of fatty acids in the infants diet are vital for adequate calorie intake and for the maximum absorption of fat soluble vitamins, calcium and essential fatty acids (EFA). In general, as a complete substitute for human milk, an infant formula should provide protein at 7-16 per cent and fat at 30-54 per cent of calories, the remaining calories being supplied by carbohydrates[1]. Infant diets containing less than 0.1 per cent total calories as linoleic acid lead to EFA deficiency[2]. EFA are involved in many metabolic processes and are necessary for the synthesis of brain lipids. The brain and the rest of the nervous system undergo rapid growth during early infancy. Myelin is still to be laid among the axons and the dendritic connections at birth and fat is an important constituent of myelin[3]. Therefore, inadequate intake of EFA could produce a long lasting effect on growth of the nervous system. In addition, high levels of saturated fatty acids in dietary fat may predispose the individual to atherosclerosis[4]. With this background and in view of the lack of data on the fatty acid profile of infant and weaning foods, the present study was undertaken.

Seven commercial infant foods and three weaning foods were purchased from the local market. Contents of 5 different tins of the same food were pooled together and an aliquot taken for analysis.

Five commonly used home weaning foods were prepared as described elsewhere[5]. The fat content of all the samples was estimated by the Soxhlet method[6]. The lipids were extracted by the method of Folch *et al.*[7] and methylesters prepared by the method of Christopherson and Glass[8]. The methyl esters were analyzed by gas liquid chromatography (GLC) using a 9 ft by $\frac{1}{4}$ in. stainless steel column packed with 10 per cent EGSSX on chromosorb WHP. The operating conditions used were as follows: carrier gas dry nitrogen at a pressure of 1kg per sq.cm. and at a flow rate of 50 ml per min temperature of injector and detector were 300°C while the column temperature was 200°C.

Standard mixtures of fatty acids were used to calibrate the GLC response and identify the fatty acid peaks of the samples analysed. The weight of each fatty acid in the food was calculated by multiplying the per cent of fatty acid in the lipid by the weight of the total fatty acids in 100 g.

The fat content of commercial infant formulae ranged from 6 to 24 per cent corresponding to 13 to 43 per cent of the total calories (Table 1). All the samples except one contained 16:0 as the predominant fatty acid and significant levels of 14:0, 18:0 and 18:1. Similar observations had been made by Iguchi *et al.*[9] and Hanson and Kinsella[10], for commercial infant formulae marketed in U.S.A. The high levels of 16:0 and 18:0 have adverse influence on calcium absorption[11] and the possibility of predisposing the individual to atherosclerosis[12].

It was also observed that the concentration of medium chain fatty acids (8:0 – 14:0) was around 14 to 20 per cent except in one sample where 55 per cent of the total fatty acids were of medium chain length. The predominant fatty acid in this sample was lauric acid, 35.3 per cent, indicating the presence of coconut or palm kernel fat. As fats containing medium chain acids are more easily absorbed, this would be an advantage.

Essential fatty acids are necessary for the normal function of all tissues. The linoleic acid content of human milk fat is 4-5 times greater than that of cow's milk[13]. Only in 3 formulae, the concentration of 18:2 was similar to that of human milk fat, in other, 18:2 levels were significantly lower. In one of the samples, no 18:2 or 18:3 was detected.

The values for 18:2 observed by us are lower than 10.9 – 15.2 per cent reported by Schacky and Weber[14] for 11 conventional infant formulae and by Hanson and Kinsella[10] who reported that 6 to 26 per cent of calories from linoleic acid were supplied by the commercial infant formulae.

The present data show that only 3 infant formulae had sufficient EFA to supply the recommended 3 per cent of total calories. With 1.3 to 1.8 per cent of total calories from linoleic acid present in three other formulae, the skin changes of EFA deficiency may not be observed, but it is doubtful whether the infant's requirements will be fully met.

