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The Effect of Some Processing Variables on the Dehydration of Pregelled Yam Pieces

O.O. AJIBOLA, B.I. ABONYI AND O. ONAYEMI
Department of Agricultural Engineering,
University of Ife, Ile – Ife, Nigeria.

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The effects of size, blanching time and drying temperature on the drying characteristics of pregelled yam pieces were investigated using a 2^3 factorial experiment. Page's model was adequate in describing the drying behaviour. Cassava pieces diced to 0.8 cm blanched for 20 min and dried at 70°C reached a safe moisture content of 10% (dry basis) in 12 hr.

Yams (*Dioscorea* spp) are a very important staple in Nigeria and many other tropical countries. Of the many species consumed, white yam, *D. rotundata* is the most important, being consumed mostly as 'pounded yam'. Traditionally, pounded yam is prepared by boiling peeled yam in water until it is cooked and then pounding it in a mortar to form a thick paste of uniform consistency.

The technology of processing yam into instant yam flour is now being developed. This process consists mainly of dicing the tuber to pieces, blanching to pregelatinize the starch, drying and then milling to flour of suitable fineness. This technology was developed with a view to obtaining a high quality final product without changing its sensory quality. The yam flour thus produced will, upon mixing with hot water reconstitute into a paste similar to pounded yam in colour, texture and flavour.

One of the problems hindering wider acceptability of instant yam flour is its high market price which is due to the high cost of production. Drying cost represents a substantial part of the production cost. The drying rate of an agricultural material is, however, affected by the pretreatment it receives prior to drying.

Pregelatinizing yam by blanching, although a very energy-intensive operation, is a necessary step which obviates the need to cook the yam after reconstitution. There are conflicting reports in the literature on the effect of blanching on the rate of drying of starchy products.[1] This makes it difficult to predict the effects of some processing conditions on the drying of a starchy material like yam.

The present study was undertaken to investigate the effect of piece size, blanching time and drying temperature on the final moisture content, drying rate and the drying time of dried pregelled yam pieces.

Materials and Methods

Preparation of material and determination of drying rates: Yam (*Dioscorea rotundata*) tubers obtained from the local market were peeled, and diced into desired sizes with a Hallde dicing machine (A.B. Hallde, Maskiner, Sweden). A 2^3 factorial experiment was performed to investigate the effects of size, blanching time and drying temperature on the drying characteristics of the yam pieces. Each factor was investigated at two levels yielding eight treatment combinations. The sizes were 0.8 and 2.0 cm, the blanching times were 10 and 20 min and drying temperatures were 50 and 70°C. Each run was replicated twice.

Relatively low drying temperatures were used because it is known that at 80°C and above dextrinisation of yam starch proceeds rapidly resulting in case hardening and the development of a yellowish off-colour. The diced yam pieces were immediately transferred into a 0.4 per cent sodium meta-bisulphite solution for about 10 min to prevent browning. The pieces were then blanched in a steam blancher (Dixie blancher, Dixie corp) at atmospheric pressure for the required length of time. For the measurement of drying characteristics one layer of about 20 g of pregelled yam pieces was spread on stainless steel trays and placed in shelves of a Gallenkamp moisture extraction oven OV-440 (in which the air changed about 3 times per min) equipped with a thermostat. The temperature of the oven was regulated via the thermostat and was checked occasionally with a mercury bulb thermometer. The weight and moisture content of the material were determined just before

drying, and the loss in weight during drying was determined by weighing the trays at intervals. Drying was deemed to be complete when there was no further loss in weight over a 24 hr period. The final moisture content of the pieces was then determined

The moisture contents of the samples were determined by grinding in a mortar and drying in a Carter Simon Rapid Moisture meter at 150°C for 15 min. This method was found to be adequate when compared with oven drying at 103°C for 72 hr.

Statistical analysis: The results obtained from the drying experiments for different piece sizes, blanching times and drying temperatures were fitted into the Page's model[2] and analyzed statistically for significance of the factors. The data were used to predict the drying rates and explain some phenomena occurring during drying.

Results and Discussion

The plots of moisture content *versus* time for different conditions are shown in Fig. 1 and 2. The rate of drying decreased with the time of drying until a constant moisture content was reached. The data were analysed by converting moisture content to moisture ratio defined as:

$$MR = \frac{M - M_e}{M_i - M_e} \quad \text{...... (1)}$$

where

MR = moisture ratio and M, Mi and Me are respectively, the moisture contents (dry basis) at any time; initialy and finally

Equilibrium moisture content was taken to be the final moisture attained by the pieces at the conditions of drying. Page's model, which has been found to be most suitable for describing the thin layer drying characteristics of several agricultural products[3,4,5]. The model is of the form:

$$MR = \exp(-Kt^N) \quad \text{....... (2)}$$

where

t = time of drying (hr); K, N constants evaluated by linear regression after logarithmic transformation to the form:

$$\ln(-\ln MR) = \ln K + N \ln t. \quad \text{....... (3)}$$

The correlation coefficients obtained for all the drying runs were greater than 0.98. Fig 1 and 2 show a comparison of measured and predicted values using the evaluated model constants.

The results of the experiments and the constants for the model for all the conditions tested are presented in

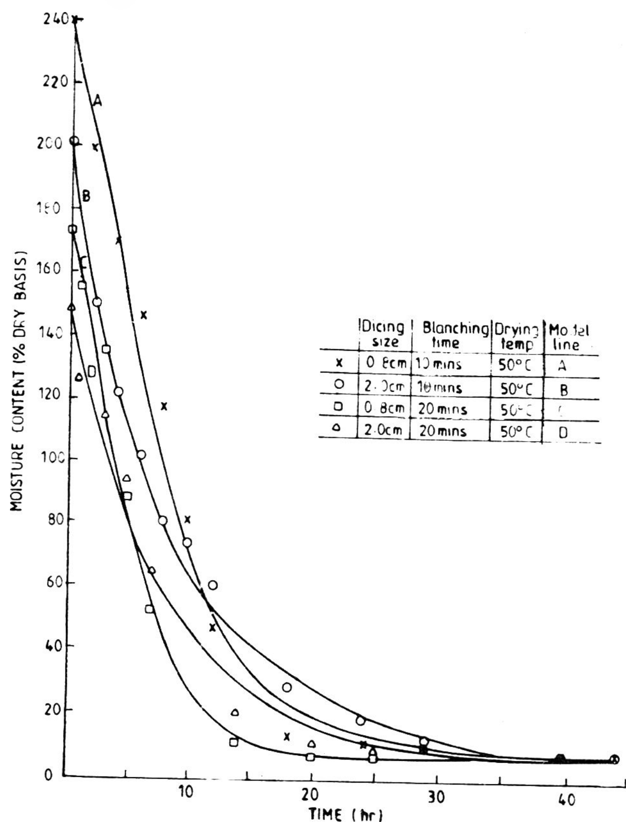

Fig. 1: Effects of dicing size and blanching time on drying behaviour at 50°C.

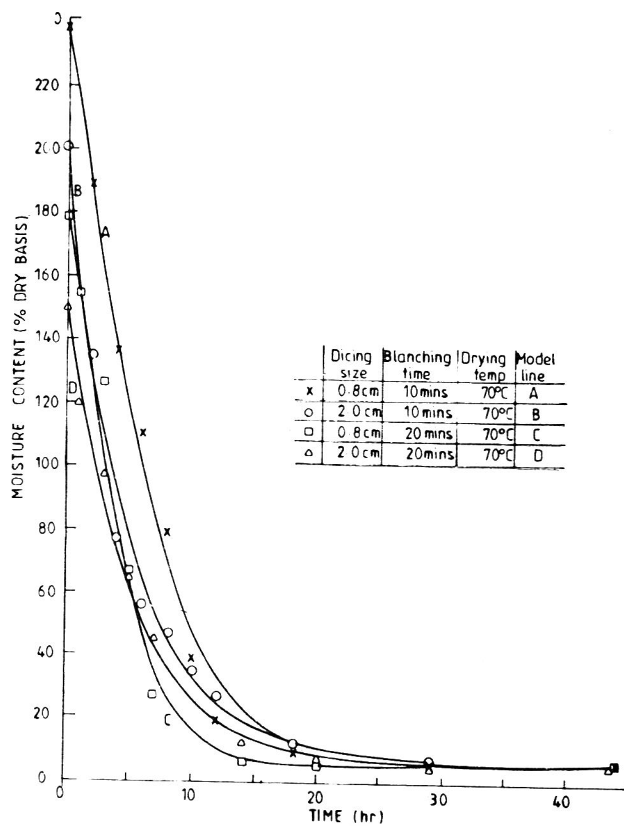

Fig. 2: Effects of dicing size and blanching time on drying behaviour at 70°C.

TABLE 1. RESULTS OF DRYING EXPERIMENTS AND PARAMETERS EVALUATED FROM PAGE'S MODEL

Size (cm)	Blanching time (min)	Drying temp. (°C)	Moisture content after blanching (% d.b.)	Moisture content after drying (% d.b)	Model parameters K	Model parameters N	Model parameters $t_{1/2}$ (hr)	Drying time* (hr)
0.8	10	50	240	10	0.058	1.36	6.16	44.0
2.0	10	50	201	9	0.142	0.94	5.38	46.0
0.8	20	50	173	8	0.089	1.36	4.51	17.5
2.0	20	50	149	8	0.119	1.06	5.30	29.0
0.8	10	70	237	6	0.100	1.25	4.75	19.6
2.0	10	70	201	6	0.239	0.92	3.16	20.4
0.8	20	70	179	6	0.119	1.39	2.55	12.0
2.0	20	70	150	6	0.182	1.05	3.56	17.0

$t_{1/2}$ = time of half response;
*Calculated to 10% moisture content (d.b.)

Table 1. In general, it was observed that the moisture content of the yam pieces was substantially less after blanching. Similar observations have been reported for potato and carrots[6] and for dewatered cassava mash[7]. Analysis of data indicates highly significant effects of size and blanching time on the moisture content after blanching. The longer the blanching time the lower was the moisture content after blanching. This was due to the drying capacity of the steam used for blanching.

The analysis of the effects of the processing factors shows that only the drying temperature had a significant effect on the final moisture content. An increase in drying temperature from 50 to 70°C decreased the final moisture content by about 2.8 per cent, because as expected, equilibrium moisture values are always lower at higher temperatures. The effects of the processing factors on the rate of drying of pregelled yam pieces can be evaluated by considering the time of half response ($t\frac{1}{2}$). The time of half response is defined as the time required to remove the first half of free moisture. This corresponds to the time required to reach a moisture ratio of 0.5. The data show that only drying temperature had a significant effect on the time of half-response. In the yam flour production line, the time required to complete drying is, of course, important. Table 2 indicates how the three factors affect the time required to dry the pieces to a moisture content of 10 per cent. The three factor interaction was assumed as error and the two factor interactions were compared with this. Then, the non-significant interactions were pooled together with the three factor interactions to make a composite value for testing the main effects. Both drying temperature and blanching time have significant effects on the drying time. An increase in drying temperature reduced the drying time because it increased the rate of drying while an increase in the blanching time reduced the drying time because it reduced the moisture content of the pieces after blanching. There was also a significant interaction between blanching time and drying temperature (Table 2). Analysis of this interaction shows that the effect of drying temperature was more pronounced for the yam pieces blanched for 10 min than those blanched for 20 min. It is concluded that the drying data were adequately represented by Page's model. Increase in blanching time reduced the drying time of pregelled yam pieces. These results would be helpful in selecting processing factors for yam flour production.

TABLE 2. ANALYSIS OF VARIANCE TABLE SHOWING THE EFFECTS OF FACTORS ON THE DRYING TIME

Source of variation	Effect	Sum of squares	D.F.	Mean square
Average	25.7			
Main effects				
Size (S)	4.8	45.56	1	46.56
Blanching times (B)	−13.6	371.28	1	371.28***
Drying temp. (T)	−16.9	569.53	1	569.53***
Interactions				
BT	8.1	132.0	1	132.0**
SB	3.4	23.46	3	11.46
ST	−1.9	7.41		
SBT	−1.3	3.51		

*** Significant at 1% level; **Significant at 5% level

Acknowledgement

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References

1. Corling, P. Physical phenomena during the drying of foodstuffs. in Fundamental Aspects of the Dehydration of Foodstuffs. *Proceedings of a Conference of the Society of Chemical Industry*, Aberdeen, March 1958, 136.

2. Page, C., *Factors Influencing the Maximum Rates of Air Drying Shelled Corn in Thin Layers*, 1949, M. S. Thesis, Purdue University.

3. Chhinnan, M.S., Evaluation of selected mathematical models for describing thin-layer drying of in-shell pecans. *Trans. Amer. Soc. agric Engr* 1984, **27**, 610.

4. Li, H. and Horey, R.V., Thin layer drying of yellow dent corn. *Trans Amer. Soc. agric Engr.*, 1984, **27**, 581.

5. Syarief, A.M., Morey, R.V. and Gustafson, R.J., Thin-layer drying rates of sunflower seed. *Trans. Amer. Soc. agric. Engr.*, 1984, **27**, 195.

6. Ede, A.J., 1958. Some physical data concerning the drying of potato strips. in Fundamental Aspects of the Dehydration of Food stuffs, *Proceedings of a Conference of the Society of Chemical Industry*, Aberdeen, March 1958, 136.

7. Ajibola, O.O., Ige, M.T. and Makanjuola, G.A., Preliminary studies on a new technique of cassava mash gelatinization. *J. Agric. Eng. Res.*, 1987, **36**, 97.

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Electrochemical Studies of Aluminium with Model Solutions and Vegetables

R. NARESH, M. MAHADEVIAH AND R.V. GOWRAMMA
Central Food Technological Research Institute, Mysore-570013, India

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Electrochemical studies of plain and lacquered aluminium sheet have been carried out with model solutions and a few vegetables. Variation in galvanic current and potential readings were noted during storage upto 100 hr. Influence of some corrosion accelerators and inhibitors was studied. The results of these studies are useful to predict the performance of aluminium cans with different vegetable products and the influence of various accelerators and inhibitors of corrosion.

Although tinplate can is considered as an ideal material for packing processed food products, aluminium containers have been successfully introduced on a commercial scale for packing many food products like meat and fish products, soft drinks, carbonated beverages and beer.

Lopez and Jimenz[1] evaluated the suitability of aluminium cans for canning a number of fruit and vegetable products. Generally, tinplate cans performed better with acid foods than coated aluminium cans. With low acid foods, coated aluminium cans compared well with tinplate containers. Uncoated aluminium cans were rapidly corroded by the majority of the products tried. Corrosion was not a problem with coated aluminium cans, but some of the coatings affected the taste and colour of the products.

Hugony[1] found that foods and beverages in contact with aluminium foil or cans generally absorbed less aluminium than their natural aluminium content. For certain fruits and juices, a lacquer or anodic oxide film is advisable to prevent corrosion. Vegetables with pH below 5.2 should not be canned in plain cans.

Jacobson and Mathiesen[2] reported that fruit jams are less corrosive than sugar syrup in aluminium cans and corrosive action is affected by storage temperature. The quality and the characteristics of aluminium plate required for cans have been discussed by Althen.[3]

The corrosion behaviour of aluminium has been reviewed by Godard[4] who discussed the factors responsible for corrosion. Wood[5] has described the various production methods for rigid containers from aluminium alloys and the factors determining the selection of the correct alloys.

In India, a few canning factories are using imported aluminium cans for packing fish products. In view of the shortage of tin metal and the high cost of tinplate containers, a few can manufacturers have made an attempt to produce aluminium cans for packaging food products. Results of investigations on the corrosion behaviour of such aluminium cans with some model solutions and vegetables are reported in this publication.

Materials and Methods

Following electrochemical studies were carried out.

Corrosion cell: It is a cell with internal diameter of 5 cm and 22 cm length open at both ends. Three standard joints are fixed as shown in Plate 1. Beading is provided at both ends. The ends are flattened uniformly for fixing in the stand.

Specimens: Aluminium cans of 77 × 90 mm (0.3 mm thickness) size with 99 per cent purity were cut into suitable size (8 × 8 cm) and degreased with carbon tetrachloride. These plates were fixed on one side of the corrosion cell (Plate 1). Model solutions of the

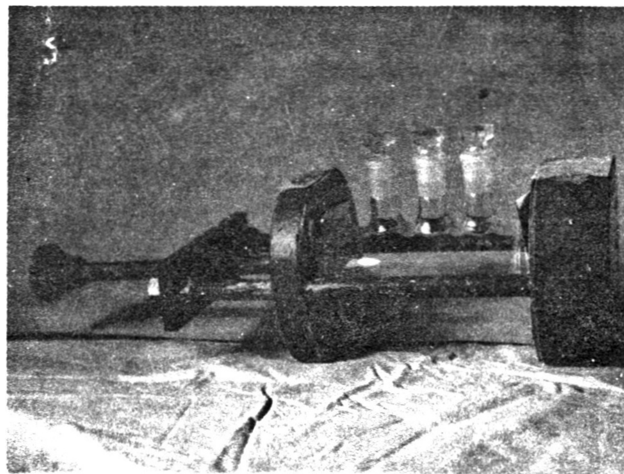

Plate 1. Corrosion cell

vegetables mentioned below were filled separately and bubbled with nitrogen for about 15 min to remove oxygen. Galvanic current readings were noted periodically at ambient temperature (25-30°C), using a digital multimeter.

Media: Buffer solutions of pH 4.0 with 0.3 per cent acidity were used as model solutions and accelerators or inhibitors were incorporated into these. Vegetables such as beans, carrot and potato were cut into small pieces, filled into the corrosion cell and covered with 2 per cent hot brine.

Results and Discussion

The periodical change in galvanic current during storage for 100 hr are discussed below.

Corrosion of plain and lacquered aluminium: The corrosion behaviour of plain and lacquered aluminium was studied with sodium citrate and citric acid buffer solutions of pH 4.0. Variation in corrosion current with pH 4.0 during storage is shown in Fig.1.

The galvanic current with plain aluminium raised gradually and reached upto 300 μA after 80 hr. Initially, the reaction was less due to the oxide layer on aluminium. For lacquered aluminium, the current was low (which is not shown in the Fig.)

Influence of organic acids on corrosion of aluminium: pH 4.0 buffer solutions were prepared separately with citric acid, tartaric acid, malic acid and oxalic acid maintaining the acidity of 0.3 per cent. The corrosion current was high for oxalic acid followed by citric acid, malic acid and tartaric acid. (Fig. 2). Upto 60 hr. there was a steady increase in current and thereafter it gradually declined.

The corrosion behaviour of aluminium with organic acids appears to be similar to tinplate corrosion as calculated by Sherlock and Britton[6] based on the stability constant. Similar results have been obtained by Mahadeviah[7] with tinplate in model experiments and polarisation technique.

Influence of corrosion accelerators: The influence of nitrite and nitrate which are considered as potential accelerators of corrosion of tinplate has been studied with aluminium. The accelerators (25 ppm) were mixed separately with citrate buffer solution of pH 4.0.

The galvanic current increased from 100 to 410 μA upto 40 hr of storage and then remained constant upto 100 hr. This indicates that till all the nitrite was utilised the corrosion reaction accelerated and thereafter there was uniform corrosion. Corrosion current was slightly less with NO_3 as compared to NO_2 (Fig. 3).