TABLE 1. FAT CONTENT AND FATTY ACID COMPOSITION OF COMMERCIAL INFANT AND WEANING FOODS

Type of food	Fat (g/100 g)	% Cal from fat	Fatty acid (% of total fatty acids) 8:0	10:0	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	Cal from EFA (%)
Infant food														
	16.0	32.0	0.8	1.2	2.4	15.7	2.5	33.7	3.7	10.3	26.0	3.2	0.9	1.3
	18.0	32.7	0.9	1.3	1.2	11.9	2.0	34.3	3.2	8.0	23.9	11.2	1.8	4.2
	19.0	36.8	0.9	1.3	2.5	8.9	1.9	38.9	3.1	8.5	22.6	9.8	1.6	4.2
	24.0	43.1	1.1	1.6	3.1	14.2	2.3	39.4	3.5	9.6	21.2	1.9	1.9	1.6
	6.0	13.2	2.6	2.1	2.7	12.2	2.2	41.7	3.3	7.9	11.9	5.9	7.4	1.8
	19.0	35.3	6.8	4.9	35.3	8.7	–	11.7	–	2.6	17.2	11.8	0.8	4.5
	17.0	34.0	1.4	2.3	3.3	11.4	1.9	40.5	3.3	10.5	25.2	–	–	–
Human milk														
	–	–	NA	2.5	8.0	11.0	NA	25.0	2.0	6.2	30.0	9.2	0.9	4.5
Weaning foods-Commercial														
	9.0		1.4	1.0	1.0	8.3	1.6	38.9	2.9	10.0	23.6	9.7	1.0	
	1.0		21.5	7.0	1.7	2.5	0.7	51.5	–	–	6.8	8.3	–	
	6.0		1.3	2.6	3.4	10.4	2.1	41.2	2.4	13.4	22.9	–	–	
Home made weaning foods														
Sheera	1.0		1.2	4.4	3.9	12.8	2.2	32.9	2.5	11.8	23.9	3.0	1.1	
Rice Kheer	3.0		1.4	2.7	3.2	13.6	2.3	34.0	3.2	12.7	23.0	2.3	1.6	
Rava Kheer	2.0		1.4	2.8	3.3	11.7	1.9	32.0	3.2	13.0	26.0	2.5	1.6	
Khichadi	0.5		12.3	15.8	18.5	9.3	1.4	27.0	2.1	1.8	11.8	–	–	
Dalia	2.0		13.4	8.8	4.4	12.1	2.6	47.8	–	–	10.6	–	–	

The fat content of weaning foods varied from 1 to 9 per cent in the commercial samples and 0.5 to 3 per cent in home made foods. Significant concentrations of 14:0, 16:0 18:0 and 18:1 were present in all weaning foods except two.

Linoleic acid could not be detected in one commercial and two home made samples, while the levels in other weaning foods ranged from 2.3 to 9.7 per cent. Only one of the commercial foods had EFA content comparable to human milk. Linolenic acid too was absent in four of the weaning foods. In otners, it varied from 1 tc 1.6 per cent.

Ideally milk substitutes should match the fatty acid composition of human milk from well nourished mothers. It is of concern that only 3 out of 7 commonly used infant formulae analyzed contain sufficient EFA to provide more than 3 per cent of total calories. The manufacturers must increase the EFA content of the infant formulae marketed.

The authors thank the Hindustan Lever Limited (Research and Development) for their assistance in fatty acid analysis.

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BOOK REVIEWS

Post-harvest Physiology of Vegetables: Edited by Weichmann, J. Series Food Science and Technology, Marcel Decker, Inc. 270, Madison Avenue, New York; 1987; pp: 597; Price: $150 (US & Canada) and $180 (other countries).

The publication of the book on post harvest physiology of vegetables fills in the long felt need. The book is divided into VI parts: Part I, vegetables, classification and definition of the physiological state; Part II, basic post-harvest physiology; Part III, Influence of post harvest factors on post harvest reactions; Part IV, post-harvest diseases and injuries; Part V, post-harvest quality changes and part VI, the post harvest physiology of certain vegetables. Each part is divided into several chapters and each chapter is written by an expert in that area. Thus, the book contains 31 chapters covering 597 pages, giving extremely useful information.

In order to get a complete idea of the book, I consider it better to list out the topics of each chapter. Part I is divided into two chapters, Chapter I, an introductory one giving the definition, classification and physiological maturity of vegetables while Chapter II deals with the biochemical and physiological changes during the harvest period.

Part II consists of 4 chapters and covers respiration and gas exchange of vegetables, hormonal alterations during post harvest period, membrane changes and consequences for the post harvest period and the last one on transpiration, water stress and gas exchange.

Part III comprises eight chapters covering – temperature: effects on metabolism and energy flux in stores, water vapour pressure, low oxygen and high carbondioxide effects on plant organs, ethylene and non-ethylene volatiles, effects of adding carbon-monoxide to controlled atmosphere and finally, the air movement effects in storage of vegetables.

Part IV on post harvest diseases and injuries gives a good account of chilling injury, frost damage and freezing injury, host-parasite relationship, bacterial diseases of vegetable crops, fungal diseases and includes an important chapter on mycotoxins and phytoalexins in stored crops (all covered under six separate chapters).

Part V on post harvest quality changes deals with sensory quality, vitamins, carbohydrates, amino-acids and nitrogenous compounds and minerals in five chapters. The last one, Part VI on the post harvest physiology of certain vegetables starts with a general account of market preparation methods and shelf life of vegetables followed by individual crops-Brassica, Asparagus, Fruits, Root vegetable and finally bulbs and tubers, distributed into 5 different chapters.

Each chapter is prepared with great care giving the latest basic information in a precise and effective way along with illustrations and a number of cross references. At the end of each chapter, a complete list of useful references cited in the text is added. The text of most chapters is ended with conclusions bringing the state of knowledge uptodate.

Quoting from the editor's preface, to say a few additional words on the need and quality of the book, it can be said that there are extensive losses in vegetables during post harvest both in the developing and developed countries that call for the development of modern technologies. A thorough understanding of the basics of post harvest physiology is vital for the developments in post harvest technologies of vegetables. This book written by several well known scientists summarises the latest knowledge of post harvest physiology of vegetables and provides information that is comprehensive and complete in all respects. The printing, illustrations, get up and the binding of the book are good.

In conclusion, I believe that this book not only fills in the need of all those working on vegetable crops-the advisers and managers, students as well as teachers, those in actual practice or engaged in research at universities and research institutes, but it may also remain as a very useful book for many years to come. It can easily be hoped that a book of this type will invigorate the development of modern technologies to curtail crop losses during post harvest.

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

Directory of Indian Food Machinery and Packaging Equipments: Central Food Technological Research Institute, Mysore; 1987; pp: 207; Price: Rs.100.

Information on Food Machinery and Packaging equipment was not readily available at a single point so far and such information was required by the industry very badly. Besides, there is a vast change in production and products of the Indian Food Industry. Foods of different types and in different packaging

material are now available in the Consumer Market. This directory of Indian Food Machinery and Packaging Equipments is therefore a good reference book for all those engaged in the food business, both in domestic and International markets.

The main feature of this Directory is that it provides more than 3000 classifications of Machinery and Equipment on their functional and utility basis also indicating the available sources. Further, turn-key project capabilities in specific areas has also been given for the benefit of the user.

This Directory is classified into three parts as under:

1. Names, Addresses, Telephone and Telex numbers of the companies,
2. Functional and utility including available sources,
3. The turn-key project capabilities in specific areas.

This is a very useful directory to the entrepreneurs who are interested in starting new ventures in foods besides existing extrepreneurs.

ANAND G. NAIK KURADE
SUMAN FOOD CONSULTANTS
NEW DELHI

Hand Book of Dietary Fibre – An Applied Approach: by Mark L. Dreher, Marcel Dekker Inc, New York; USA; 1987; 468; Pp: 468; $99.75 (US & Canada), $119.50 (all other countries).

A number of publications have been brought out on the subject of dietary fibre (DF), but for the first time information on various aspects of DF has been put into one hand book.

In 12 chapters, the author has provided up-to-date information on basic research, analytical methodology, applications, fibre containing products and current approaches in physiological and medical research on DF.

The opening chapter deals with historical and current perspectives and nomenclature associated with fibre. It also contains information on trends in the DF content of Western diets over the last 70 years. A few thoughts on future challenges in the field have been expressed.

In addition to the detailed information on the various conventional and unconventional components of DF, chapter 2 contains a clear presentation on the classification of various DF components, their main structural features and variations.

Methodology involved in fibre estimation is discussed in the third chapter. With a brief introduction and historical perspective on the development of various procedures for measuring DF and its fractions, the author has included full details of the key methods. Lot of background information on different steps involved, enzymes used and composition of reference material for analytical and nutritional studies has also been provided. The chapter ends with suggestions for further research needs for the improvement of existing methodology.

An over-view of DF intake, actual intake figures for selected countries and variations in intake are given in Chapter 4. Methods of assessing DF intake and factors to be considered for food intake study have also been discussed.

Fifth chapter deals with the physico-chemical properties of DF in relation to bowel function. An over-view of soluble DF and their functional properties as related to food uses have been presented. Methods for determining physico-chemical and functional properties of DF have been given.

Importance of DF and its role in human physiology has been appreciated with the advances in our understanding of its role. In chapter 7, results of studies on the physiological effects of DF on the intestinal tract, glucose tolerance and cholesterol levels have been reviewed.