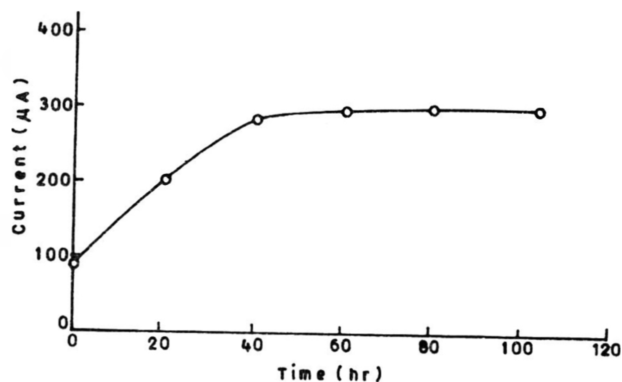

Fig. 1. Corrosion behaviour with pH 4 buffer

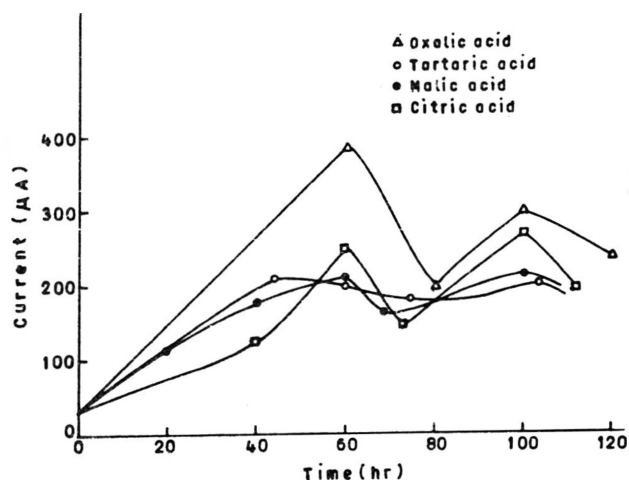

Fig. 2. Influence of organic acids

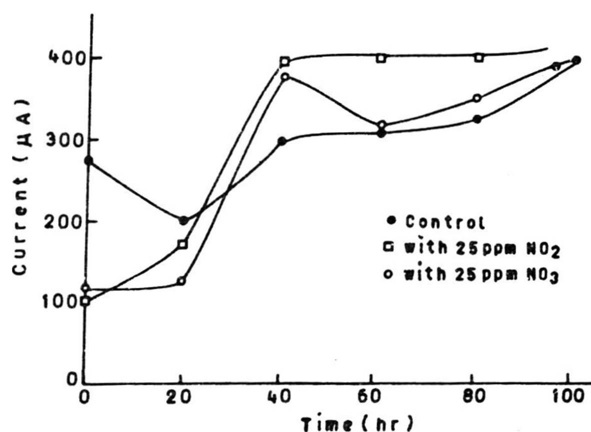

Fig. 3. Influence of corrosion accelerators

These results indicate that nitrite and nitrate have the same effect on aluminium as on tinplate.

Influence of polyphenols: The influence of polyphenols like gallic acid, tannic acid and pyrocatechol present in some of the fruits and vegetables was studied. Polyphenols at 0.5 per cent

level were incorporated in buffer solution of pH 4.0 and the variation in current with aluminium was noted during storage.

The corrosion current was more with pyrocatechol indicating the acceleration effect of pyrocatechol as compared to gallic acid and tannic acid. Gallic acid and tannic acid showed less corrosion than control (Fig. 4). This is contrary to the results obtained by Mahadeviah *et al*[8] with tinplates, for gallic acid which was found to be present in mango peel.

Influence of inhibitors: In order to find the possibility of reducing corrosion of aluminium, the influence of a few thickening agents has been tried. Thickening agents such as carboxymethyl cellulose, gelatin and agar agar were separately incorporated at concentration of 0.5 per cent to the citrate buffer solution of pH 4.0. The corrosion current readings indicated that aluminium showed less corrosion in the presence of carboxymethyl cellulose and gelatin as compared to buffer free from inhibitors (Fig. 5). However, agar agar gave more galvanic current than the control and other thickening agents. Thus, it may be inferred that by incorporating carboxymethyl cellulose or gelatin in low concentrations in the media, the corrosion of aluminium can be inhibited. This is similar to the results obtained by Mahadeviah *et al*[8], for carboxymethyl cellulose in reducing corrosion of tinplate with mango nectar.

Corrosion of aluminium with vegetables gave the following results.

Potatoes in brine: Corrosion behaviour of plain and lacquered aluminium sheet was studied in the presence of potatoes in brine. The galvanic current of plain aluminium went upto 350 μA after 20 hr and did not show much further change upto 100 hr. For lacquered aluminium, the galvanic current was very low and it ranged from 0 to 0.004 μA. The results indicate that for packing potato, plain cans may not be suitable.

Carrots in brine: Galvanic current was comparatively less in both plain and lacquered cans as compared to potatoes in brine. Current was 320 μ A after 40 hr in plain aluminium and then there was no significant change upto 100 hr. In lacquered aluminium plate the galvanic current was below 100 μ A throughout (Fig. 6).

Green beans in brine: The results indicate that plain aluminium cans may not be suitable for canning green beans, because of high current. The above electrochemical results with vegetables in the presence of both plain and lacquered aluminium correlated with results obtained by Lopez and Jimenz[1] in vegetable products canned in plain and lacquered aluminium cans.

The results of these electrochemical studies give an indication about the corrosion behaviour of aluminium in the presence of accelerators, inhibitors and a few vegetables. However, it is necessary to confirm these results by actual canning trials.

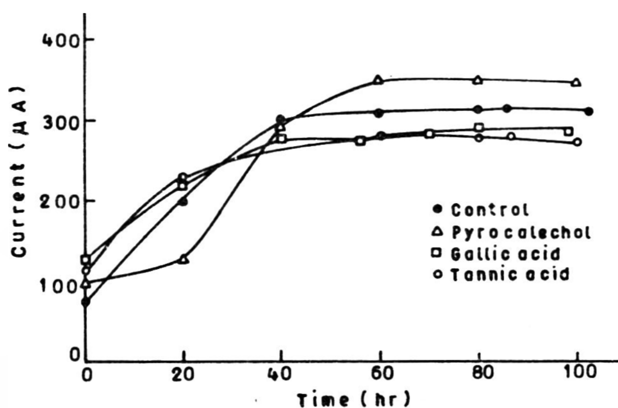

Fig. 4. Influence of polyphenols

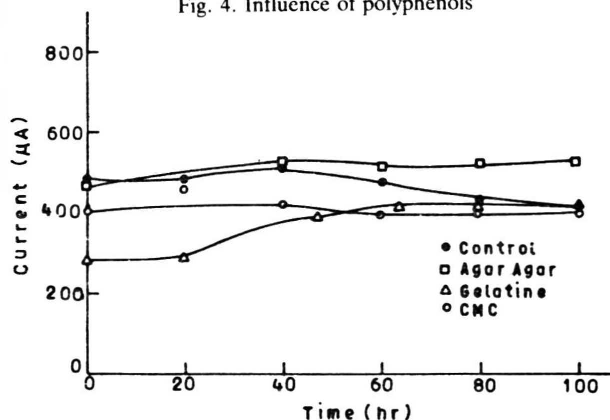

Fig. 5. Influence of corrosion inhibitors

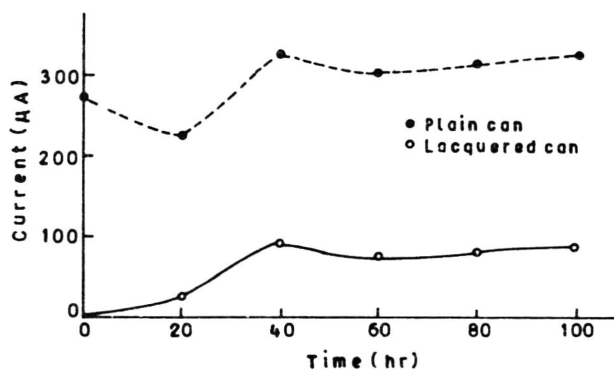

Fig. 6. Carrots in brine

References

1. Lopez, A and Jimenz, M.A., Canning fruit and vegetable products in aluminium containers. *Fd. Technol.*, 1969, **23**, 1200.

2. Jacobsen, F. and Mathiesen, E., Corrosion of containers for canned foods. *Skrifterutg, ov. Det Norske Videnskaps - Akad. Oslo, I Mat. Naturev. Klasse*, 1946, No. 5.

3. Althen, P.E., The aluminium can. *Fd. Technol.*, 1965, **19**, 784

4. Godard, H.P., The corrosion behaviour of aluminium, *Corrosion*, 1955, **11**(12), 1.

5. Wood, G.C., Aluminium alloy for rigid containers. Sheet Metal Industries, January 1975, Alcan International Ltd. Banberry, -0.

6. Sherlock, J.C. and Britton S.C., Complex formation and corrosion rate for tin in fruit acids, *Br. Corrs. J.*, 1972, **7**, 180.

7. Mahadeviah, M. *Internal Corrosion of Tin Plate Container with Mango and Orange Products*, Ph.D. Thesis, University of Mysore, 1974.

8. Mahadeviah, M., Gowramma, R. V. Eipeson, W.E. and Sastry, L.V.L., Internal corrosion of tinplate container in canned mango nectar. *J. Sci. Fd. Agric.*, 1975, **26**, 821.

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Comparison of the Effect of a Putrefactive *Pseudomonas* sp. Inoculated into Muscle Tissue and Beef Fat

R.B. BABIKER*, J.T. PATTERSON AND A.P. DAMOGLOU
Queen's University of Belfast, Department of Agricultural and Food Bacteriology, Newforge Lane BT 9 5 PX.

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The effect of a putrefactive, *Pseudomonas* II sp. inoculated into muscle tissue and beef fat was studied. This species was observed to follow a different mode of attack on glucose, lactic acid and free amino acids in both meat and fat. Marked difference was noted in the pattern of utilization of amino acids in meat and fat. Rise in the pH of inoculated meat and fall in the pH of inoculated fat was noticed. The ability of *Pseudomonas* II sp, to spoil the fat earlier than the lean meat showed the importance of fat in the spoilage of meat stored aerobically at refrigeration temperature.

The aerobic spoilage flora of fresh meat stored at chill temperatures is usually dominated by *Pseudomonas* sp, although *Acinetobacter, Moraxella, Enterobacter* sp. and *Brochothrix thermosphactum* may be present[1]. The reason for the importance of *Pseudomonas* lies on the fact that some of them can grow faster on meat than the other previously mentioned species at temperatures between 2 and 15°C[2].

Patterson and Gibbs[3] listed several types of odours produced by pure bacterial strains from normal meat spoiled aerobically at 4°C. Daud *et al.*[4] showed that off-odours produced by the genus *Pseudomonas* included 'fruity' (evaporated milk) and sulphide-like.

The fat surfaces of carcasses are usually kept too dry to allow bacterial growth. However, in packages, the fat surfaces are bathed in the drip from the muscle. In a domestic refrigerator, fat surface will often be kept moist by the periodic entrance of warmer air. Under these conditions, bacteria can grow and cause spoilage.

Although microbial spoilage of fat has received considerable attention, there has been little research on microbial spoilage at the fat surface. Gill and Newton[5] followed the development of a non-proteolytic, non-lipolytic *P. fluorescens* on lamb adipose tissue. In the present study, the effect of a putrefactive *Pseudomonas* on meat and fat surfaces was studied.

Materials and Methods

The organism used in this study is a lipolytic non-proteolytic *Pseudomonas* II sp. isolated earlier by the authors[6] from beef allowed to spoil at 15°C.

The bovine striploin (*M. longissimus dorsi*) used to prepare sterile meat samples had the following characteristics: extract release volume, 68 ml using Jay technique[7]; pH 5.7 with pH meter (Corning EEL Model 10); titrable alkalinity 1.3 determined by the method of Shelef and Jay[8]. The fat cover (pH 7.15) of the *M. longissimus dorsi* was removed and sterile pieces of meat and fat (5 – 10 g) were prepared as described by Sharp[9].

A pure culture of *Pseudomonas* II sp. was grown on 5 ml sterile nutrient broth overnight at 25°C. Using a dropper pipette, one drop (0.02 ml) was subcultured into 10 ml of sterile nutrient broth and incubated for 24 hr at 25°C. Cells were harvested by centrifugation in a Bench Centrifuge (BTL) at 1400 G for 15 min and washed twice with 9 ml sterile distilled water. Decimal dilutions were made from the concentrated cell suspension in 9 ml sterile distilled water. Aliquots of 0.02 ml from suitable dilutions were inoculated carefully onto the surface of the sterile meat and fat pieces. Ten fold dilution was made from the original suspension in sterile peptone water. From suitable dilutions, 0.1 ml was inoculated on previously prepared nutrient agar plates to obtain the required number of viable cells for further inoculation. Plates were counted after 3 days incubation at 25°C.

The organism was inoculated onto 12 sterile pieces of each of fat and meat. DTNB (5,5 – dithiobis-2-nitrobenzoic acid) and LA (lead acetate) (one of each) papers were aseptically placed in the head space above the inoculated samples in such a manner that they did not come into contact with the

**Present address:* Department of Microbiology, Food Research Centre, Khartoum North, P.O. Box 213, Sudan.

samples. Samples were incubated at 4°C and growth of the bacteria was followed. Analyses for total count, pH, odours produced and changes in carbohydrates and amino acids were carried out at appropriate times.

The method described by McMeekin *et al*[10]. was followed for the preparation of DTNB papers to detect methanethiol and possibly other thiols. Lead acetate papers were prepared similarly to detect hydrogen sulphide only.

At each sampling time, two inoculated fat and one inoculated meat samples were removed. One sample each (fat and meat) was used to prepare an initial ten fold dilution in sterile distilled water. Each sample was homogenized in a homogenizer (MSE) at full speed for 2 min. The homogenate was used to prepare decimal dilutions in 0.5 per cent (w/v) sterile peptone water. From suitable dilutions, 0.1 ml was taken and spread on previously poured and dried nutrient agar plates. Plates were incubated at 25°C for 3 days before the total count was determined. The pH was measured on the original 10^{-1} dilution using a pH meter (Corning EEL Model 10).

The second of the two fat samples was treated with 6 per cent (w/v) perchloric acid, homogenized for 2 min at full speed in a homogenizer and the homogenate centrifuged at 2000 × G (0°C) for 20 min in a centrifuge (MSE HS-21). The resulting supernatant was adjusted to pH 6.5 with 20 per cent (w/v) potassium hydroxide[11]. The clear supernatant was used to assay total glucose (glucose plus glucose-6-phosphate), lactic acid and amino acids. For the meat samples, half of the original meat homogenate (meat in distilled water) was used to extract the carbohydrates in a manner similar to that used for the fat samples. Bochringer Mannheim kits were used to determine the quantity of free glucose and lactic acid[12]

Determinations of the free amino acids in the fat samples were carried out using the same solution prepared for the analyses of lactic acid and total glucose. The solution was rotary evaporated to dryness. The residue was taken up in 1 ml lithium citrate buffer (pH 2.2) containing a known concentration of norleucine (as an internal standard). Amino acids were estimated using a LKB Model 4400 amino acid analyzer. In the meat samples, proteins were precipitated by the method described by Sutherland *et al*[13]. The method used for determination of free amino acids in meat was similar to that described for fat.

Results and Discussion

Results of carbohydrate analyses are shown in Fig 1 and 2. *Pseudomonas* II sp. utilized glucose and lactic

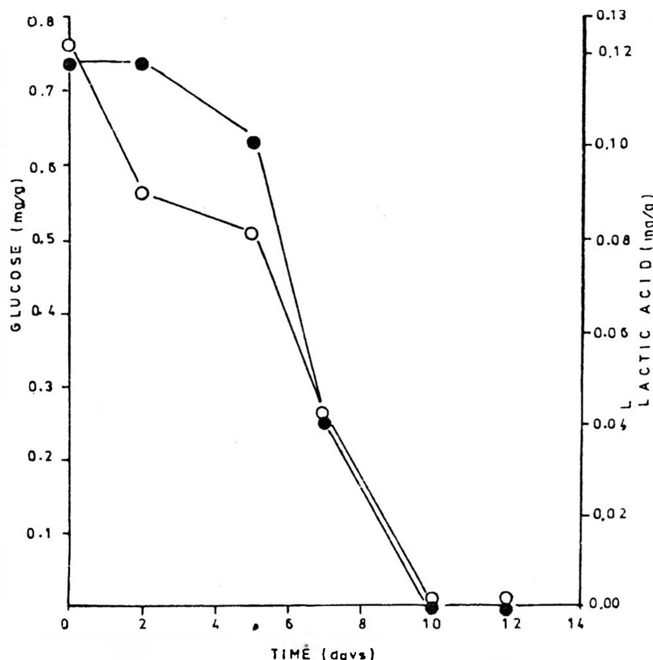

Fig. 1. Changes in the concentration of Glucose (●) Lactic acid (O) in fat inoculated with *Pseudomonas II*

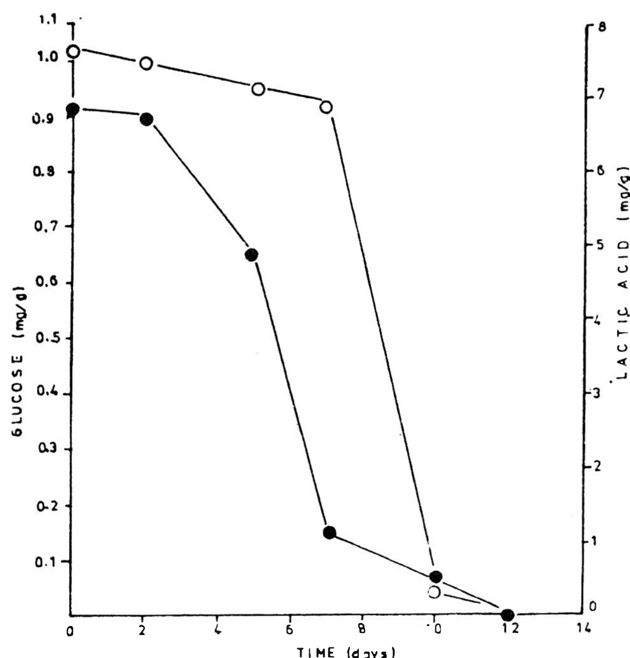

Fig. 2. Changes in the concentration of Glucose (●) Lactic acid (O) in fat inoculated with Pseudomonas II

acid simultaneously in fat (Fig. 1). However in meat, lactic acid was utilized only when glucose was near zero level (Fig. 2). The preferential utilization of glucose by *Pseudomonas* has been reported by Jacoby[14] in liquid culture, by Gill[11] and Shelef[15] in meat and by Gill and Newton[5] in fat. Effects of *Pseudomonas* II sp. on free amino acids in lean meat

and fat surfaces are presented in Table 1. Changes in asparagine, ornithine and cystine are shown in Fig. 3. In meat, asparagine and cystine were brought to zero level in about 7 and 10 days, respectively. However in fat, asparagine and cystine were depleted after only 2 days. The majority of amino acids in meat were observed to increase initially before being utilized e.g. aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine. The mode of attack of *Pseudomonas* II sp. on fat was different from that on most amino acids. It started and continued to utilize several amino acids at 2 days storage e.g. aspartic acid, serine, proline, alanine (Table 1). The decrease or increase of amino acid concentration depends upon the relative rates of two opposing processes, free amino acid release as a result of proteolysis, and amino acid consumption by the bacteria[11].

Pseudomonas II sp. required twice as many organisms and three times as long to change the amino acid concentrations in meat than in fat (log 10^7 in 7 days) and (10^3 in 2 days) respectively.

Results in Table 2 show rise in pH of inoculated meat and fall in the pH of inoculated fat. The rise in the pH of meat could be attributed to production of ammonia. Jay and Kontou[16] found that low molecular weight compounds in meat provided the substrates which the meat spoilage organisms converted to ammonia and hydrogen sulphide. The fall in the pH of inoculated fat may be due to acids obtained from the hydrolysis of fats. Slightly acidic and acidic odours were detected in inoculated fat at the 7th and the 12th day respectively. The acidic odours could be attributed to the deamination of glycine, alanine and valine. Sutherland *et al*[13] reported that the deamination of glycine, alanine and valine can lead to the formation of volatile carboxylic acids; acetic acid from glycine, propionic acid from alanine, isobutyric acid from valine although other complex pathways might be involved. *Pseudomonas* II sp. in meat was able to utilize glycine, alanine and valine at 7 days when the bacterial load was $\log_{10}$ 5.75, yet in fat it started to attack the same amino acids at 2 days when the bacterial count was only $\log_{10}$ 3.38 (Table 2).

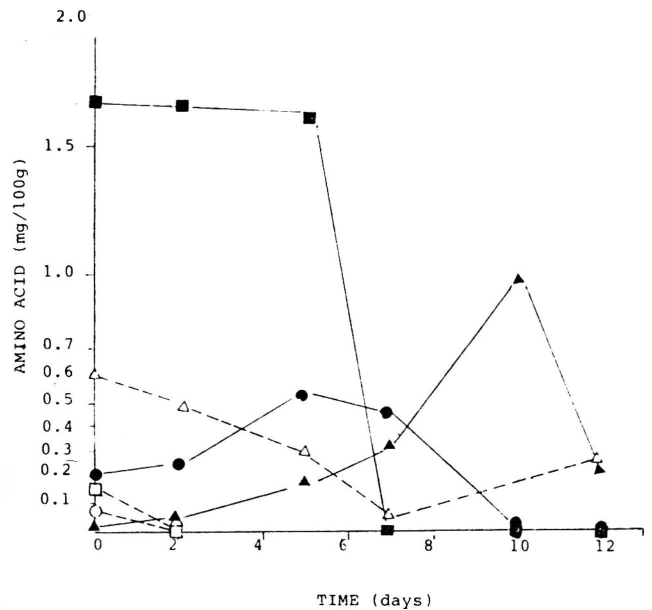

Fig. 3. Changes in the concentrations of Asparagine ■ □) Ornithine ▲ △ and Cystine ●○ in meat and fat inoculated with *Pseudomonas* II. Sp. solid line, meat; dashed line, fat.

The inoculated meat exhibited positive DTNB and lead acetate reactions: DTNB was positive on the 10th and 12th day and the lead acetate on only the 12th day. These positive reactions were accompanied by production of putrid odour. Positive DTNB suggests the release of methane-thiol and possibly other thiols from methionine. Decomposition of methionine to methanethiol was reported by Segal and Starkey[17].

TABLE 1. CONCENTRATION OF AMINO ACIDS (mg/100 g) IN MEAT AND FAT INOCULATED WITH PSEUDOMONAS II SP.

	Concn. of amino acids in meat						Concn. of amino acids in fat				
	0 hr	2d	5d	7d	10d	12d	Ohr*	2d*	5d*	7d*	12d*
Urea	3.0	3.1	4.5	4.1	3.6	4.1	5.3	3.9	2.6	2.0	1.5
Aspartic acid	0.2	0.3	0.5	1.3	0.1	0.1	3.1	2.4	1.9	1.8	0.8
Threonine	1.7	1.8	3.6	3.0	1.5	0.2	1.5	1.3	1.0	1.0	0.4
Serine	1.9	2.5	5.0	3.8	0.2	0.1	1.9	1.6	1.4	1.2	0.9
Glutamic acid	2.4	5.1	10.3	8.2	0.2	0.2	2.3	2.6	2.8	1.0	1.0
Proline	1.6	1.5	2.7	2.1	0.0	0.0	1.8	1.4	1.3	1.1	0.6
Glycine	1.0	1.1	2.0	1.9	0.6	0.1	2.5	2.1	2.0	1.9	0.6
Alanine	3.6	3.8	7.5	6.5	0.9	0.1	4.8	4.0	3.7	3.6	1.3
Valine	1.6	1.8	3.7	3.0	1.2	0.3	1.3	1.1	1.0	1.0	0.4
Methionine	0.6	0.8	1.6	1.3	0.3	0.2	0.1	0.1	0.3	0.2	0.08
Tyrosine	1.1	1.3	2.6	2.2	0.8	0.1	0.7	0.6	0.5	0.5	0.2
Ammonia	0.8	0.9	1.9	2.1	0.9	1.5	0.1	0.1	0.1	0.3	0.04
Carnosine	30.0	32.0	57.0	51.0	41.0	37.0	0.0	2.9	10.4	0.4	0.0

d: days

TABLE 2. ACTION OF *PSEUDOMONAS* II sp. ON MEAT AND FAT

	Meat					
	Ohr	2 d	5 d	7 d	10 d	12d
log_{10} bacterial no./g	4.05	4.26	5.38	7.18	9.18	9.46
pH	5.70	5.71	5.65	5.75	7.15	7.50
	Fat					
log_{10} bacterial no./g	2.96	3.38	7.54	3.64	-	8.28
pH	7.00	7.00	6.68	6.65	-	6.60

d : days

Methionine is first deaminated and the product demethylated to form methanethiol. Utilization of methionine in meat is shown in Table 2. McMeekin *et al*[10] reported that cultures giving positive result with DTNB were judged to have attacked only methionine. Positive lead acetate reactions suggests the release of hydrogen sulphide. The putrid or the sulphide-like off-odour detected in meat on the 10th and 12th day could be attributed to the release of hydrogen sulphide.

Pseudomonas II sp. spoiled the fat earlier than the meat (Table 2). This reflected the importance of this type of microorganisms in initiating the spoilage of fat surfaces thus causing the spoilage of meat held in domestic refrigerators.

References

1. Ingram, M. and Dainty, R.H., Changes caused by microbes in spoilage of meat. *Appl. Bacteriol.*, 1971, **34**, 21.
2. Gill, C.O. and Newton, M.G., The development of aerobic spoilage flora on meat stored at chill temperatures. *Appl. Bacteriol.*, 1977, **43**, 189.
3. Patterson, J.T. and Gibbs, P.A. Incidence and spoilage potential of isolates from vacuum packaged meat of high pH value. *Appl. Bacteriol.*, 1977, **43**, 25.
4. Daud, H.B., McMeekin, T.A. and Thomas, C.J. Spoilage association of chicken skin. *Appl. Environ. Microbiol.*, 1979, **37**, 399.
5. Gill C.O. and Newton, M.G. Development of bacterial spoilage at adipose tissue surfaces of fresh meat. *Appl. Environ. Microbiol.* 1980, **39**, 1076.
6. Babiker, R.B., *Some Aspects of Aerobic Bacterial Degradation of Meat.* 1982. Ph.D. Thesis. The Queen's University of Belfast.
7. Jay, J.M., Release of aqueous extracts of beef homogenates and factors affecting release volume. *Food Technol.*, 1964, **18**, 1633.
8. Shelef, L.A. and Jay, J.M., Use of titrimetric method to assess the bacterial spoilage of fresh beef. *Appl. Microbiol.*, 1970, **19**, 902.
9. Sharp, J.G. Aseptic autolysis in rabbit and bovine muscle during storage at 37°C. *J. Sci. Fd. Agric.*, 1963, **14**, 468.
10. McMeekin, T.A., Gibbs, P.A. and Patterson J.T., Detection of volatile sulphide - producing bacteria isolates from poultry-processing plants. *Appl. Environ. Microbiology*, 1978, **35**, 1216.
11. Gill, C.O., Substrate limitation of bacterial growth at meat surface. *Applied Bacteriol.*, 1976, **41**, 401.
12. Skjelkvale, R., Fyhn, P.G., Slinde, E. and Hoyem, T., Process control in the production of fermented sausages. *Proceedings of the European Meeting of Meat Research Workers*, 1980, **26**, 116.
13. Sutherland, J.P., Gibbs, P.A., Patterson, J.T. and Murray J.G., Biochemical changes in vacuum-packaged beef occurring during storage at 0-2°C. *Fd. Technol.*, 1976, **11**, 171.
14. Jacoby G.A. The induction and repression of amino acid oxidation in *P. fluorescens*. *Biochem. J.*, 1964, **92**, 1.
15. Shelef, L.A. Effect of glucose on the bacterial spoilage of beef. *J. Fd. Sci.*, 1977, **42**, 1172.
16. Jay, J.M. and Kontou, K.S., Fate of free amino acids and nucleotides in spoiling beef. *Appl. Microbiol.*, 1967, **15**, 759.
17. Segal, W. and Starkey, R.L., Microbial decomposition of methionine and identity of the resulting sulphur products. *J. Bacteriol.*, 1969, **98**, 908.

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Application of Gel Consistency Test to Parboiled Rice

K.R. UNNIKRISHNAN AND K.R. BHATTACHARYA
Discipline of Grain Science and Technology, Central Food Technological Research Institute, Mysore, India

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Mobility of an alkaline rice-flour gel increased upon parboiling of the rice and with increasing severity of parboiling. Gel mobility of variously parboiled samples in a variety increased with increasing concentration of alkali, finally reaching a common value for all samples (including raw rice of the same variety), but then again seemed to diverge among processing conditions in a reverse order with further rise in alkali strength. Gel mobility was proportional to the room-temperature hydration power of whole-grain rice as well as to sediment volume of an aqueous flour dispersion, the proportionality among processing conditions being identical in different varieties. Gel mobility appeared to be related only to the degree of starch gelatinization, unaffected by starch reassociation, and hence could be a good test for the extent of gelatinization in parboiled rice.

Cagampang *et al.*[1] devised a new test for rice quality, called the gel consistency test. Rice flour was dispersed in dilute alkali, and the resulting 4.4 per cent (dry basis) alkaline gel was allowed to flow by laying the tube horizontally. The distance to which the gel flowed gave an index of its consistency, which was found to correlate well with the inherent cooking and eating qualities of rice[2]. The shorter the gel flow, the harder the variety after cooking, and *vice versa*. This test has not been applied to parboiled rice, except in a preliminary study with indifferent results[3], which led to the present work.

Materials and Methods

Five varieties of rice ('Rathnachudi', 'IR 20', 'Intan', 'IR 8', and 'MR 301') were collected from the University of Agricultural Sciences Experiment Station at Mandya, air-dried, cleaned, fumigated and stored in the laboratory under ambient conditions (25 30°C) for about 6 months and then in the cold (4-6°C) until use.

Paddy was soaked in warm water overnight[4], drained and then steamed under different pressures for different times to obtain rice parboiled to different degrees. For dry-heat parboiled rice, the soaked paddy, instead of steaming, was heated with two times its weight of sand in a hand-operated electrical coffee roaster at different temperatures for different times[5]. Paddy parboiled in either way was air-dried and then milled with laboratory McGill equipment to about 8 per cent degree of milling, the broken grains being separated and discarded.

To study the effect of starch reassociation on the properties of parboiled rice[5], distilled water was mixed with dry-heat parboiled paddy to raise its moisture content to approximately 26 per cent on wet basis (w.b.) and the paddy was held in closed bottles, fully filled, for 2-3 days to promote starch reassociation. It was then air-dried and milled as before.

Milled rice was ground in a Buhler laboratory grinder (type MLI 301) and then in a Raymond hammer mill in sequence to pass a 60 mesh screen. Portions (100-200 mg) of the above flour were ground in a Wig-L-Bug amalgamator for 30-120 sec, the amount of flour and the time were so adjusted that each sample gave approximately a uniform 100-mesh powder. The powder was exposed to 27°C and 65 per cent RH for a day for moisture equalization (about 12 per cent moisture, w.b.).

The gel consistency test was carried out as prescribed by Cagampang *et al.*[1] and Perez.[2]. An alkali concentration of 0.2 N KOH and 100 mg of flour, as prescribed, were used except when otherwise stated. The flour, after initial wetting with 0.2 ml ethanol containing thymol blue, was mixed with 2.0 ml of alkali in a 13 x 100 mm test tube in a vortex mixer. The tube was heated in a vigorously boiling water bath for 8 min, cooled in air for 5 min and then in an ice water bath for 20 min, and then laid horizontally for 1 hr. Tubes of 100 mm length as originally prescribed were initially used. Since many samples showed a gel length of 100 mm, 150 mm tubes were used subsequently. As defined by the above authors, the longer the gel travels, the lower is its consistency, and *vice versa*. There has, therefore, been a little confusion in the use of the term 'gel consistency' in the literature, in as much as perceived *high* consistency is expressed by a *low* numerical value (of gel travel) and

vice versa. To avoid this confusion, the length of gel travel has been called 'gel mobility' in the present work.

Equilibrium moisture content attained by milled rice when soaked in water at room temperature (EMC-S) was determined as described before[6] and expressed on wet (w.b.) or dry (d.b.) basis as appropriate. Moisture content of paddy was determined by drying at 105°C for 24 hr and adding a correction factor of 1 percentage point (d.b.) to the per cent loss in weight[6]. Sediment volume was determined as per Bhattacharya and Ali[7] by dispersing 2.0 g of 60-mesh rice flour in 20 ml 0.05 N HCl and then allowing it to stand for 4 hr. All analyses were generally done in duplicate.

Results and Discussion

Parboiling led to an increase in gel mobility of rice (Table 1), apparently due to its pregelatinization. This parameter could, therefore, be used as a test for parboiled rice.

Bhattacharya and Ali[8] have recently shown that the two opposing phenomena of starch gelatinization and starch reassociation occur during the parboiling process, and that differential testing of the two phenomena would be very useful for a better characterization of parboiled rice. Therefore, it is of considerable interest that starch reassociation induced in dry-heat parboiled rice by wetting and holding did not result in appreciable change in its gel mobility, although its hydration power clearly fell (Table 1). These results suggest that gel mobility is probably a reflection of starch gelatinization alone, unaffected by starch reassociation. If true, this would be a very valuable test for differential testing of the precise degree of starch gelatinization in parboiled rice.

All subsequent work was done using 150 mm tubes to be able to distinguish gel mobility values beyond 100 mm.

Gel mobility was inversely related to the quantity of flour taken for the test, even though the inter-sample gradation remained unaltered (Table 2).

Gel mobility was strongly affected, positively, by the alkali concentration (Fig. 1). There was some varietal difference in the pattern of the results, the meaning of which is not clear at this time. One difficulty was that when the gel reached 150 mm or thereabouts, the observed mobility value was no longer reliable, for the gel might well have flowed more had the tube been longer. On this basis, considering the data of Intan variety, it looks as though the gel mobility values of raw and processed samples of a variety first converge to a common value at a particular alkali concentration and then again diverge but in the reverse order to that shown by more dilute alkali. This reversal, repeatedly verified with 0.4N KOH on Intan variety, cannot be explained at this time, except noting that it is reminiscent of the

Table 1. Gel mobility of variously parboiled rice, and effect of starch reassociation

Steaming[a]		Dry heating[a]			Gel mobility[b,c] (mm)		EMC-S (% w.b.)	
Pressure (kg/cm^2)	Time (min)	Temp. (°C)	Time (sec)	Final paddy moisture (% w.b.)	As such	Retro-graded	As such	Retro-graded
				Ratnachudi				
Raw					47	—	—	—
0	2				65	—	—	—
	10				72	—	—	—
	60				100+	—	—	—
1	60				100+	—	—	—
		250	60	18.0	90	87	62.6	41.7
				IR20				
Raw					40	—	—	—
		250	40	19.9	76	75	50.2	39.0
			120	14.5	79	77	61.5	41.3
		300	30	19.9	65	61	51.7	38.9
			80	13.8	76	77	64.4	43.2
		350	10	19.6	46	42	58.4	42.7

[a]Paddy was soaked in warm water overnight to about 30% moisture (w.b.), then either (i) steamed or (ii) dry heated with two times the weight of sand under conditions indicated.

[b]Tested in 13 mm × 100 mm test tubes. 100+ mm indicates that the gel reached the end of the tube and would presumably have flowed more.

[c]Test conducted with 100 mg flour for 'Ratnachudi' and with 125 mg flour for IR20 (for, with 100 mg, all samples gave a value of 100+mm).

[d]Parboiled paddy was moistened and held to promote starch reassociation, then dried and milled.

Table 2. Effect of flour concentration on gel mobility of parboiled rice (Intan variety)

Rice[a]	Gel mobility (mm) at different flour wt. (mg)			
	90	100	110	120
Raw	103	71	50	35
2(0)	114	83	61	40
10(0)	128	92	73	46
60(0)	141	110	90	60
60(1)	150+	125	103	71

[a]The numbers stand for parboiled samples: First number shows the time (min) and the number in parenthesis, the pressure (kg/cm^2) of steaming.

precisely similar opposite orders of reaction of whole-grain raw, mild and severe parboiled rice in very dilute and concentrated alkali seen earlier[9]. When immersed in very dilute alkali, raw rice grains remain unaffected, but parboiled grains are degraded in proportion to the severity of processing; when immersed in more concentrated alkali, on the other hand, raw rice grains seem to be attacked the most and the severely parboiled grains the least[9].

Gel mobility in three varieties was closely related to the equilibrium moisture content attained by whole-grain milled rice when soaked in water at room temperature (EMC-S) as well as to the sediment volume of rice flour when dispersed and then allowed to settle in water[7] (Fig. 2). We have shown earlier that the viscosity of an aqueous slurry of parboiled-rice flour too was proportional to its EMC-S and sediment

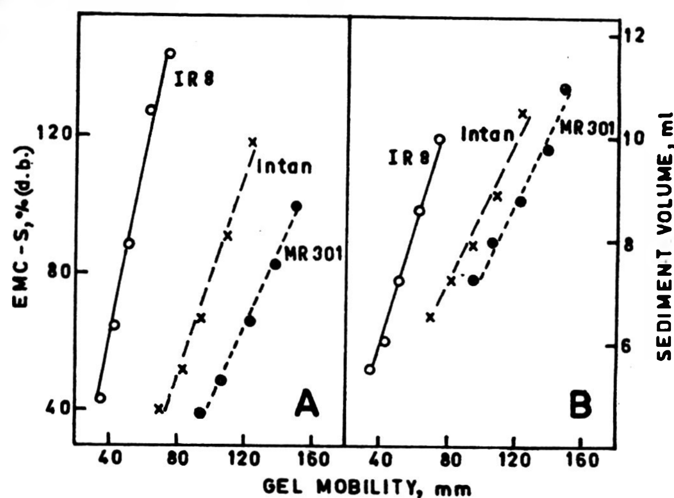

Fig. 2. Interrelationship between gel mobility, room temperature hydration power (A) and aqueous-slurry sediment volume (B) of rice in three varieties. The samples from the lowest to the highest value are: Raw, PB-2(0), PB-10(0), PB-60(0) and PB-60(1) (see code in Table 2).

Fig. 1. Effect of alkali concentration on gel mobility of raw and variously parboiled rice (codes of parboiled rice as in Table 2).

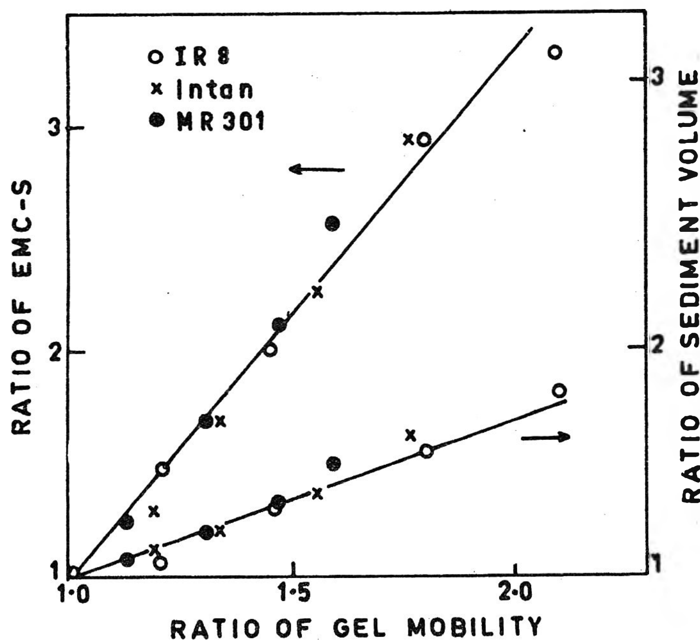

Fig. 3. Relationship between the ratio of gel mobility to ratios of hydration power and of sediment volume of parboiled to raw rice in three rice varieties.

volume[10]. Clearly all these properties are mutually interrelated.

Even though the curves for the three varieties for the individual properties in Fig. 2 were not parallel, the ratios of a parameter for a given degree of parboiled rice to that of raw rice were virtually constant in all the three varieties. This is shown in Fig. 3 where the ratios of the values of the processed samples to that of the respective raw rice for both EMC-S and sediment volume are plotted against the ratios of gel mobility, and all the three varieties are found to fall in a single straight line each. This is an additional indication of the likely usefulness of gel mobility test for parboiled rice. The excellent reproductibility of the test was testified by the fact that the gel mobility values of Intan raw and parboiled rices obtained on different days (Fig. 1 and 2, Table 2) were not significantly different as tested by Cochran's Q Test[11].