Occurrence of some diseases has been linked to the deficiencies of DF in the diet. Chapter 8 deals with such diseases where lack of fibre appears to be a causative factor based on known physiological action of fibre. The diseases discussed include obesity, bowel disorders, cancer of the large bowel, gall stones, diabetes mellitus and atherosclerosis.

Parallel to the beneficial effects of DF in reducing the risk to certain diseases, there may be a possibility of deleterious effects associated with high levels of DF in the diet. In this context the author, in Chapter 9, has reviewed the reported potential adverse effects of DF on mineral and vitamin availability and other toxic effects.

Chapter 10 presents data on the content of total fibre and its components in various foods. Effects of storage and processing conditions on the DF content of fruits and vegetables, specialized fibre containing products and new opportunities for the latter have been discussed briefly.

Information on a variety of sources of DF like cereals, vegetables, fruits and their by-products and their utilization in cereal based bakery products, jams, jellies and other items has been given in Chapter 11. In addition, an appendix listing sources and suppliers of DF ingredients is also given.

Health claims regarding high fibre foods have been on the increase. Permitting such claims in the absence of evidence may be misleading to the public. In Chapter 12, the author deals with the regulatory aspects of DF, DF levels in food and maximum usage

levels of isolated DF ingredients, health messages on food labels, nutritional labelling of DF etc. The chapter includes information on tests for identification of hydrocolloids.

This hand book covers various aspects of DF and contains up-date information in the areas, along with actual data and all relevant literature references. The information on diverse aspects has been presented in a concise and lucid manner. This is a very useful hand book for those working in the field of food technology, nutrition, product development, medicine and food regulations. Although major portion of the hand book is more useful to areas where the diets are deficient in DF, it can serve as a guideline for methodology and data on the ill-effects of high fibre for the other areas.

KANTHA SHURPALEKAR
C.F.T.R.I., MYSORE.

Environmental Health Criteria 58 – Selenium: WHO Publication; 1987; Pp: 306; Price: SW. fr. 24 or US $9.60.

The publication on Selenium – Environmental Health Criteria 58 – is well presented. Section I deals with summary of the findings of the WHO task force and identifies areas for further research. Sections 2 and 7 cover analytical methods, sources of transport, levels in environmental media, human exposure, and metabolism of selenium. These are very well covered supported by exhaustive literature survey. However, in Section 7, the mechanism of toxicity could have been dealt in a little more elaborate manner. The monograph brings out the controversy regarding selenium as to its carcinogenic and anticarcinogenic potential depending on the levels of intake. The book also contains specific areas for further research especially on the need to establish the dose response relationship in over-exposed population, on Keshan disease & Kashin Beck Disease. This is a very useful book on Selenium with 772 references.

LEELA SRINIVAS
C.F.T.R.I., MYSORE

Food Product Package Compatibility – Proceedings: Edited by J. Ian Gray, Brue R. Harte and Jesephu Mitz, Published by Technomic Publishing Company Inc., 851, New Holland Avenue, Box 3535, Lancaster, Pensylvania 17604, USA; Pp: 286; Price: S.Fr. 150.

School of Packaging, Michigan State University, East Lansing is one of the pioneer institutions to start regular graduate and post graduate courses in packaging. Also, the school has been very active in promoting packaging research and development.

Food packaging is largely responsible for providing adequate and good nutritious food to mankind by optimising preservation and distribution. The present level improvement in the quality of life, especially in developed countries is a direct consequence of the rapid growth of packaging industry. Hence it is appropriate that the School of Packaging sponsored a seminar on food packaging (July 14-16, 1986), covering all the allied fields.

The book, under review, represents the proceedings of the above seminar. The seminar has been well attended not only by well known scientists in the field of food packaging and food engineering, but also other allied areas to the nutrition and food safety disciplines which are closely inter-linked with food packaging, in addition to experts from packaging industry.

The book is a collection of papers from sixteen reputed authors covering divergent fields like (i) interaction of food components during packaging, (ii) migration of low molecular weight species from packaging materials, (iii) interaction between metal can and food products, (iv) modified atmosphere packaging, (v) packaging for micro-wave oven foods, (vi) tamper evident packaging, (vii) high barrier plastics and (viii) adhesives lamination purposes.

Each presentation has been adequately supported by extensive bibliography, graphs and tables and thus providing complete data necessary for stimulating further reading and cross references.

This compendium of up-to-date knowledge in the field of packaging effectively fills the gap of covering voluminous data in a single book. It will definitely serve as a source of reference as well as data to all the scientific/technical persons engaged in research and development of adequate and safe food packaging for food and should find a place in such institutions.