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References

1. Cagampang, G.B., Perez, C.M. and Juliano, B.O., A gel consistency test for eating quality of rice, J. *Sci. Fd. Agric.*, 1973, **24**, 1589.
2. Perez, C.M., Gel consistency and viscosity of rice. In *Chemical Aspects of Rice Grain Quality*, International Rice Res. Inst., Los Banos, Laguna, Philippines, 1979, 293.
3. *Annual Report for 1979*, International Rice Research Institute, Los Banos, Laguna, Philippines, 1980, 36.
4. Bhattacharya, K.R. and Indudhara Swamy, Y. M., Conditions of drying parboiled paddy for optimum milling quality. *Cereal Chem.*, 1967, **44**, 592.
5. Ali, S.Z. and Bhattacharya, K.R., Starch retrogradation and starch damage in parboiled rice and flaked rice. *Sarke*, 1976, **28**, 233.
6. Indudhara Swamy, Y.M., Ali, S.Z. and Bhattacharya, K.R., Hydration of raw and parboiled rice and paddy at room temperature. *J. Food. Sci. Technol.*, 1971, **8**, 20.
7. Bhattacharya, K.R. and Ali, S.Z., A sedimentation test for pregelatinized rice products. *Lebensm. Wiss. Technol.*, 1976, **9**, 36.
8. Bhattacharya, K.R. and Ali, S.Z., Changes during parboiling and properties of parboiled rice. *Adv. Cereal Sci. Technol.*, 1985, **7**, 105.
9. Ali, S.Z. and Bhattacharya, K.R., An alkali reaction test for parboiled rice. *Lebensm. Wiss. Technol.*, 1972, **5**, 216.
10. Unnikrishnan, K.R. and Bhattacharya, K.R., Cold-slurry viscosity of processed rice flour. *J. Texture Stud.*, 1983, **14**, 21.
11. Cochran, W.G., In Biometry: *The Principles and Practices of Statistics in Biological Research* by Robert B. Sokal and F. James Rolhf (Ed.), W.H. Freeman and Co., San Francisco, 1969, 654.

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Cooking Quality and Nutritional Evaluation of the Rice-Bean (*Vigna umbellata*)

CHARANJEET K. HIRA, JASBIR K. KANWAR, NEETA GUPTA AND ANITA KOCHHAR
Department of Foods and Nutrition, Punjab Agricultural University, Ludhiana-141004, India

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Five varieties of ricebean were tested for cooking quality and compared with common varieties of green gram, black gram and cowpea. All the varieties were also analysed for their nutritional quality i.e. protein, total sulphur, S-containing amino acids, lysine, calcium, iron, phytin phosphorus, phenols and *in vitro* digestibility of proteins. The results indicated that nutritional quality and acceptability of ricebeans were comparable to other commonly consumed pulses.

Grain legumes have a high protein content. Although they are deficient in sulphur containing amino acids methionine and cystine, they are rich sources of lysine[1] and thus a natural supplement to cereals. Traditional pulses grown in India are unable to meet the pulse requirement of the country and new sources are being exploited by Agricultural Universities and Research Institutes for cultivation in plains. Among new crops, rice-bean is a minor crop and is found growing wild in India, Central China and Malaysia. It possesses a broad range of genetic variability and can be grown in a wide range of agroclimatic condition in tropics and subtropics[2], but is confined to local areas and does not play a role in the cropping system. To encourage its production, breeding trials have also been going on in Punjab Agricultural University and a variety 'RBL-I' of rice bean has been released. In the present study, five varieties of rice-bean have been tested for their cooking quality and nutritional value and compared with other popular pulses consumed in the region viz., green gram, black gram and cowpea.

Materials and Methods

Five varieties of rice-bean ('RBL-1', 'RBL-2', 'RBL-4' 'RBL-5' and 'RMG-1') and one each of green gram *Vigna radiata* ('ML-267'), black gram (*Vigna mungo*) ('UG-218') and cowpea (*Vigna sinensis*) ('CL-1') were procured from the Department of Plant Breeding, Punjab Agricultural University, Ludhiana. To test the cooking quality of pulses, 5 g of sample was put in a test tube with excess of water and kept in boiling water till done. Grains were tested for doneness in between two glass slides and cooking time noted. The grains were then separated from excess of water and put in between the layers of filter paper to soak excess of water. These grains were then weighed and per cent water absorption calculated. Excess water left behind after removing the grains were put in preweighed crucibles and oven-dried at 110 ± 1°C and weight of the residue noted. Per cent solid dispersion was calculated as follows:

$$\text{Solid dispersion (per cent)} = \frac{\text{Wt of the residue}}{\text{Wt. of the sample cooled}} \times 100$$

For sensory evaluation of pulses, 50 g of each was cooked in a pressure cooker for 15 min in triplicate. Sensory evaluation was done by seven panelists for appearance, taste, texture, doneness and overall acceptability. The mean scores were calculated for each of the tested parameters. The results were analysed by analysis of variance for individual parameters.

For chemical analysis, the whole samples were finely ground to 100 mesh, defatted, dried and analysed in duplicate. Protein was estimated using micro-kjeldahl method[3]. Sulphur was estimated after wet digestion using gravimetric method[4]. Cystine and methionine were estimated using colorimetric methods of Liddle and Saville[5] and McCarthy and Sullivan[6], respectively. Lysine was estimated according to Hocquellet[7]. Total phenols were estimated using the method of Swan and Hillis[8]. Calcium was estimated by the titrimetric method of AOAC[3] and iron by atomic absorption spectrophotometry after triple acid digestion[9]. Phytic phosphorus was estimated using the method of McCance and Widdowson[10] as modified by Snook[11]. *In vitro* digestibility of proteins was determined by the method of Akeson and Stahman[12].

Proteins were extracted by the Osborne solubility method as modified by Nagy *et al*[13], with four solvents successively and Kjeldahl N in the extract was multiplied by 6.25. All the samples were analysed in

duplicate and average values were recorded. The level of significance was tested using analysis of variance. Coefficients of correlation (r) were worked out between total protein and total sulphur and total sulphur and sulphur containing amino acids and compared to Table values to test their significance.

Results and Discussion

The results in Table 1 indicate that cooking time for ricebean varieties was significantly higher than that of green gram ('ML-267') but comparable to black gram ('UG-218'). Cooking times of 60 min for kidney beans[14] and between 27-49 min for green gram and 50-74 min for black gram have been reported[15]. Water absorption and per cent solid dispersion were selected as criteria of cooking quality because higher the values for these parameters better is the liking for the cooked pulse. Personal observations have shown that separate grains without solid dispersion are generally not preferred by the consumer. Water absorption during cooking was more for ricebean varieties (94-107 per cent) compared to green gram and black gram probably due to more time taken for cooking. The values were within the range of water uptake for red gram varieties (52.0-150 per cent) reported by Narasimha and Desikachar[16]. The values of water uptake for green gram and blackgram were slightly lower than those reported by Vimala and Pushpamma[15] probably due to varietal and ecological differences. Narasimha and Desikachar[16] have reported wide range of water uptake in different varieties of red gram. The solids dispersed in the medium during cooking were significantly higher than for green gram but values for ricebean were comparable to cowpea and black gram.

Sensory evaluation of cooked pulses (Table 2) showed that overall acceptability of whole ricebean was comparable to that of green gram, black gram and cowpea and the differences among various pulse varieties were non-significant. The results further indicated that in terms of appearance, ricebean variety 'RMG-1' scored significantly higher ($P<0.01$) than mashbean and cowpea but was comparable to green gram. In appearance, all other ricebean varieties were comparable to the commonly consumed pulses. In terms of texture, all ricebean varieties scored significantly higher ($P<0.01$) than cowpea. In addition, ricebean varieties 'RBL-5' and 'RMG-1' also had higher score for texture ($P<0.01$) than green gram and 'RMG-1' also had higher score for texture ($P<0.01$) than green gram and 'RMG-1' scored higher ($P < 0.01$) than black gram. In terms of taste, all pulses were comparable to one another except for 'RBL-5' and 'RMG-1' which scored higher ($P<0.01$) than cowpea. Thus, it may be concluded that in sensory characters, ricebean is comparable to green gram, black gram and cowpea.

TABLE 1 COOKING CHARACTERISTICS OF RICE BEAN, GREEN GRAM, BLACK GRAM AND COW PEA VARIETIES.

Variety	100 kernel wt. (g)	Cooking time (min)	Water absorption (%)	Solid dispersion (%)
RBL-1	4.70 ±0.3	53 ±2.1	107 ±13	6.2 ±0.3
RBL-2	4.78 ± 0.2	52 ± 3.4	106 ± 17	4.2 ± 0.3
RBL-4	4.66 ± 0.2	55± 2.9	94 ± 10	8.4 ±0.4
RBL-5	4.55 ± 0.4	54 ± 4.1	100 ± 12	5.8 ± 0.4
RMG-1	4.70 ± 0.3	55 ± 1.9	104 ± 11	6.6 ± 0.3
ML-267	2.98 ±0.4	41 ± 3.2	85 ± 9	11.3 ± 0.5
UG-218	4.40 ± 0.2	51 ± 3.3	86 ± 8	6.4 ±0.3
CL-1	3.90 ± 0.2	49 ± 2.4	93 ± 10	6.1 ± 0.4

Mean of 6 samples ± SD

TABLE 2. SENSORY EVALUATION OF RICEBEAN, GREEN GRAM, BLACK GRAM AND COW PEA VARIETIES

Variety	Appearance	Texture	Taste	Flavour	Overall acceptability
RBL-1	6.0	6.0	6.1	6.1	6.1
RBL-2	6.0	6.0	6.0	6.0	6.0
RBL-4	6.1	6.1	6.0	6.0	6.0
RBL-5	6.5	6.4	6.5	6.5	6.5
RMG-1	7.0	7.0	6.4	6.4	6.4
ML-267	6.5	5.6	6.0	6.0	6.0
UG-218	6.0	6.0	6.0	6.0	6.0
CL-1	6.0	5.0	5.0	6.0	6.0
F-ratio	104.63**	11.14**	5.76**	0.82	0.82
CD at 1%	0.81	0.66	1.90		

**Significant at 1 % level; others not significant

Key to score: Strongly favourable 7: favourable 6; mildly favourable 5; undecided 4; mildly unfavourable 3; unfavourable 2; strongly unfavourable 1

Protein content of the rice-bean varieties was about 20 per cent and was comparable to green gram but significantly lower than black gram (23.62) and cowpea (24.06) (Table 3). A similar protein value for ricebean strains has been reported by Jha *et al*[2]. In another study, Singh *et al*[17]. have reported a range of 17.81-22.00 per cent except for one strain 'GRRS-S' with a protein content of 25.12 per cent. The data on protein fractions indicate that albumin in ricebean varieties ranged from 8.2-10.3 per cent and was comparable to other pulses. Globulins formed the predominant protein fraction and prolamins were the lowest. Similar values for protein fractions have been reported by Singh *et al*[17]

The data in Table 4 show that total sulphur (S) content for rice-bean varieties was slightly higher than the other pulses. The S content of the pulses was found to be negatively correlated to protein content ($r=0.78$, $P< 0.05$). The cystine content of ricebean

TABLE 3. PROTEIN FRACTIONS OF RICEBEAN, GREEN GRAM, BLACK GRAM AND COW PEA VARIETIES

Variety	Total protein (%)	Albumin (%)*	Globulin (%)*	Prolamin (%)*	Glutelin (%)*	Residue (%)*	Recovery (%)
RBL-1	20.82	9.83	44.41	3.65	7.57	18.74	84.30
RBL-2	19.42	10.30	46.06	2.75	6.45	19.62	85.18
RBL-4	20.91	8.20	50.25	2.65	6.37	18.34	86.21
RBL-5	19.51	9.35	48.62	1.65	6.37	22.88	88.87
RMG-1	20.12	9.00	47.04	1.65	6.45	21.24	85.38
ML-267	20.12	11.60	44.58	2.10	6.45	20.74	85.47
UG-218	23.36	8.75	49.17	2.75	6.21	18.24	85.12
CL-1	24.06	11.00	48.50	1.70	7.73	18.50	87.43

* % of total protein.

TABLE 4. LYSINE AND SULPHUR AMINO ACIDS IN RICE BEAN, GREEN GRAM, BLACK GRAM AND COWPEA VARIETIES

Variety	Sulphur (%)	Lysine (g/16 gN)	Cystine (g/16gN)	Methionine (g/16gN)	Total S-amino acids (g/16gN)
RBL-1	0.12	7.34	0.89	1.15	2.04
RBL-2	0.14	7.04	1.31	1.24	2.55
RBL-4	0.12	6.88	0.94	1.20	2.14
RBL-5	0.13	6.88	1.13	1.09	2.22
RMG-1	0.13	6.72	1.23	1.19	2.42
ML-267	0.12	6.88	0.97	1.18	2.15
UG-218	0.12	6.40	0.80	1.24	2.04
CL-1	0.11	7.68	0.94	1.08	2.02
FAO/WHO (1973)	–	5.40	–	–	3.50

TABLE 5. PHYTIN PHOSPHORUS, PHENOLS, CALCIUM AND IRON CONTENT OF PULSES

Variety	Phytin P (%)	Total phenols (%)	Calcium (%)	Iron (mg %)	Protein digestibility (%)*
RBL-1	0.11	0.23	0.36	5.0	83.86
RBL-2	0.14	0.19	0.47	5.7	84.45
RBL-4	0.10	0.14	0.37	4.0	85.56
RBL-5	0.12	0.11	0.37	7.1	87.08
RMG-1	0.13	0.16	0.46	5.9	90.30
ML-267	0.18	0.10	0.20	6.6	72.47
UG-218	0.17	0.10	0.20	7.4	88.53
CL-1	0.14	0.17	0.20	7.3	91.65

*In vitro

varieties ranged between 0.89 and 1.31 g/16 gN and was comparable to green gram and cowpea but ranged between 0.89 and 1.31 g/16 gN and was comparable to green gram and cowpea but significantly higher than that of black gram (Table 4). The present values for cystine corresponded to the values (0.95-1.43 g/16 gN) reported by Singh *et al*[17] but higher than that of Jha *et al*[2]. The cystine content of the pulses was found to be positively correlated to total S content ($r=0.91$, $P<0.01$). The methionine content of ricebean varieties ranged between 1.09 and 1.24 g/16 gN. The values were higher than those of cowpea but comparable to those of green gram and black gram. Singh *et al*[17]. have reported a range of 0.51-1.18 g/16 gN whereas, Jha *et al*[2]. have reported an average value of 1.22 g/16.gN. The methionine content of the pulses was found to be non-significantly related to total S content of the pulses ($r=0.41$). The total S amino acid content of ricebean ranged between 2.04 and 2.55 g/16gN and was higher than that of black gram and cowpea. A highly significant correlation was observed between total S content and S-amino acids ($r=0.94$, $P<0.001$). The total S-amino acid content of all the ricebean varieties was, however, lower than the value of 3.5 g/16 gN of the provisional scoring pattern of FAO/WHO[18].

Lysine content of all the ricebean varieties was higher than that of black gram and only in one variety it was lower than green gram. However, lysine content of all the ricebean varieties was low compared to cowpea. Singh *et al*[17] have reported a range of lysine from 5.60-5.73g/16gN for ricebean. The lysine content of all the ricebean varieties was found to be much higher than the value of FAO/WHO provisional pattern of amino acids[18].

The data regarding calcium and iron content of different legume varieties are given in Table 5. Calcium content of all the ricebean varieties was found to be higher than the other three pulses but iron content was lower. A high calcium content of ricebean was also reported by Singh *et al*[19]. Phytin phosphorus of ricebean varieties was lower than in the other three legumes whereas total phenolic content was higher than green gram and black gram but comparable to cowpea (Table 5). *In vitro* protein digestibility in ricebean varieties ranged between 83.86 and 90.30 per cent which was higher than green gram and comparable to black gram and cowpea.

Thus, the nutritional and cooking quality of ricebean is comparable to black gram, green gram and cowpea. In addition to its high calcium content, it is well adapted to different soil types, fairly tolerant to

drought and highly resistant to pests and diseases [2]. Therefore, the crop has a good scope to be sown in different parts of India to fulfil the needs of the population especially in Northern India where people are well adapted to consume whole pulses.

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References

1. *Legumes in Human Nutrition*, FAO Nutritional Studies No. 19, 1964, FAO, Italy.
2. Jha, P.K., Nair, S. and Kumar, R.S.: Protein rich crops for tropics. *Sci. Rep.* 1984, **21**, 390.
3. *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington 1980, 13th Edn.
4. Raghuramulu, N., Madhavan Nair, K. and Kalyanasundaram, S., *A Manual of Laboratory Techniques*, National Institute of Nutrition, ICMR, Hyderabad, India, 1981, 148.
5. Liddle, H.F. and Saville, V., Colorimetric determination of cystine. *Analyst*, Lond., 1959, **84**, 188.
6. McCarthy, T.E. and Sullivan, M.X., A new and highly specific colorimetric test for methionine. *J. biol. Chem.*, 1941, **141**, 1871.
7. Hocquellet, P., Determination of lysine in foods. *Ann. Fals. Chim.* 1968, **61**, 155.
8. Swain, T. and Hillis, W.E., The phenolic constituents of *Prunus domestica*. 1. The quantitative analysis of phenolic constituents. *J. Sci. Fd. Agric.*, 1959, **10**, 63.
9. Piper, C.S., Soil and Plant Analysis. Interscience publication Inc., New York 1950, 212.
10. McCance, R.A. and Widdowson, E.M., Phytin in human nutrition. *Biochem.J.* 1935, **29**, 2694.
11. Snook, L.C., Cereal grains a source of nutritionally useful phosphorus.*emp. J. exp. Agric.*, 1938, **6**,20.
12. Akeson, W.E. and Stahman, M.A., A pepsin-pancreatin digest index of protein quality evaluation. *J. Nutr.*, 1964, **83**, 257.
13. Nagy, D., Weildein, W. and Hixon, R.M., Factors affecting the solubility of corn proteins. *Cereal Chem.*, 1941, **18**, 514.
14. Bueno, E.C, Narasimha, H.V. and Desikachar, H.S.R., Studies on the improvement of cooking quality of kidney beans (*Phaseolus vulgaris*). *J. Fd. Sci. Technol.* 1980, **17**, 235.
15. Vimala, V. and Pushpamma, P., Storage changes in cookability of pulse from three regions of Andhra Pradesh. *J. Fd. Sci. Technical.*, 1987, **24**, 155.
16. Narasimha, H.V. and Desikachar, H.S.R., Objective methods for studying cookability of tur pulse (*Cajanus cajan*) and factors affecting varietal differences in cooking. *J. Fd. Sci. Technol.*, 1978, **15**, 47.
17. Singh, S.P., Misra, B.K., Sikka, K.C., Chandel, K.P.S. and Pant, K.C. Studies on some nutritional aspects of ricebean (*Vigna umbelleta*) *J. Fd.Sci. Technol.*, 1985, **22**, 180.
18. Ad hoc Expert Committee. *Energy and Protein Requirements* WHO, Technical Report Series No. 522, 1973 FAO Nutrition Meetings Report Series No. 52, 1973, WHO, Geneva, FAO, Rome.
19. Singh, S.P., Misra, B.K., Chandel, K.P.S. and Pant, K.C., Major food constituents of ricebean (*Vigna umbelleta*) *J. Fd. Sci. Technol.*, 1980, **17**, 238.

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Studies on Canned Strained Baby Foods Based on Vegetables. I. Carrots

P.V. MRUDULA KALPALATHIKA, A.M. NANJUNDASWAMY AND M. V. PATWARDHAN
Central Food Technological Research Institute, Mysore-570013, India.

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A process for the preparation of canned strained carrot baby food (CBF) was developed. Thermal process time calculations indicated that processing for 59 min at 115.5°C was necessary to achieve commercial sterility. The canned product retained the original colour and flavour. Thermal processing caused a loss in ascorbic acid (17.5%), thiamine (69%), arginine (26.6%) and lysine (18.7%). CBF was found to be as effective as milk in promoting growth in young weaning rats.

Strained baby foods based on fruits and vegetables are significantly helpful in promoting the health of infants. They are natural supplements of vitamins and minerals, which are essential for promoting growth, generation of RBC and retention of nitrogen. A judicious combination of vegetable and fruit based baby foods would therefore, make an infant's diet nutritionally complete.

Methodologies for the preparation of various fruit and vegetable based baby foods have been developed and studied, specially with apples, pumpkin, peas and carrots by earlier workers[1-4]. The technology of preparation of baby foods does not differ basically from that of canned foods. Studies on the proximate composition of baby foods made with mango, banana, apple, carrots, peas, etc. indicated them to be good sources of proteins minerals and vitamins[5-8]. But, thermal processing of these foods have been found to lower certain amino acids, specially lysine and certain vitamins like ascorbic acid and thiamine[9-12]. Nutritive value of baby foods evaluated by rat bio-assay methods was found to be satisfactory[13,14].

Carrot is a natural supplement of vitamins and minerals with sweet taste and good aroma which, if processed, would make a good supplementary food for infants. Investigations were, therefore, carried out (i) to standardize conditions for preparation of strained carrot puree; (ii) to evolve thermal process schedule for canned strained carrot puree; (iii) to study the nutritional profile of the canned strained carrot baby food; and (iv) to evaluate its nutritional quality by rat bio-assay method.

Materials and Methods

Tender and succulent carrots were procured from the local horticultural society and used in all the experiments.

Preparation of strained carrot puree involved the following steps.

Peeling: Carrots were peeled mechanically in an abrasion peeler under continuous spray of water and sliced in a Stephan universal machine with a slicer attachment.

Carrots were peeled by dipping in boiling lye (1 per cent sodium hydroxide) solution for 2 min and washed thoroughly, dipped in citric acid (0.5 per cent) solution for 4 min which was found to be optimum for complete neutralization of surface residual alkali and washed thoroughly to remove traces of acid. Lye peeled carrots were sliced as described before.

Steaming: Carrot slices were cooked by steaming under pressure at 10 psig and at atmospheric pressure. Cooked carrot slices were then blended in a Stephan universal machine with a blender attachment.

Straining: The carrot puree was passed through a pulper fitted with a 60 mesh stainless steel sieve to remove coarse fibre.

Following thermal process evaluation studies were done.

Heat penetration studies: Heat penetration rate into cans of 8 oz capacity (301 × 206 size) filled with hot strained carrot puree (85°C) was measured using an Ecklund-non-projecting plug-in needle type thermocouple. Lead wires from the thermocouples were connected through a selector switch to a manually operated Leeds and Northrup potentiometer. The temperature during heating and cooling was recorded every two min. Four cans were used for each run and three runs were made.

Process time calculations: Process time for strained

carrot puree was evolved by the general method[15] to achieve the classical F_o values of 3 to 5 recommended for baby foods[16,17]. The values thus obtained were cross checked with those obtained by the formula method of Ball[18].

Inoculated pack studies: The validity of the process time calculated to achieve commercial sterility was cross checked by inoculated pack studies as described in the NCA manual[15].

Physical methods: Reflectance colour was measured by the weighted ordinate method between 415 and 685 nm at an interval of 30 nm in a Bausch and Lomb Spectronic 20 spectrophotometer with reflectance attachment[17,19]. Vacuum in cans was determined by using a vacuum gauge[17]. Gross weight of the product was obtained by weighing the cans before opening. Particle size of the puree was determined by passing a known amount of the puree over a standard sieve (250 mμ mesh) for a known time. Weight of the sieved puree was used to calculate per cent retention of puree on the sieve.

Chemical methods: Moisture, protein (N × 6.25, micro-Kjeldahl method) titratable acidity, total ash, crude fibre, ether extractives and starch (acid hydrolysis method) were estimated by the AOAC methods[19]. Reducing and total sugars were estimated according to the modified Somogyi method[20]. Minerals like calcium, iron, magnesium, manganese, sodium, potassium, copper, zinc and lead were analysed by atomic absorption spectrophotometry using instrumentation laboratory as/ae spectrophotometer, 751 model. Phosphorus was estimated by Fiske and Subba Rao method[21]. Tin was estimated as described by Ranganna[18].

Total carotene, β-carotene, ascorbic acid and thiamine were analysed as described under AVC methods[22]. The amino acid composition was determined in a LKB α-amino acid analyser equipped with a programmer and integrator. Sample for analysis was prepared according to the procedure of Moore and Stein[23]. Non-enzymatic browning was expressed as the absorbance of alcoholic extracts (60 per cent) of strained and canned carrot puree measured at 420 nm.

Peroxidase activity in cooked carrot slices was determined as described by Ranganna[17] in which pronounced reddish brown colouration and scattered brown markings were considered as positive tests.

Microbial counts: Total bacterial, *E. coli*, and yeast and mould counts were done following standard methods[18,24].

Supplementary value: Supplementary value of canned strained carrot baby food was determined using 28 day old weaning rats (10 rats in each group) weighing between 35 and 40 g, distributed in a completely randomized block design equally according to sex and body weight and housed individually in cages. The control diet with 10 per cent protein level consisted of a commercial infant milk food (45.5 g) corn starch (44.5 g) and sugar (10 g), cooked with 5.5 times water to gelatinize the starch. The experimental diet contained canned strained carrot baby food at 20 per cent supplementation level (on dry weight basis) to the milk diet. Rats were fed *ad libitum* with food and water for a period of 8 weeks. Daily food records and weekly weight records were maintained. After the expiry of the experimental period rats were sacrificed. Haemoglobin content and RBC count were determined on blood drawn from incision in the heart[25,26]. Livers of the rats after removal were wiped free of blood and weighed. The data were analysed statistically by student's 't' test.

Results and Discussion

Peeling: Mechanical and lye peeling techniques were evaluated for peeling of carrots. Peeling loss was found to be comparatively less in lye peeling (14.0 per cent) but the puree obtained by lye peeling was not only coarse in texture but also sour to taste with a pH value of 4.6. This might be due to absorption of citric acid during lye peeling process. In contrast, the puree obtained by abrasion peeling was smooth with the characteristic carrot taste and flavour with a pH of 5.9. However, loss was comparatively high (21.5 per cent).

Steaming: Initial cooking of carrot slices was essential to soften the vegetable tissues to facilitate easy pureeing. Carrot slices were subjected to steaming (atmospheric as well as pressure) for various periods and the effect on peroxidase inactivation, retention of carotene, texture and pureeing characteristics were studied (Table 1). During atmospheric steaming of carrot slices, a negative test for peroxidase was obtained after 12 min of steaming. A negligible decrease in the per cent retention of total carotene was observed with an increase in steaming time. The percentage of coarse pulp that retained on the sieve decreased with an increase in steaming time, thus resulting in better yield of smooth puree. Despite obtaining a negative test for peroxidase activity at 5 min of pressure steaming, the retention of total carotene was found to be poor, which might be due to the heat damage caused to carotenoids during the high pressure steaming operation. Loss of total carotene also affected the overall colour of the puree remarkably.

Based on these observations, abrasion peeling and atmospheric steaming of carrot slices for 15 min were considered to be optimum for the preparation of puree for strained carrot baby food.

TABLE 1. EFFECT OF STEAM PRESSURE AND TIME ON THE INACTIVATION OF PEROXIDASE ACTIVITY, CAROTENE, RETENTION, TEXTURE AND YIELD OF STRAINED CARROT PUREE.

Time (min)	Peroxidase activity	Carotene retention (%)	Texture of slices	Retention of coarse pulp (%)*
Atmospheric steaming				
5	+ve	99	undercooked	–
10	+ve	99	slightly undercooked	66
12	–ve	98	Cooked	60
15	–ve	95	Soft	20
20	–ve	92	Soft	15
Pressure steaming (10 psig)				
5	–ve	53	Soft	30
7	–ve	51	Soft	18
10	–ve	44	Soft	14
12	–ve	36	Mushy	10
15	–ve	27	Mushy	10

*When sieved on 250 mμ sieve

Thermal process schedule for strained carrot puree: The thermal process time calculated by both the graphical and formula method indicated that carrot puree packed hot (85°C) in 301 × 206 cans and retorted at 115.5°C required a processing time of 59 min to ensure F_0 value of 4. Process time of 55 and 63 min were found to be necessary to achieve F_0 values of 3 and 5, respectively. Inoculated pack studies confirmed the commercial sterility of the calculated process time as growth of the test organism was absent in the sub-cultures made from processed carrot puree.

Hereafter, the canned strained carrot puree should be referred to as canned strained carrot baby food (CBF).

Product profile of CBF during processing stages: The product profile and cut out analysis of CBF are shown in Table 2. The CBF was found to be free flowing in nature with smooth consistency, bright yellow colour and characteristic carrot flavour.

The reflectance colour data (Table 3) indicated that despite a decrease in the brightness of colour (Y percent) in the heat processed carrot puree, the colour of the fresh carrots, strained puree and CBF remained in the yellowish orange region of the chromaticity diagram. The colour absorbance of both the strained puree and CBF did not differ much indicating that heat processing of the puree did not lead to significant browning.

TABLE 2. PRODUCT PROFILE OF CANNED STRAINED CARROT BABY FOOD (CBF) AT VARIOUS STAGES OF PREPARATION

Particulars	Strained puree*	CBF**
pH	5.8	5.6
Vacuum	–	12.0
Gross wt (g)	–	240.0
Particle size (mμ)	250.0	250.0

*Puree is free flowing, smooth textured, deep yellowish orange with carrot aroma and sweet taste.

**Puree is free flowing, smooth textured, bright yellowish orange with carrot aroma and sweet taste.

TABLE 3. REFLECTANCE COLOUR DATA OF CBF AT VARIOUS STAGES OF PREPARATION

Particulars	Reflectance colour data			
	x	y	Y%	Dnm
Carrot fresh	0.3661	0.3578	36.61	583.0
Strained puree	0.3635	0.3534	32.47	584.2
CBF	0.3617	0.3517	31.24	584.2

The total count of CBF at the end of 72 hr of incubation was found to be 40,000/g, as against the Indian Standards Institute specification of 50,000/g The *E. coli* and yeasts and mould counts were found to be nil. Thus, CBF was microbiologically safe.

Nutrient composition of CBF during processing stages: The proximate constituents of fresh carrots, strained puree and CBF are presented in Table 4. Of the proximate constituents, total ash and acidity decreased during pureeing and thermal processing. Both pureeing and thermal processing caused a marginal decrease in the mineral constituents present in fresh carrots. Considerable loss of vitamins was also observed during the processing of carrots. Thermal processing had destroyed ascorbic acid (15 per cent) and thiamine (65 per cent) similar to the observations made by earlier workers[10,12]. However, β-carotene was found to be stable during pureeing and thermal processing unlike the other vitamins.

Thermal processing led to the loss of arginine (26.6 per cent) and lysine (13.7 per cent), an observation similar to those made by Golenkova[92].

Supplementary value: Data on the effect of supplementing CBF (at 20 per cent level) to milk diet, are presented in Table 5. At the end of feeding for 4

TABLE 4. EFFECT OF PROCESSING ON THE PROXIMATE COMPOSITION OF CBF AT VARIOUS STAGES OF PREPARATION

Constituents[a] (g/100 g DM)	Fresh carrot	Strained puree	CBF
Protein (N × 6.25)	10.80	9.36	9.36
Ether extractives	1.32	1.08	1.06
Total ash	10.47	8.24	8.14
Ash soluble in HCl	8.81	6.79	6.44
Titratable acidity (as anhy. citric acid)	0.70	0.68	0.44
Sugar (total)	54.86	57.93	58.29
Sugar (reducing)	26.07	28.87	28.64
Starch	7.33	8.18	8.19
Crude fibre	7.99	6.18	6.14

a: Each value is the mean of triplicate analysis

DM: Dry matter

TABLE 5. EFFECT OF SUPPLEMENTING MILK DIET WITH CBF AT 20% LEVEL ON THE GROWTH, HAEMOGLOBIN CONTENT, RBC COUNT AND LIVER WEIGHT OF WEANING RATS

Particulars[a]	Control	Experimental	S.E.M.
Initial Wt (g)	39.80	39.80	
Daily food intake (g)	12.50	11.00	
Body wt gain in 4 wk	69.50	72.70	± 0.55
Body wt. gain in 8 wk	138.80	129.20	± 0.58
Haemoglobin (g/dl)	15.42	16.19	± 1.71
RBC count (mil/dl)	7.37	6.63	± 1.36
Liver wt (g)	6.90	7.05	± 0.75

a: Each value is the mean of 10 rats
S.E.M. at 18 d.f.
Values for experimental are not significantly different from controls.

weeks, the rats fed with CBF gained 72.7 g weight compared to that of control group (69.5 g); after feeding for 8 weeks, the experimental group gained 129.2 g weight, while the control group gained 138.8 g weight. However, the low weight gain in the experimental group after 8 weeks was not significantly different from that of the control group. Thermal processing of strained carrot puree was found to affect the biological value irrespective of the amino acid pattern, similar to the observations made by Ponomarenko *et al*[28]. The growth trend in the control and experimental groups was almost similar. Liver weight, haemoglobin content and RBC count of both control and experimental groups were comparable.

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References

1. Mrozewski, S., and Nowakowska, T., Fruit puree baby foods. *Przemysl spozywczy*, 1970, **24**, 195. (*Fd. Sci. Technol. Abstr.* 1970. **2**, 11G, 385).
2. Maczynska, D., Rembowski. E., and Gorecka, H. New production technologies for baby foods based on fruit. *Prace Instytutow Laboratoriow Bradawczych Prazemyslu sponzywcgo*, 1970, **20**, 195. (*Fd. Sci. Technol. Abstr.*, 1971, **3**, 8G 276).
3. Crncevic, V., Manufacture of fruit and vegetable puree products for babies". *Hrna i. Ishrana*, 1973, **14**, 208 (*Fd. Sci. Technol. Abstr.*., 1974. 6, 7G 937).
4. Karadschov, I., Rationalized process for manufacture of canned foods for children. *Lebensmittel-Industrie.*, 1976, **23**, 447.
5. Ranganna, S., *Studies of Strained Baby Foods*, 1958 Associateship thesis, Central Food Technological Research Institute, Mysore, India.
6. Siddappa, G.S. and Ranganna. S., 'Strained baby foods Part-I. Proximate, mineral and vitamin composition of some canned fruit and vegetable pulps and fruit custards. *Fd. Sci.*, 1960, **10**, 29.
7. Blegan, E., Riise, E., and Reinton, R., Contents of some nutrients in Norwegian Commercial baby foods, *Tidsskrift for Hermetikindustri*, 1972, **58**, 229. (*Fd. Sci. Technol. Abstr.*, 1973, **5**: 2A 80).
8. El-Ashwah, E.T., Sedky, K.A.H., Zaki, S.A., Sedky, H.A., *Chemical Composition and Nutritional Evaluation of Canned Vegetable Baby Foods*. Research Bulletin No. 1305: Faculty of Agric. Adn. Shams Univ. 1980.
9. Golenkova, S.P., Effect of stabilization on the contents of amino acids in pumpkin puree with rice. *Trudy Vsesoyuznyi Nauchnoiesledovatel' skii Institute Konservnoi in Ovoshchesushil' noi promyshlennosti.*; 1975. No. 23, 34, (*Fd. Sci. Technol. Abstr.*, 1978, **10**, 7G 411.)
10. Gasztonyi, M. and Horvath, D., Changes in the vitamin C content of fruit containing baby foods during processing. *Elelmi zesi Ipar.*, 1979, **33**, 191.
11. Harvey, T.C., Can J. R. and Margart, T.H., Papaya puree and concentrate: Changes in ascorbic acid, carotene and sensory qualities during processing. *J. Fd. Sci.*, 1975, **40**, 701.
12. Rakowska, M., Rutkowska, U., Szkilladziowa, W., Nadolna., I., Suckewer, A. and Trzebska-Jeske, I., Nutritive value of commercial infant foods of the Bobo-vita and Bobo-fruit types, *Roczniki Panst Wowego Zakladu Higieny.*, 1974, **25**, 329.
13. Baardseth, P., Protein quality in Norwegian commercial baby foods. *Tidssikrift for Hermetikindustri.*, 1972, **58**, 267. (*Fd. Sci. Technol. Abstr.*, 1973, **5**, 2G 81)
14. Khovaeva, L.A., Markh, Z.A., Leichenko, L.I., Afana's seva, N.A. and Evstatrova, N.V., Nutritional value of meat cereal canned foods with added vegetables, herbs and vitamins intended for infant nutrition. *Gigiene i Sanitariya.*, **11**, 18. (*Fd. Sci. Technol. Abstr.*, 1983, **15**, 10G, 745).
15. National Canner's Association Laboratory Manuel for Food Canners and Processors, The AVI Publishing Co., West Port., 3rd ed. 1968, Vol. 1.
16. Felmingham, J.D., Canning practice - process evaluation and conditions, *Fd Manuf.*, 1967, **42**, 23.
17. Ranganna, S., *Manual of Analysis of Fruits and Vegetable Products*, Tata McGraw Hill Book Co, New Delhi, 1977.
18. Ball, C.O., in *An Introduction of Thermal Processing of Foods* by Goldblith, S.A., Joslyn, M.A. and Nickerson, J.T.R., (Eds) The AVI Publishing Co., West Port, 1961.
19. Association of Official Analytical Chemists., *Official Methods of Analysis*, William Horwitz., (Ed) Washington D.C., 1975. 12th Edn.
20. Nelson, N. A photometric adoptation of the Somogyi method for the determination of glucose., *J. Biol. Chem.*, 1944, **153**, 375.
21. Fiske, C.H. and Subba Rao, Y. The colorimetric determination of phosphorus., *J. Biol. Chem.*, 1925, **66**, 375.

22. *Methods of Vitamin Assay*. The Association of Vitamin Chemists, Interscience Publishers., New York., 1966, 3rd Edn.

23. Moore, S., and Stein, W.H., Chromatographic determination of amino acids by the use of automatic recording equipment., *Methods Enzymol.*, 1963, **6**, 819.

24. *Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedure*. Difco Laboratories Inc., Detroit, I. Michigan, 1953, 9th Edn.

25. Van Kampen, E.J., and Zijlstra, W.G., Standardization of hemoglobinometry. II. The hemoglobincyanide method. *Clin. Chem. Acta*. 1961, **6**, 538.

26. Biggs, R., and MacMillan, R. L., The error of the red cell count, *J. Clin Pathol.*, 1984, **1**, 288.

27. *Specifications for Protein Rich Food Supplements for Infants and Preschool Children*. Indian Standards Institution, New Delhi, IS:7021-1973.

28. Ponomarenko, W., Jablonski, E., and Rafalski, H., Evaluation of nutritive value of infant and children's food and their contribution to the protein calorie requirements., *Frezemyl sponzywczy*., 1975, **29**, 173, (*Fd. Sci. Technol. Abstr.*, 1975, **7**, 11G,705.)

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Optimisation of Processing Parameters in the Manufacture of Paneer

S. SACHDEVA AND S. SINGH
National Dairy Research Institute, Karnal-132001, India

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As per Prevention of Food Adulteration (PFA) standards for paneer, a minimum of 5.8% fat is required in buffalo milk having 9.5% solids-not-fat (SNF). A fat: SNF ratio of 1:1.65 in milk is necessary for paneer making. High heat treatment of milk improved the solids recovery, yield, flavour and body and texture characteristics of paneer. A heat treatment upto 90°C without any holding is recommended. Coagulation of milk at 60°C resulted in paneer having a very weak and loose body unsuitable for frying. Coagulation at 70°C produced the desirable body and texture characteristics. Higher temperatures of coagulation induced greater solids recovery but lower yield due to increased moisture expulsion. For coagulation of milk at 70°C, the optimum pH was 5.30 to 5.35. The concentration of citric acid solution that resulted in the best product was 1%.

Paneer is a popular indigenous variety of soft cheese. It is obtained by the acid precipitation of milk at high temperatures. Precipitation involves the formation of large structural aggregates of proteins in which milk fat and other colloidal and soluble solids are entrained with whey. The information available on paneer manufacturing technology relates mostly to small scale production. In order to adopt this technology on a large scale, every step has to be standardized with respect to simplification of production process, recovery of solids, energy conservation and adaptability for mechanisation. In view of the rapid growth of organised dairying in India, with greater emphasis on the production of indigenous dairy products, it is essential to look into the various technological aspects of paneer manufacture.