A.N. SRIVATSA
D.F.R.L. MYSORE

Statistical Procedures in Food Research: Edited by J.R. Piggott, Elsevier Applied Science Publishers Ltd., New York 10017, 1986; Price £50.

The 1984 Weurmann symposium on "Statistical Methods" exposed that the participants were not familiar with the methods and an "User's guide" to

these methods was suggested as a solution. This has resulted in the form of this book which consists of ten chapters, 62 tables and 113 illustrations each written by an expert.

The contributors who are either practising food scientists or statisticians involved in food research have tried to explain the most common and most useful multivariate methods, after the introduction of basic principles of statistical methodology and terminology. Emphasis is laid on understanding the principles of various methods, their applications and the interpretation of the results. Each method has been copiously illustrated with examples drawn from Food Research and detailed computer programmes are provided.

The first chapter on "Aspects of Experimental Design" examines the characteristics of good design and the problems that are likely to be faced and provides some solutions.

The second chapter on "Univariate Procedures" briefly describes the main summary statistics, graphical representations, main distribution, notions of estimations and testing of hypotheses, classical analysis of variance and the problem of outliers.

Selection of an emperical form which may be invested with biological meaning has been discussed very much in detail under the chapter "A practical Approach to Regression".

Response surface methods, under Chapter IV deals with obtaining a reliable polynomial model and to test this model to investigate the response surface.

Under the chapter "Discriminant analysis", the following points have been discussed. Main features of different methods of discriminant analyses, illustrations with examples, problems associated with discriminant analyses in general and each of the discriminant analyses methods, advice of precautions which need to be taken to minimise such problems and to forewarn the prospective user of pitfalls to be avoided either in carrying out the analyses or in interpreting the results.

To help in the interpretation of complex multivariate data, the chapter on "Methods to aid interpretation of multidimensional data" discusses the most commonly used multivariate procedures like principal component analyses. An exposure to principal coordinates analysis and factorial correspondence analysis, which are used for similar purposes and which are of very recent developments are covered.

Under the chapter on "The use of generalised procrùster techniques in sensory analysis", the method of Generalised procruster analysis has been discussed, which is a valuable statistical tool in sensory profiling.

A brief description of the basic concepts of multi-dimensional scaling along with mathematical techniques that are used to interpret the geometric representations derived by this technique has been provided in the next chapter entitled "Multidimensional scaling and its interpretations".

The more recently developed partial least squares regression technique, which is very useful both for calibration and for general data interpretation has been very widely discussed in the next chapter as partial least squares regression. Illustrations are mainly taken from various food research data and especially for studies of sensory-chemical relationships. References to other applications are also given.

The recent developments of Applied Cluster Analysis are discussed in the last chapter. It also enlists Food Science related examples of Applied Cluster analysis with corresponding references.

Further, each chapter is followed by a good list of references and suggestions for further reading, and also contains lot of tables and figures for illustration purposes and useful computer programmes. The get up and printing are excellent, and it will be a very useful addition to the library.

B.S. RAMESH
C.F.T.R.I., MYSORE

ERRATUM

In this journal of Vol.25 No.4, 1988 in page 206, in first column, 5th line should read as: "plunger *into the flesh of the pared apple to a depth of* 7/16 of an inch", instead of plunger *of 5/16 of an inch diameter. On each fruit two* 7/16 of an inch.

Association News

Jabalpur Chapter

The Annual General Body Meeting was held on 6th June 1988. The names of the new executive members are as below:

President	: Prof. Y.K. Sharma
Vice President	: Dr. A.K. Mandloi
Hony. Secretary	: Prof. S.K. Sharma
Jt. Secretary	: Prof. A. Mishra
Hony. Treasurer	: Er. C.M. Abroal

Bombay Chapter

President	: Dr. P.J. Dubash
Vice Presidents	: Mr. S.V. Krishnaswamy
	Dr. M.R. Vora
Hony. Secretary	: Dr. A.S. Gholap
Hony. Jt. Secretary	: Dr. S.M. Gaonkar
Hony. Treasurer	: Dr. S.R. Padwal-Desai

SYMPOSIUM

The Association of Food Scientists and Technologists (India) is proposing to organise a Symposium on "ENVIRONMENT AND POLLUTION IN FOOD INDUSTRY" AT C.F.T.R.I., Mysore during February/March 1989.

INSTRUCTIONS TO AUTHORS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Research Papers

Research Notes

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