Materials and Methods

Buffalo milk obtained from the dairy farm of the National Dairy Research Institute, Karnal was used in the present investigation. Paneer was prepared from standardized milk according to Bhattacharya *et al*[1]. Each lot was prepared from 40 l of milk. The fat content in milk and paneer whey was determined by the Gerber method[2] and the total solids by the standard gravimetric method[3]. Moisture was determined by the ISI method[4] for cheese with slight modification which involved addition of 5 ml of hot distilled water to break the curd and disperse it uniformly. The method described for determination of fat in chhana[5] using Mojonnier apparatus was used for paneer. Ash content in paneer was determined according to the method for chhana[6]. The pH values were measured at the temperature of coagulation (70°C) with the help of a portable pH meter with a glass electrode. The total nitrogen content of paneer was estimated by the semi-micro kjeldahl method of Maneffee and Overman[7]. A qualitative disc electrophoretic study was made using a Metrex Gel Electrophoretic Apparatus (100 × 5 mm gel tubes) and following the method of deJong[8] as for cheese. The samples for electrophoresis were prepared by dissolving 0.1 g of paneer in 10 ml of Tris HCl buffer of pH 8.5 in presence of 8M urea, followed by filtration to remove any suspended matter and addition of two drops of mercaptoethanol to the filtrate. Hardness was determined by a precision penetrometer (Central Ignition Co., London).

Paneer was cut into cubes of approx. $1'' \times \frac{3''}{4} \times \frac{1''}{2}$ size. Frying of paneer cubes was done in refined groundnut oil heated to a temperature of 175 - 185°C. The fried paneer was cooked in double the quantity of water with 1.5 per cent salt by boiling for 5 min. Sensory evaluation of paneer in raw, fried and cooked form was done on a 9 point hedonic scale by a selected panel of 6 judges. Four replications were done for each test.

Results and Discussion

Fat level in milk: The respective average composition of paneer obtained from buffalo milk with fat adjusted to different levels (SNF 9.5 per cent) is given in Table 1. The moisture content in paneer varied inversely with the fat level in milk. The fat content in paneer increased with the increase in fat level of milk while the protein and carbohydrate percentages decreased.

TABLE 1. EFFECT OF FAT CONTENT OF MILK ON PANEER COMPOSITION

Fat in milk (%)	Moisture (%)	Fat (%)	Protein (%)	Carbo-hydrates (%)	Ash (%)	FDM* (%)
5.5	54.6	22.2	18.9	2.5	1.9	48.7
5.8	54.1	23.5	18.2	2.4	1.8	51.2
6.0	53.2	24.9	17.9	2.3	1.7	53.4

*Fat and dry matter basis
Each value is the mean of four replicates

To meet the PFA standards for paneer[9] which require a minimum of 50 per cent fat on dry matter basis, a minimum of 5.8 per cent fat in buffalo milk having 9.5 per cent SNF is essential. A fat: SNF ratio of 1:1.65 has to be maintained. Any value higher than 5.8 would result in unnecessary economic loss to the paneer trade. Higher fat content in milk results in lower moisture retention in paneer and therefore, a loss in terms of yield. It was also noted that paneer of good quality could never hold moisture beyond 60 per cent and thus the value of 70 per cent as the maximum limit for moisture in paneer as stipulated in PFA standards appears to be too high.

Final temperature of heating: Milk was subjected to heat treatment ranging from 70-90°C. Thereafter it was cooled to 70°C and protein was coagulated with 1 per cent hot (70°C) citric acid solution. The moisture content in paneer thus obtained ranged from 51 to 54 per cent without any definite correlation with the extent of heat treatment (Table 2). The yield and total solids recovery increased with the increase in heating temperature while solids in whey decreased. The absorption of moisture after dipping the pressed paneer in cold water was inversely proportional to the temperature of heating. Temperatures beyond 90°C caused deposition of milk solids on the surface of the vat resulting in an overall solids loss and were, therefore, not considered for this experiment.

The flavour score of paneer made from pasteurised milk was the lowest and it was criticized for raw milk type flavour which is uncharacteristic of paneer. The flavour scores increased with the increase in heat treatment. Paneer from pasteurised milk had a very weak and soft body that was particularly unsuitable for frying. The best body and texture was observed in raw paneer made from milk heated to 90°C. Fried and cooked samples behaved similarly.

The improvement in flavour at higher temperatures could be due to the heat induced changes occurring in milk resulting in a number of flavourful compounds, more particularly sulphur compounds viz. those with SH groups and hydrogen sulphide which are responsible for cooked flavour[10]. The improvement in body and texture could be due to more preponderant and intensive heat induced protein–protein interaction[11]. The increase in yield and total solids recovery may also be due to complex formation between whey proteins and casein. Thus, at higher temperatures casein acts as a scavenger for serum proteins which are otherwise lost in the whey. Muller *et al*[12] and Dzurec and Zall[13] utilized this very principle in increasing the yield of cottage cheese.

Holding time: Milk heated to 90°C in the cheese vat was held at this temperature for varying lengths of time ranging from 0 to 15 min before cooling to 70°C and coagulating with 1 per cent citric acid solution. Table 3 shows that the moisture in paneer did not bear any definite relationship with the holding time. The yield of paneer was directly correlated with the moisture content. The total solids recovery was reduced on holding for 15 min due to deposition of solids on the vat surface. The rate of moisture absorption increased with the increase in holding period. Holding time at 90°C did not appreciably alter the sensory quality of paneer.

Bhattacharya *et al*[1]. recommended a maximum heat treatment of only 82°C for 5 min which resulted in a maximum solids recovery of 60.8 per cent whereas our results show that a total solids recovery of 66.8 per cent could be effected by heating milk to 90°C without any holding.

Temperature of coagulation: Milk heated to 90°C was coagulated at different temperatures ranging from 60 to 90°C with 1 per cent citric acid solution. The moisture and yield of paneer decreased consistently with an increase in temperature of coagulation (Table 4). However, the recovery of total solids increased

TABLE 2. EFFECT OF FINAL HEATING TEMPERATURE ON YIELD AND SOLIDS RECOVERY OF PANEER

Heating temp. (°C)	Moisture (%)	Yield (%)	Total solids recovery (%)	Moisture absorption (%)	Total solids in whey (%)
72	53.9	19.5	57.9	9.1	7.9
80	51.1	20.2	64.0	8.4	6.1
85	51.4	20.7	65.0	7.2	5.9
90	52.1	21.3	66.5	7.0	5.8

Values are average of four replicates

TABLE 3. EFFECT OF HOLDING TIME AT 90°C ON YIELD AND SOLIDS RECOVERY OF PANEER

Holding time (min)	Moisture (%)	Yield (%)	Total solids recovery (%)	Moisture absorption (%)	Total solids in whey (%)
0	54.3	21.5	66.7	7.0	6.0
5	53.1	21.0	66.8	8.0	5.9
10	54.7	21.6	66.3	9.5	5.9
15	57.4	21.8	62.7	10.5	5.9

Values are average of four replicates

TABLE 4. EFFECT OF COAGULATION TEMPERATURE ON YIELD AND SOLIDS RECOVERY OF PANEER

Coagulation temp. (°C)	Moisture (%)	Yield (%)	Total solids recovery (%)	Moisture absorption (%)	Total solids in whey (%)	Penetrometer reading
60	59.5	22.8	59.9	6.2	6.2	172
70	55.1	21.7	63.0	6.8	6.0	133
80	49.9	20.1	65.3	8.9	5.9	105
90	48.8	20.0	66.3	10.2	5.8	95

Values are average of four replicates

TABLE 5. EFFECT OF pH OF COAGULATION ON YIELD AND SOLIDS RECOVERY OF PANEER

Coagulation pH	Moisture (%)	Yield (%)	Total solids recovery (%)	Total solids in whey (%)	Penetrometer reading
5.40	58 6	24.8	65.8	6.5	183
5.35	55.2	23.5	67 6	6.3	137
5.30	52 7	22.3	67.6	6.3	129
5.20	51 4	21.5	67.1	6.3	116
5.10	50 0	20.8	66.5	6.4	114

Values are average of four replicates

directly with the coagulation temperature while the solids loss in whey decreased. The moisture absorption increased with the coagulation temperature. The penetration values decreased markedly with increasing temperatures of coagulation indicating increase in hardness of paneer.

Paneer obtained on coagulation at 60°C was criticised for flat flavour while that obtained at 90°C was opined to have a slightly coarse flavour. The scores were, however, in a close range of 6.9 to 7.4. The effect of coagulation temperature was more conspicuous on the body and texture of paneer. Coagulation of milk at 60°C resulted in paneer with a very soft, loose and weak body and scored the minimum (5.9). The cubes lost their size and shape on cooking. Coagulation at 70°C produced desirable body and texture characteristics in paneer and got maximum score (7.6). Coagulation temperatures higher than 70°C resulted in hard and dry paneer. Paneer obtained on coagulation at 60°C had free moisture and thereby a lower score for appearance while the rest of the samples were similar in appearance. The quality attributes of raw paneer were reflected in the respective fried and cooked samples as well.

Fig. 1 depicts the electrophoretic pattern of proteins in paneer obtained at different coagulation temperatures in increasing order. The area under alpha-S and beta casein enlarged with higher coagulation temperatures suggesting greater complex formation between whey proteins and casein and therefore, higher solids recovery when higher

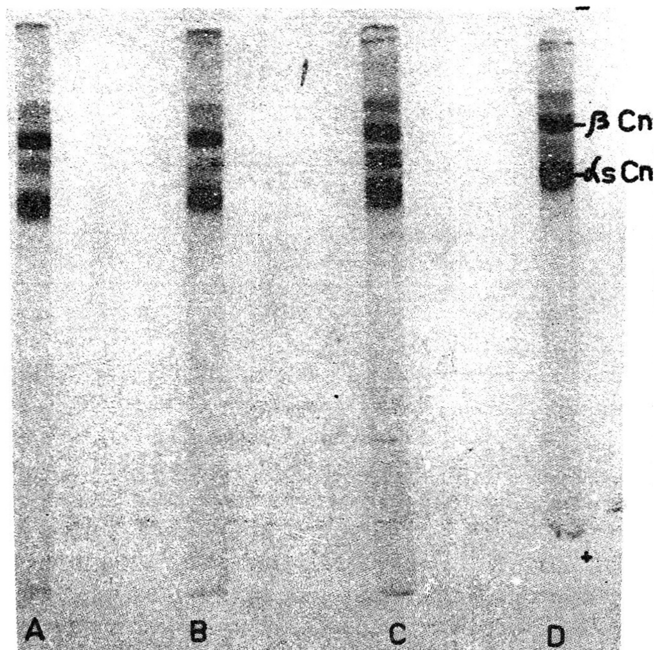

Fig. 1. Electrophoretic pattern of proteins obtained on Coagulation at different temperatures.
A. 60°C B. 70°C C. 80°C D.90°C

temperatures of coagulation were employed. It was also noted that the greater complex formation slightly reduced the electrophoretic mobility of the alpha-S and beta caseins.

pH of coagulation: The effect of pH of coagulation on the quality, yield and solids recovery of paneer is reported in Table 5. The moisture retention in paneer decreased with the fall in pH and consequently, the yield also decreased. The solids recovery was higher in the pH range of 5.30 – 5.35 and tended to decrease on either side of this pH range. Total solids lost in whey varied accordingly. The hardness of paneer measured in terms of penetration value, ranged from 183 to 114 in direct relation with the decrease in pH from 5.4 to 5.1.

The maximum sensory scores for flavour, body and texture and appearance were awarded to paneer obtained on coagulation at a pH of 5.35. Paneer obtained on coagulation at pH 5.4. was criticized for flat taste while that obtained at pH 5.1 had a coarse flavour. However, the sensory scores were not much affected. The body and texture scores were less for the two pH extremes. The product had a soft, weak and crumbly body when coagulation was terminated at pH 5.4 while it was hard when pH 5.1 was employed. Except for the paneer made on coagulation at pH 5.4, which had free moisture, all the samples were similar in appearance.

Strength of citric acid: Citric acid solution of 1 per cent concentration has been conventionally used in the manufacture of paneer[1]. There are reports mentioning the use of stronger solutions in the manufacture of

TABLE 6. EFFECT OF THE CONCENTRATION OF CITRIC ACID SOLUTION ON YIELD AND SOLIDS RECOVERY OF PANEER

Coagulant								
Concn.	pH	Titratable acidity %	Amount used (1/100) 1 milk)	Moisture (%)	Yield (%)	Total solids recovery (%)	Total solids in whey (%)	Penetrometer reading
1.0	2.5	1.5	21.0	57.2	23.9	66.1	5.8	137
2.0	2.3	2.7	14.0	53.1	21.2	64.3	6.4	106
3.0	2.2	3.9	12.0	52.1	20.5	63.5	6.8	101

Values are average of four replicates

chhana and other sott cheeses. The optimum concentration of citric acid solution required for the manufacture of paneer was ascertained using solutions of varying concentration (Table 6). The amount of citric acid solution required for coagulation decreased with increasing strength. The moisture, yield and total solids recovery in paneer varied inversely with the concentration of the solution. The penetrometer values showed an increase in hardness with increase in concentration of citric acid.

The sensory scores were maximum for paneer made with 1 per cent solution and decreased with increase in concentration. Stronger solutions resulted in paneer with slightly acidic taste and harder body. Solutions lower than 1 per cent concentration would increase the bulk of the contents posing problems in handling.

The present study has shown that to get a good quality paneer, it is necessary to standardise it to a fat: SNF ratio of 1:1.65, heat it to 90°C without any holding, coagulate at 70°C at a pH between 5.30-5.35 with 1 per cent citric acid. Optimisation of processing parameters may lend itself to mechanisation and finally automation which is an urgent need for large scale production.

References

1. Bhattacharya, D.C., Mathur, O.N., Srinivasan. M, R. and Samlik, O., Studies on the method of production and shelf life of paneer. *J. Fd. Technol.*, 1971, **8**, 117.

2. *Determination of Fat by the Gerber Method Part I: Milk*, IS: 1224-1977. Indian Standards Institution, New Delhi.

3. *Methods of Test for Dairy Industry Part II. Chemical analysis of Milk*, IS: 1479-1961. Indian Standards Institution. New Delhi.

4. *Specification for Natural Cheese (Hard Variety). Processed Cheese, Processed Cheese Spread and Soft Cheese* IS: 2785-1979. Indian Standards Institution, New Delhi.

5. Standard Methods for the Examination of Milk and Milk Products Other Than Ghee. I.C.A.R. Bulletin No. 70. 1951.

6. *Specification for Chhana*, IS: 5162-1980, Indian Standards Institution, New Delhi.

7. Meneffee, S.C., and Overman, O.R., A semi-micro Kjeldahl, method for the determination of total nitrogen in milk. *J. Dairy Sci.*, 1940, **23**, 1177.

8. deJong, L., A quantitative electrophoretic method of studying cheese ripening. *Neth. Milk Dairy J.*, 1975, **29**, 162.

9. *P.F.A. Act: Commentary on the Prevention of Food Adulteration Act.* Jain Law Agency, Chandigarh, 1976, 605.

10. Hartman G.H.J.R., Swanson, A.M. and Stahmann, M.A., Effect of pH and sulphydryl blocking agents on interaction between B-lactoglobulin and k-Casein. *J. Dairy Sci.*, 1965, **48**, 780.

11. Richert, S.H., Current milk protein manufacturing processes. *J. Dairy Sci.*, 1975, **58**, 985.

12. Muller, L. L., Hayes, J.F. and Snow, N., Studies on coprecipitates of milk proteins. Part I. Manufacture with calcium contents. *Aust. J. Dairy Technol.*, 1967, *22*, 12.

13. Dzurec, D.J., and Zall, R.R., Effect of heat treatment, cooling and storage of milk on casein and whey proteins *J. Dairy Sci.*, 1982, **65** (Suppl 1), 55.

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Studies on Differentiation of Beef from Meat of Other Species of Animals. I. Comparative Specificity and Sensitivity of Serum Raised in Buffalo Calves Against Cattle Antigens

A.K. BANSAL AND USHA V. MANDOKHOT
Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences, Haryana Agricultural University, Hisar-125 004, Haryana

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Antisera to differentiate cattle meat from that of the other species of animals were raised by injecting cattle antigen into buffalo calves and rabbits. The anti cattle buffalo (BAC) serum when tested by double immuno diffusion (DID), immunoelectrophoresis (IE) and counterimmunoelectrophoresis (CIE), reacted only with homologous cattle antigens by forming two to three precipitation lines. The anti cattle rabbit (RAC) serum could be made monospecific by absorbing with cross reacting antigens. Both the sera were found to be equally specific as they reacted only with 146 cattle but not with any of the heterologous 114 field antigens derived from buffalo, sheep, goat, pig, chicken and donkey in DID and CIE tests. The minimum possible cattle protein (antigen) that the BAC serum could detect from the adulterated meats was 396 μg/ml while the RAC serum detected 198 μg/ml in some instances. The use of BAC serum to identify cattle meat is recommended.

Cattle slaughter is not allowed under law in some of the states in India. Cattle meat is, therefore, sold at a far cheaper rate than any other meat and finds its way as an adulterant in other meats. Religious sentiments apart, adulteration of meat of one species of animal with meat of any other species is also not allowed by the Prevention of Food Adulteration Act. Of the various methods tried for differentiation of these two kinds of meats, the serological methods are more commonly used and the antibodies required for these tests are produced in phylogenetically distinct animals like rabbits. Such an antiserum reacts nonspecifically with antigens from many heterologous species, unless cross-reacting antibodies contained therein are removed by absorption with tissue proteins. Elimination of crossreacting antibodies especially of closely related species like cattle *vs* buffalo or sheep *vs*. goat by absorption is, however, not always successful and quite often the serum titre due to dilution is lowered to such a level that negative results are obtained even with the homologous antigen. Such a problem might be avoided by producing antiserum in genetically closer species. Therefore, a study was undertaken to find out the feasibility of raising antiserum in buffalo calves against cattle antigen and to compare its specificity and sensitivity with the serum raised in rabbits.

Materials and Methods

Preparation of antigens: Freeze dried (FD) skeltetal muscle extract antigens of cattle (CFD), buffalo (BFD), sheep (SFD) and goat (GFD) prepared by the procedure of Warnecke and Saffle[1] were procured from the Department of Veterinary Public Health and Epidemiology, Hisar. The dried extracts were placed in air tight containers, labelled and stored at -20°C until used for serological tests and for raising antisera. For serological tests, the FD antigens were dissolved in normal saline to obtain 50 per cent solutions from which further two fold dilutions were prepared. Field samples of muscles as also the organs from cattle, buffaloes, sheep, goats, donkeys, chicken and pigs were collected and stored at -20°C until processed for the preparation of field antigens (saline extract antigens). To prepare field antigens, 25 g sample of skeletal muscles/organs was blended with 75 ml of physiological saline (25 per cent meat extract) in a homogeniser for 15 min. The slurry was centrifuged at 5000 rpm for 15 min at room temperature and filtered through Whatman filter paper No. 4. To the filtrate, thiomersol (merthiolate) was added (final concentration of 1 in 10,000) and was stored at -20°C till used.

The protien concentration in freeze dried antigens and saline muscle extracts was estimated by the method of Lowry *et al.*[2] using a Spectronic-20 to determine the optical density at 620 nm. Bovine serum albumin (BSA) was used as standard.

Production of hyperimmune serum: The freeze-dried cattle antigen was reconstituted in physiological saline to make 75 mg/ml solution and was filtered through

Whatman filter paper No. 4, followed by 0.22 mm millipore filter to make it bacteriologically free. The filtrate was emulsified with an equal volume of either Freund's complete (FC) (Difco Laboratories, Detroit, Michigan) or incomplete (FI) adjuvant (one part of hot melted lanolin and nine parts of light liquid paraffin emulsified with a syringe) and used for immunization.

Apparently healthy rabbits of 4 to 6 months age (body wt. 1.25 to 1.5 kg) and 6 to 7 months old buffalo calves were used for raising hyperimmune sera as per the procedure of Warnecke and Saffle[1]. Presence of antibodies against FD antigens of cattle, sheep and goat in pre-immunization sera of both buffalo calves and rabbits was ruled out before immunization. The rabbits and buffalo calves were divided into two groups of three and one, respectively. To one group, all the antigen injections given were emulsified in FC adjuvant and to the other only first injection of antigen given was emulsified in FC and the rest in FI adjuvant. Rest of the procedure of immunization schedule is given in Table 1. In rabbits, one week after the last injection of immunization schedule, blood was collected at weekly intervals for 90 days and from the buffalo calves after 4 days of the 4th injection and thereafter every 4th day upto 42 days. The blood after the 6th injection was collected on every 4th day thereafter upto another 42 days. Blood sera were collected, pooled species wise and preserved with thiomersol (1:10,000). The pooled serum was distributed in 8 to 10 ml quantities, heated in a water bath at 56°C for 30 min, centrifuged at 5000 rpm and supernatant stored at –20°C till used for different serological tests.

Absorption of antisera with cross reacting antigens: Rabbit anticattle (RAC) serum was absorbed with buffalo, sheep and goat antigens to make it cattle specific, the details of which are shown in Table 2. The antigen antibody mixture was incubated at room temperature for 4 hr with repeated shaking, followed by incubation at 4°C for 12 hr after which it was centrifuged at 3000 rpm for 15 min to remove the precipitate containing cross reacting antibody antigen complexes.

Table 1. Immunnization schedule for rabbits and buffalo calves

Animal sp.	Periodicity of injections (days)	Dose/animal* mg antigen	Dose/animal* ml quantity	Injection dose/hind leg
Rabbits	0,7,14,21	150	4 ml+	2 ml
Buffalo Calves	0,2,24,44, 86,95	300	8 ml‡	4 ml

*injected intramuscularly

+2 ml antigen 2 ml adjuvant

‡4 ml antigen 4 ml adjuvant

Table 2. Absorption of RAC serum with heterologous antigens

Cross reacting antigens added (mg/ml)	Precipitation reaction in DID with antigens: Cattle	Buffalo	Sheep	Goat
None	+	+	+	+
8, BFD	+	W	+	+
8, GFD	+	W	+	–
8, SFD	+	W	W	–
4, BFD	+	–	W	–
2, SFD	+	–	–	–
6, each of BFD, SFD and GFD	+	W	W	–
8, BFD; 8, SFD; 6, GFD	+	W	W	–
10, BFD; 8, SFD; 6, GFD	+	W	–	–
12, BFD; 8, SFD; 6, GFD	+	–	–	–

W, weak precipitation reaction

Concentration of buffalo anticattle (BAC) serum: Buffalo anticattle serum (BAC) was concentrated by ammonium sulphate precipitation by following the procedure of Campbell *et al*[3]. and was preserved as in the case of RAC serum.

To ensure monospecificity, the absorbed RAC and the unconcentrated and concentrated BAC sera were tested in double immunodiffusion tests (DID) against FD and saline muscle extract (ME) antigens of cattle, buffalo, sheep and goat. The FD and ME antigens were prepared as explained earlier. Serum reacting only with the homologous antigen (cattle) but not with the heterologous antigens (buffalo, sheep and goat) was considered to be monospecific. These monospecific sera were used to identify 146 cattle field antigens comprising muscles (42), liver (18), kidney (18), spleen (18), heart (18), lung (18) blood serum (10), adrenal gland (4) and to differentiate 114 heterologous field antigens from buffalo, sheep, goat, chicken, donkey and pig. The same sera were also used to detect adulterant cattle meat in buffalo, sheep and goat. Adulteration of one species of meat with another was achieved by mixing 25 per cent saline skeletal muscle extracts of the desired species in different proportions.

Serological tests: The three serological tests employed to identify and differentiate cattle antigen from those of other species of antigens were DID[4], IE[5] and CIE[6].

Results and Discussion

Irrespective of the type of serological test used for differentiation of meats, the antibody used for detection of species specific antigen had always been produced in phylogenetically distinct animals such as rabbits[1,7-14]. Such a serum, apart from containing homologous antibodies also contained cross-reacting antibodies and required absorption with cross-reacting antigens to make it species specific. The absorption with cross-reacting antigens was not always successful[10,13,15] because either the cross-reacting antibodies were not completely removed or the serum titre was too low to give positive precipitation reaction even with the homologous antigen. To overcome these difficulties, the use of the serum raised in phylogenetically related animals was suggested[10]. In the present study, therefore, efforts were made to raise the serum against the CFD antigen in both a phylogenetically closer animal such as buffalo and in a relatively distant animal such as rabbit and to compare their suitability in identification of cattle meat, especially from buffalo and also from the meat of other species of animals like sheep, goat, pig, donkey and chicken using different serological tests like DID, CIE and IE.

The immunization in phylogenetically related animals yielded very encouraging results. The undiluted and unconcentrated BAC serum was found to be monospecific on testing by the DID test. It did not cross-react with sheep, goat, chicken, pig, donkey and even with very closely related buffalo antigens (Fig. 1). The absence of cross-reacting antibodies in the BAC serum was probably because of the generation of immune response by phylogenetically related animals to only fine antigenic differences that existed between the isoantigens, The same serum, however, after ten times concentration with

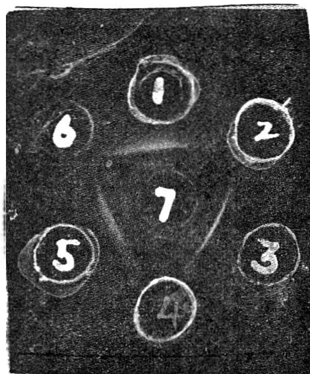
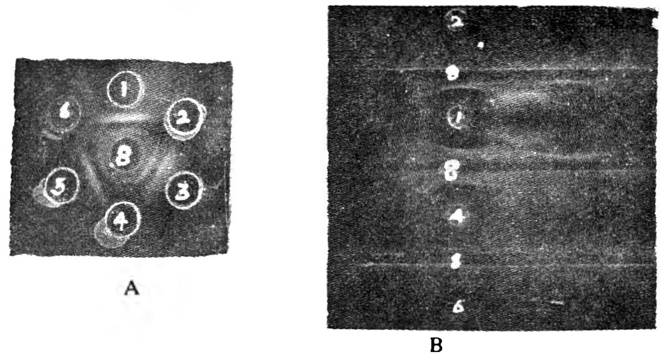


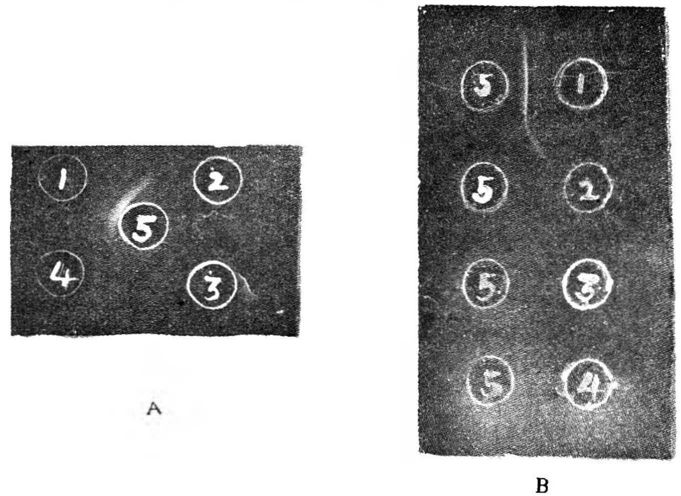
Fig. 1. Species specific precipitation reaction using undiluted and unconcentrated BAC serum in DID test

Antigens (50 mg/ml)	: 1, 3, 5, CFD, 2, BFD 4 GFT, 6, SFD
Anti serum	7. BAC (unconcentrated), 8. BAC (concentrated)



A B

Fig. 2: Non-specific precipitation reaction between concentrated BAC serum and heterologous antigens in (A) DID and (B) IE tests. Legend for numbers as under Fig. 1.



A B

C

Fig. 3. Species specific precipitation reaction between cattle antigen and BACM serum.
(A) DID test, (B) CIE test, (C) IE test.
Antigens (50 mg/ml): 1, CFD; 2, BFD; 3, GFD; 4, SFD
Antiserum: 5 BACM.

ammonium sulphate reacted, though weakly, with the sheep and the goat antigens as revealed by the formation of a faint precipitation line against GFD/GME antigens in the DID (Fig. 2A) and against GFD/GME and SFD/SME antigens in the IE tests (Fig. 2B). This cross-reaction, however, was so weak that it disappeared completely (Fig. 3A, 3B and 3C) when the serum was diluted to 1:2 with NSS or was stored at 4°C for 15 days. This serum did not produce visible precipitation reaction in the DID, IE and the CIE tests with 50, 25, 12.25, 6.125, 3.06, 1.8 and 0.9 per cent concentration of the heterologous FD and 25 per cent ME antigens of sheep, goat and buffalo. The species specificity of the produced serum was further proved when it elicited precipitation reaction with all the 146 homologous cattle field antigens but not with any of the 114 heterologous antigens tested in the DID or the CIE test. The monospecificity of the BAC serum thus produced avoided the complications associated with absorption of serum with cross-reacting antigens.

Besides the monospecificity of the BAC serum, the other advantage of raising antiserum by cross immunization was the quantum of antiserum which could be obtained from a single animal allowing for concentration of the same thereby increasing the titre. On the other hand, to obtain sufficient quantity of the antisera in rabbits, a battery of these comparatively delicate animals was required.

The weak serological cross-reaction between the sheep and goat antigens and the concentrated BAC serum (Fig. 2A and 2B) indicated that at least one antigen each of sheep and goat is common to the cattle antigen, but not to the buffalo antigen. The results further revealed that the cross-reacting antigen was either not as potent as other species specific antigens of cattle or was present in too low a concentration to elicit strong immune response in buffalo calves even after hyperimmunization with CFD antigen.

The undiluted and unconcentrated BAC serum formed 1 to 2 precipitation lines and the concentrated BAC monospecific serum (BACM) formed three lines with the CFD or CME antigens in the DID test (Fig. 1, 3A). In the IE and CIE tests, the BACM serum formed three precipitation arcs with CFD/CME antigens (Fig. 3C, 3B). These results indicate that there were probably three species specific antigens of cattle. However, all the three species specific antigens did not appear to be present in all the tissues/organs or body fluids of cattle or if present were not in equal concentrations because the BACM serum in the DID and the CIE tests reacted with cattle lung and serum antigens by forming only one and two precipitation lines, respectively. With spleen, kidney, heart, adrenal glands, liver and muscle antigens, three precipitation lines were formed. These species specific proteins (antigens) of cattle appeared to be negatively charged as all the three precipitation arcs formed in the IE test were towards the anode (Fig. 3C).

In contrast to the BAC (unconcentrated) serum, the RAC unabsorbed serum cross-reacted strongly with buffalo, sheep and goat but not with pig, chicken and donkey antigens in the DID (Fig. 4A, 4B), IE (Fig. 4C) and the CIE (Fig. 4D) tests. With the homologous CFD and the CME antigens in the DID tests, it formed at least seven precipitation lines; of these, two did not give reaction of identity with any of the heterologous cross-reacting antigens (Fig. 4A) indicating these two lines to be species specific. However, they disappeared when the dilution of the serum was more than 1:16 or when the concentration of the CFD antigen used was lowered to 25.0 mg/ml.

Bubloz[8], Ramadass[9] and Sherikar *et al.*[13] reported the differentiation of the cattle meat from that of the buffalo, goat and sheep meats on the basis of number of precipitation lines formed by using unabsorbed RAC serum in the DID test. Pandey and Pathak[10] and Reddy[15] did not observe any difference in the number of precipitation lines formed with the homologous antigen (cattle) and with the heterologous (buffalo, sheep and goat) antigens using unabsorbed RAC

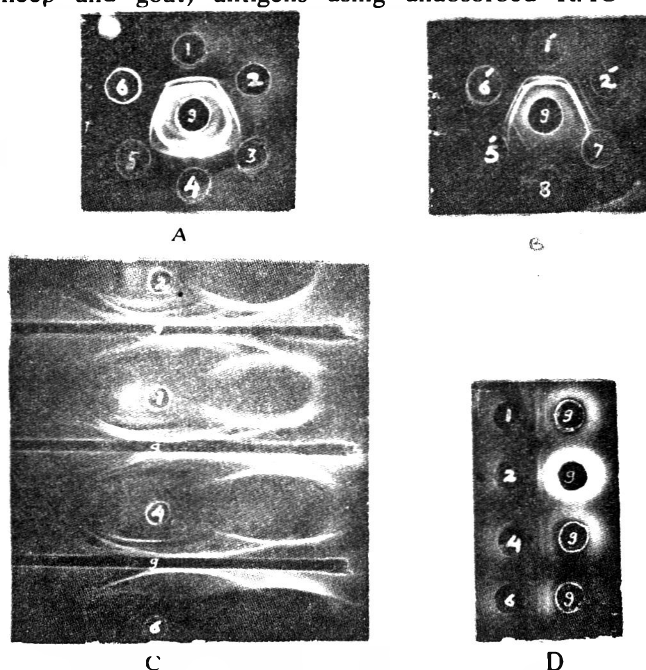


Fig. 4. Non-specific precipitation reaction between sheep; goat, buffalo antigen and unabsorbed RAC serum.
(A) DID test; (B) DID test; (C) IE test; (D) CIE test
Antigens (50 mg/ml): 1, CFD; 2, BFD; 4, GFD; 6, SFD (12.5 mg/ml) : 3,5, CFD
:1' CME; 2', BME; 5' GME, 7' DME, 8 PME.
Antiserum :9 RAC (unabsorbed)

serum in the DID test. These conflicting results might have been due to the use of low concentration of antigen or low titre serum used in DID test or due to the differences in the potency of the antigen used for immunization.

In the IE test. the unabsorbed RAC serum formed 8 precipitation arcs with homologous cattle antigen (50 mg/ml) and a few with heterologous antigens (seven with GFD and five each wtih buffalo and sheep (Fig. 4C). While in the CIE test five precipitation lines were formed with the homologous CFD/CME antigens, only four were formed with the heterologous buffalo, sheep and goat antigens. In both the tests, no precipitation reaction was observed with the chicken, pig and donkey antigens. Ramadass and Misra[14] had differentiated the meats of cattle, buffalo, sheep, goat, pig and chicken on the basis of the number and position of precipitation arcs formed in the IE test. However, in the present study, use of unabsorbed RAC serum employing the IE test to detect the meat to its origin of species was found unreliable as the number of precipitation arcs formed varied from time to time even when the same batches of CFD/CME antigen and antibody (RAC serum) were used and the other experimental conditions were kept identical.

The RAC serum was successfully absorbed and made monospecific. The absorbed serum evoked precipitation reaction only with its homologous CME and CFD antigens (Fig. 5A, 5B and 5C) and not with the BFD/BME, SFD/SME, GFD/GME, PME and DME antigens in the DID, IE and CIE tests. The species specificity of the serum was further confirmed when it reacted positively only with the 146 field cattle antigens but not with any of the 114 heterologous antigens in DID and CIE tests. Though the RAC serum could be made species specific successfully, it was observed that the quantity of the cross-reacting antigens to be used for absorption had to be decided by trial and error (Table 2). Perhaps, this is the reason why different groups of workers reported different results[1,7,10,11,13,15]

The species specific (cattle) precipitation arc in the IE test using CFD/CME as antigen and RACM serum as antibody was always formed towards the anode (Fig. 5C). These results corroborated the earlier results observed in the IE test using BACM serum, indicating that the species specific cattle antigens were negatively charged.

The sensitivity of both the sera in detecting adulterant meat in mutton and chicken by employing either the DID or the CIE test was observed to be the same as both the tests could detect adulteration upto 2.5 per cent containing 396μg/ml of cattle protein. However, the RACM serum was found to have an

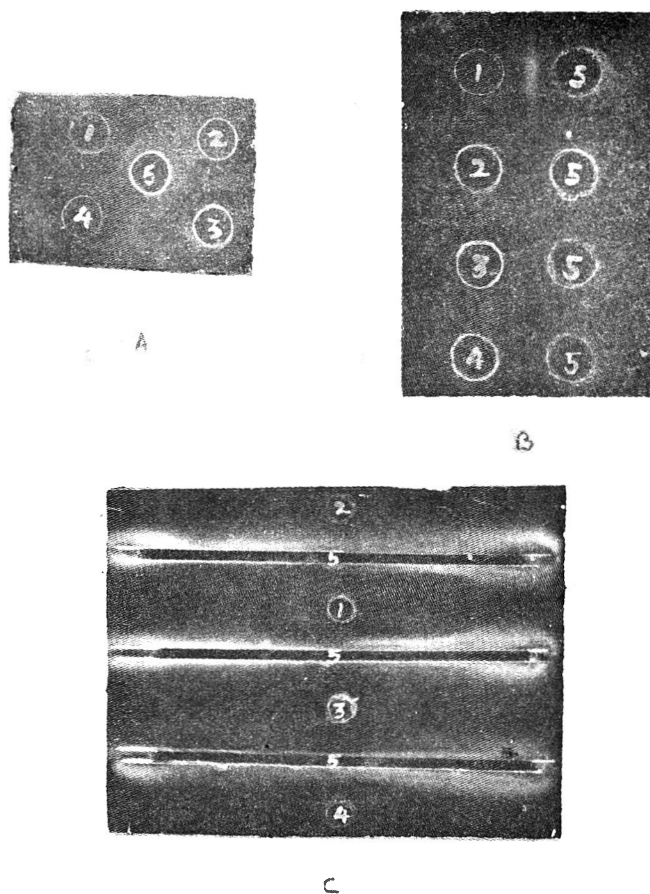


Fig 5. Species specific precipitation reaction of the absorbed RACM serum.
(A) DID test, (B) CIE test, (C) IE test,
Antigens (50 mg/ml) :1, CFD; 2, BFD; 3, GFD; 4, SFD
Antiserum :5 RACM.

edge over BACM serum in detecting adulterant cattle meat in buffalo meat since it could detect a minimum of 198μg/ml of cattle protein as compared to BACM which detected only upto 396μg/ml. The detection of 1.25 to 2.5 per cent adulteration was considered to be significant since meat when adulterated for economic reasons would always be at higher level than the detected ones.

When both BACM and RACM sera were set against cattle FD antigen, the BACM formed three precipitation lines, one of which formed a reaction of identity with the single line formed against the RACM serum. The results indicated that at least one species specific antigen had evoked strong antibody response in both the species of animals used for raising hyperimmune serum.

Warnecke and Saffle[1] investigated the various antigenic preparations and their routes of injection to determine the optimum method for producing antibodies of the desired titres and specificity and advocated multiple intramuscular injections of freeze

dried water extracts of skeletal muscle antigen emulsified in Freund's complete adjuvant. The Freund's complete adjuvant is comparatively a costly chemical and needs importation. Comparative study was undertaken to find out the efficacy of the serum produced in the buffalo calves as well as in the rabbits by giving multiple intramuscular injections of freeze dried extract of cattle skeletal muscle (CFD), all emulsified in the FCA and by giving only first injection of FD antigen emulsified in the FCA and the rest in laboratory made FIA. The hyperimmune monospecific sera produced in either species by both the methods were found to be equally good.

On the basis of the results obtained, use of cattle specific antibody produced in buffalo calves by injecting CFD antigen emulsified in FC for the first injection and in FIA for the rest of the injections is recommended for the identification of beef. However, further studies are recommended to standardize the dose of antigen, route of injection and immunization schedule to raise even more potent antisera.

References

1. Warnecke, M.O. and Saffle, R.L., Serological identification of animal proteins. 1. Mode of injection and protein extracts for antibody production. *J.Fd.Sci.*, 1968, **33**, 131
2. Lowry, O.H., Rosebrough, N.H., Farr, A.L. and Randal, R.J., Protein measurement with the folin phenol reagent. *J. biol. Chem.*, 1951, **193**, 265
3. Campbell, D.H., Garvey, J.S., Cremer, N.E. and Sussdorf, D.M., *Methods in Immunology*. W.A. Benjamin Inc., New York 1970, 2nd Edn. 128.
4. Ouchterlony, O., *Handbook of Immunodiffusion and Immunoelectrophoresis*, Ann. Arbor Science Publishers, Inc., Ann., Arbor, Mich, 1968.
5. Graber, P. and Burtin, P. *Immunoelectrophoretic Analysis*. Elsevier Publishing Co., Amsterdam, 1964.
6. Edwards, E.A., Counterimmunoelectrophoresis and double immunodiffusion. In *Rapid Diagnosis in Infectious Diseases*, M.W. Rytel, (Ed) CRC Press Inc, Florida, 1979.
7. Pinto, F.C., Serological identification of ox, buffalo, goat and deer flesh. *Br. Vet. J.*, 1961, **117**, 340.
8. Bubloz, A., Detection of adulteration of meat preparations by the addition of other species of meats. The use of double gel diffusion method. *Zbt. Vet. Med.*, 1962, 9, 961 *(Vet. Bull Abstr. 1963*, **33**, 2571.)
9. Ramadass, P., *Physico-chemical and Immunological Differentiation of Meats of Different Species of Animals*. 1972, M.V. Sc. Thesis, G.B. Pant University of Agricultural and Technology, Pantnagar.
10. Pandey, R. and Pathak, R.C., Serological relationship of taxonomically closely related domestic ruminants. Cattle, buffalo, sheep and goat. *Indian J. expt, Biol.*, 1975 **13**, 371.
11. Tagore, S.S.R., Venkatesan, E.S. and Ramamurthi, R., The differentiation of beef from buffalo flesh by immunodouble diffusion test. *Cheiron*, 1977, **6**, 134.
12. Hayden, A.R., Immunochemical detection of ovine, porcine and equine flesh in beef products with antisera to species myoglobin. *J. Fd Sci.*, 1979, **44**,494.
13. Sherikar, A.T., Ajinkya, S.M., Khot, J.B., Vaiday, A.M., Differentiation of meats by gel diffusion technique. *Indian J. Anim. Sci.*, 1979, **49**, 350.
14. Ramadass, P. and Misra, D.S., Immunoelectrophoretic differentiation of meats of bullock, buffalo, goat, sheep, pig and chicken. *Indian Vet. J.*, 1981, **58**, 978.
15. Reddy, P.M., *Studies on the Identification of Cooked Meats of Cattle, Sheep/Goat*, 1986. Ph.D. dissertation, Haryana Agricultural University, Hisar.

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Studies on Quality of Turmeric (*Curcuma longa*) in Relation to Curing Methods

S.R. Sampathu, N. Krishnamurthy, H.B. Sowbhagya and M.L. Shankaranarayana
Central Food Technological Research Institute, Mysore-570013, India.

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Dried turmeric fingers obtained under farm-processing conditions by three different treatments viz. water-cooking, alkaline water-cooking and traditional processing, were similar in quality with respect to drying characteristics, appearance, colour and oleoresin contents. Cooking and/or slicing helped to drastically reduce the drying period. Sun-dried fingers were bold in appearance, while mechanically dried fingers were shrivelled; however no difference in colour content was observed between them. Colour retention was better in mechanically dried uncooked slices than the conventionally cooked and sun-dried fingers. In laboratory trials, where the material to alkaline water ratio was higher (1:3) the loss of colouring matter was about 6% in contrast to negligible losses in farm trials where the ratio was lower (0.33). The extractability of colouring matter with acetone was significantly higher (92-94%) in uncooked samples than in conventionally cooked samples (76-84%).

India is the largest producer and exporter of turmeric. During 1984-85, India produced 2,43,200 tonnes of turmeric and exported 12,923 tonnes valued at Rs. 17.16 crores[1]. Conventional primary processing of turmeric consists of boiling the mature fresh rhizomes followed by sun-drying and polishing. For boiling, water or alkaline water has been used[2,3]. In a study on curing of turmeric, eighteen varieties were evaluated and the Erode variety gave the highest dry yield[4]. Said and Hussain[5] have recommended a method wherein the rhizomes are coated with a film of mustard oil followed by roasting in hot sand for 8-12 min. and drying in the sun. In this method, it is stated that drying is effected in less time than that required for the boiled rhizomes. In China, the cleaned rhizomes are heated in pots and dried in the sun[6]. In Jamaica, the washed turmeric is steamed in an autoclave and mechanically dried using a verti-flow grain drier[6]. Desikachar *et al*[7]. have recommended the use of lime water or 0.05-0.1 per cent solution of sodium bicarbonate or sodium carbonate, to replace unhygienic traditional practices. Some processors also adopted the practice of using an emulsion made up of castor seed paste, alum and chemichrome (a brand of lead chromate) to give an yellow coating to the turmeric fingers during polishing[7]. Desikachar *et al*[7]. also recommended the use of a solution containing sodium bisulphite and concentrated hydrochloric acid as a replacement for lead chromate in the emulsion during polishing. At present, in order to get an improved yellow colour, dried turmeric fingers are sometimes sprinkled with water and turmeric powder during polishing and no other chemical or emulsion is used. Krishnamurthy *et al*[8]. observed that there was no appreciable change in the volatile oil or colour content of turmeric obtained by different processing techniques. Also, boiling or slicing or peeling reduced the drying time considerably. However, no information is available regarding the drying time, drying yield, extractability of oleoresin and colouring matter with respect to the conventional and improved methods of primary processing. The present study was aimed at providing this information.

Materials and Methods

Fresh turmeric rhizomes (Erode variety) of full maturity (nine months growth) were obtained from a local farm. Bulbs and fingers were separated manually and used in the studies.

Farm trials: Studies were carried out on 90 kg batches. Fresh turmeric fingers were uniformally loaded into rectangular M.S. pans of size 2.5′ × 1.75′ × 1.25′ to which water (30 l) or sodium bicarbonate solution (30 l of 0.1 per cent solution) was added. One batch was also processed according to traditional method. The pans were covered with wet gunny bags and heated using firewood. Boiling was effected for 45 min when the rhizomes were soft to finger pressure and a thin pointed stick would penetrate them easily as judged by skilled workers at the farm. After boiling, the material in the pans was unloaded as a heap on drying yards and left undisturbed overnight. Next morning, the heaps were spread out as a single layer for sun-drying. The dried fingers were polished using a hand-operated batch polisher.

Laboratory trials: Fresh turmeric in 2 kg batches was subjected to the following treatments. Cooking period was 45 min for fingers and 1 hr for bulbs.

(i) fingers cooked in 6 l of water,
(ii) fingers cooked in 6 l of 0.1 per cent sodium bicarbonate solution,
(iii) fingers – no cooking,
(iv) fingers sliced by knife to 0.4 cm thickness, no cooking,
(v) fingers cooked in water and sliced as in (iv),
(vi) fingers, cooked in water, pulped in a Fryma homogeniser and shade dried,
(vii) fingers, no cooking but pulped as in (vi) and shade dried,
(viii) bulbs (mother rhizomes) cooked in water and sun-dried.

Turmeric samples obtained from treatments (i) to (v) were divided into two equal portions. One portion was sun-dried and the other portion was mechanically dried in a cross-flow drier at 60±2°C. The drying period and dry yield were recorded in each case.

Quality evaluation: (i) Physical appearance in relation to boldness, shrivelling, surface colour and colour on breaking were noted. Aroma of powdered turmeric was also evaluated.

(ii) The dried samples (about 200 g) obtained from each of the treatments were powdered to pass through 30 mesh sieve and were analysed for moisture (toluene distillation), volatile oil (Clevenger distillation) and total colour content (as curcumin) by ASTA methods[9], and for non-volatile acetone extract (NVAE) by cold percolation method. In this method, 30 g powder was packed in a glass column of internal diameter 3.2 cm and extracted with acetone giving an initial contact time of 2 hr and subsequently 1 hr. Totally, 5 extracts of 30 ml each were collected, pooled and analysed for NVAE and curcumin. For NVAE, 30 ml aliquots were used and non-volatile solids estimated according to the ASTA method for non-volatile ether extract[9]. Curcumin in the pooled extract was estimated by evaporating 5 ml aliquots on a boiling waterbath, suitably diluting the solids obtained with 95 per cent ethyl alcohol and recording the absorbance at 425 nm. The percentage recovery of curcumin by acetone extraction was calculated based on the initial curcumin content of the respective sample. The spent material left after acetone extraction was air dried and analysed for curcumin content. The oleoresin content of the samples was computed by adding up the values of volatile oil and NVAE.

Results and Discussion

Farm trials: The dried product obtained by three different treatments, namely, water cooking, alkaline water cooking and traditional method of cooking had similar appearance after polishing, and on breaking, they appeared orange yellow in colour. The drying time was eleven and fourteen days for fingers and bulbs respectively. The longer drying time for bulbs may be ascribed to their larger diameter. There was no appreciable difference in dry yield, moisture, curcumin, volatile oil and oleoresin contents among the treatments for fingers (Table 1). The dry yield of bulbs was slightly lower than that of the fingers on moisture-free basis. However, on as is basis, their dry yields were similar. The bulbs were richer in curcumin and volatile oil and oleoresin contents than the fingers. Based on these results, it is inferred that good quality turmeric can be obtained by cooking in water only and there is no need for using alkali water or for adopting traditional methods. The yield of fingers and bulbs per acre were of the order of 127 and 23 quintals when fresh and 25.4 and 4.6 quintals when dried. The ratio in both the cases was 5.5:1.

Laboratory trials: Physical appearance of the dried products obtained by different treatments were compared. Sun-dried fingers were bold while the mechanically dried samples were shrivelled in all the cases. The shrivelling may be attributed to the fast drying in the drier and would be a disadvantage during subsequent polishing. All the fingers were orange yellow in colour on breaking. Uncooked slices had an yellow surface on sun-drying while the mechanically dried slices had an orange-yellow surface. The fading of colour in the sun-dried sample can be ascribed to the increased surface area and the photosensitive nature of curcumin[10]. A similar observation and explanation holds good for the dried products obtained from cooked and sliced turmeric. Turmeric

TABLE 1. FARM TRIALS – DRY YIELD AND QUALITY OF TURMERIC

Treatment	Drying time (days)	Dry yield* (%)	Moisture (%)	Curcumin* (%)	V.oil* (% v/w)	Oleoresin* (%)
Fingers water cooked, sun-dried and polished	11	20.22	9.34	3.03	3.58	9.0
Fingers traditionally cooked, sun-dried and polished	11	19.80	9.99	3.03	3.44	8.9
Fingers sod. bicarbonate sol. (0.1%) cooked sun-dried and polished	11	20.47	9.05	3.02	3.41	8.9
Bulbs water-cooked and sun-dried	14	19.51	12.58	3.67	4.33	10.6

Average of duplicate determinations
*Moisture-free basis.

powder obtained from the pulped and shade dried treatments, cooked or uncooked was unacceptable in colour and odour.

Drying time and dry yields: The drying period for both water-cooked and alkaline water-cooked fingers was 11 days in the sun and 32 hr in the drier. Corresponding drying periods for the uncooked turmeric fingers were 30 days (sun) and 68 hr (drier) respectively (Table 2). This confirms the known fact that pre-cooking considerably reduces the drying period[7]. Mere slicing without cooking reduced the drying time considerably which was only one day in sun-drying and 7.5 hr in cross-flow drying. This was further reduced to 5.5 hr in the cooked and sliced sample. The drastic reduction in drying time can be attributed to the increased surface area and ease of removal of moisture from the cut surface and the rupturing of cells on cooking. The dry yield is 20 per cent on moisture-free basis, which was comparable for all the treatments.

TABLE 2. DRYING TIME AND YIELD OF TURMERIC FINGERS IN VARIOUS TREATMENTS

Treatment	Drying time		Dry yield*	
	Sun (days)	Cross-flow (hr)	Sun (%)	Cross flow (%)
Cooked in water	11	32	20.2	20.5
Cooked in sod. bicarbonate sol. (0.1%)	11	32	20.4	20.3
No cooking	30	68	20.5	19.9
Sliced, no cooking	1	7.5	19.5	19.6
Cooked in water and sliced	1	5.5	22.2	21.6

Average of duplicate determinations,
*Moisture-free basis

Chemical analysis: Results are presented in Table 3. The sun-dried samples had, in general, a higher moisture content (9.0 – 12.9 per cent) than the mechanically dried samples (6.3 – 10.8 per cent). The volatile oil content in fingers and slices obtained by sun-drying and cross-flow drying ranged from about 3 to 3.9 per cent. Highest volatile oil content of 4.3 per cent was noticed in the case of bulbs followed by 3.9 per cent for fingers dried without cooking. The turmeric sample which was uncooked, pulped and shade-dried had the lowest volatile oil content (1.1 per cent), while there was no loss of volatile oil in cooked, pulped and shade-dried sample.

The curcumin content in the water-cooked fingers was about 3.1 per cent for both sun-dried and cross-flow dried samples whereas in alkaline water cooking the corresponding values were 2.9 per cent. The blanched solutions left after water and alkaline water cooking were found to account for 0.2 and 0.6 per cent curcumin, respectively on the basis of the charge.

The drop in the curcumin content (about 7.6 per cent) of alkaline water cooked samples can be attributed to the destruction of curcumin in the presence of hot alkali. The sensitivity of curcumin to alkaline solution is known[11,12]. This is in contrast to our findings from the farm trials where both water and alkaline water cooked samples had the same curcumin content. The higher loss of cucumin in alkaline water cooking in the laboratory trials is obviously due to the higher ratio of turmeric fingers to alkaline water which was 1:3 as against 1:0.33 in farm trials. The higher ratio in the laboratory trials was needed to keep the fingers immersed throughout cooking. On the other hand, in the farm trials a smaller ratio of water was

TABLE 3. ANALYSIS OF TURMERIC FINGERS SUBJECTED TO DIFFERENT TREATMENTS (LABORATORY TRIALS)

Treatment	Drying method	Moisture (%)	V.oil* (% v/w)	NVAE yield* (%)	Oleoresin* (%)	Curcumin Content* (%)	Curcumin Recovery (%)	Curcumin Spent residue (%)
Cooked in water	Sun	9.54	3.21	5.22	8.43	3.16	78.90	0.60
	Cross-flow	8.50	3.17	6.15	9.32	3.16	78.76	0.61
Cooked in alkaline water	Sun	9.00	3.08	5.62	8.70	2.91	79.40	0.54
	Cross-flow	8.90	3.40	5.27	8.67	2.93	76.70	0.62
No cooking	Sun	12.88	3.91	7.50	11.41	3.46	93.41	0.12
	Cross-flow	9.86	3.33	6.44	9.77	3.19	93.30	0.19
Sliced, no cooking	Sun	9.59	3.21	5.79	9.00	2.90	94.13	0.15
	Cross-flow	6.34	2.97	5.78	8.75	3.26	92.05	0.24
Cooked & Sliced	Sun	10.79	3.36	5.74	9.10	2.84	84.76	0.38
	Cross-flow	10.49	3.35	5.91	9.26	2.77	79.91	0.49
Cooked in water, pulped & shade-dried		12.13	2.99	5.49	8.48	2.08	67.50	0.59
No cooking, pulped & shade-dried		10.13	1.10	5.25	6.35	1.89	83.10	0.28

Average of duplicate determinations
*Moisture-free basis.

sufficient wherein cooking was achieved due to a combined effect of boiling/frothing and steam; the heat being conserved with a gunny bag covering.

Uncooked, sliced and sun-dried samples had a lower curcumin content compared to the corresponding mechanical dried samples. The loss may be ascribed to the increased surface area on slicing and the photosensitive nature of curcumin. However, in the case of cooked and sliced samples, there was no appreciable difference in curcumin content between sun-dried and mechanical samples. There was a considerable drop in the curcumin contents of pulped and shade-dried samples for both cooked and uncooked treatments. The bulbs had the highest curcumin content of 3.67 per cent.

Variations noticed in NVAE values could not be related to any pre-treatment or method of dryig. The NAVE value for fingers and slices ranged from 5.2 to 6.2 per cent except in the case of uncooked fingers where the values were 7.5 and 6.4 per cent for sun-dried and mechanically dried samples, respectively (Table 3). The bulbs contained 6.3 per cent NVAE.

The oleoresin yield from fingers ranged from 8.4 to 11.4 per cent and followed a trend similar to that of NVAE. Bulbs (cooked in water, sun-dried) were richer in oleoresin (10.6 per cent) than fingers from all other treatments except uncooked sun-dried fingers (11.4 per cent). Though bulbs contain higher amounts of volatile oil and colouring matter (Table 1) and oleoresin compared to the fingers, they fetch a slightly lower price in the market, the reason for which is not clear. Probably appearance, higher moisture content and poor demand for turmeric oil may affect the price.

The recovery of curcumin using acetone as a solvent (by percolation) was drastically reduced by pre-cooking. For cooked fingers and slices the recovery (from the dried powder) ranged from 76.7 to 84.7 per cent while for uncooked fingers and slices it ranged from 92 to 94 per cent (Table 3). For the cooked bulbs, curcumin recovery was about 82 per cent, similar to that of fingers treated like-wise. The lower recovery of curcumin in the case of cooked turmeric may be due to the gelatinisation of starch which hinders the release of colouring matter during extraction. A similar trend was observed even by Soxhlet extraction for 24 hr with acetone. The extractability for uncooked fingers was 97.6 per cent while for cooked fingers it was 89.9 per cent. This was further confirmed by the analysis of spent residues left after acetone extraction. For cooked fingers and slices the values ranged from 0.4 to 0.6 per cent while for uncooked fingers and slices the corresponding values ranged from 0.1 to 0.2 per cent. Hence, for oleoresin extraction, it will be advantageous to use turmeric powder obtained without pre-cooking as there is a definite increase of 10-15 per cent in the recovery of curcumin.

References

1. Agricultural Situation in India, February 1986, Directorate of Economics and Statistics, Ministry of Agriculture, New Delhi.
2. Govindarajan, V.S., Turmeric – chemistry, technology and quality; *CRC Rev. Fd. Sci. Nutr.*, 1979, **12**, 199.
3. Pruthi, J.S., *Spices and Condiments – Chemistry, Microbiology and Technology*, Academic Press, N.Y. 1980.
4. Patil, R.B. and Sapkar, R.T., Studies on the curing percentage of some varieties of turmeric, *Indian Spices*, 1983, **20** (2), 9.
5. Said, M. and Hussain, Ch.A., Curing, processing of turmeric, West Pak., *J. Agric. Res.*, 1964, **2** (3), 22.
6. Purseglove, J.W., Brown, E.G., Green, C.L. and Robbins, S.R.J., *Spices*, Longman Group Limited, London, 1981, Vol.2, 552.
7. Desikachar, H.S.R., Srinivasan, M. and Subrahmanyan, V. Processing of turmeric, *Res. Ind.* 1959, **4**, 172.
8. Krishnamurthy, M.N., Padmabai, R., Natarajan, C.P. and Kuppuswamy, S., Colour content of turmeric varieties and studies on its processing, *J. Fd Sci. Technol.*, 1975, **12**, 12.
9. *Official Analytical Methods*, American Spice Traders Association, Inc. New York, 1968.
10. Coulson, J., Food colours – a balanced view. *Fd. Flavour. Ingred., Pckg. Process.* 1981, **3**, 14.
11. Tonneson, H.H. and Karlsen, J., Studies on curcumin and curcuminoids. V. Alkaline degradation of curcumin, *Z. Lebensm. Unters, Forsch.*, 1985, **180**, 132.
12. Tonneson, H.H. and Karlsen, J., Studies on curcumin and curcuminoids, VI. Kinetics of curcumin degradation in aqueous solutions, *Z. Lebensm. Unters. Forsch.*, 1985, **180**, 402.

RESEARCH NOTES

SPECTROPHOTOMETRIC METHODS OR THE DETERMINATION OF MORIN, QUERCETIN, TANNIN AND CHLOROGENIC ACID

C.S.P. SASTRY, K. EKAMBARESWARA RAO, D. VIJAYA, A. RAMAMOHANA RAO
Food and Drug Laboratory, School of Chemistry, Andhra University, Waltair – 530 003, India.
AND
M. VEERABHADRA RAO,
Central Food Technological Research Institute, Mysore, India
Received 19 March 1987; revised 28 May 1987

Simple spectrophotometric methods for the determination of some polyphenolic compounds have been described using two pairs of reagents: metol – Cr(VI) for morin and tannic acid and m-aminophenol – periodate for quercetin and chlorogenic acid. The methods are reproducible, accurate within ± 1.5% and are applicable for estimation in foodstuffs.

Polyphenols are important natural constituents of vegetables, fruits, wines, coffee, tea, tobacco, etc. Quercetin and tannic acid are also used in pharmaceuticals as hemostatics, whereas chlorogenic acid contributes to the taste of coffee, tea, tobacco, etc. The methods proposed earlier for their determination involve the use of cupric acetate or cadmium acetate[1] and tetraphenyl dibroxide[2] for morin (2',3',4',5,7-pentahydroxy flavone) and quercetin (3,3',4',5,7-pentahydroxy flavone), Sn(II)[3] and aluminium chloride[4] for quercetin, ferric chloride – triethanolamine – sodium dodecyl sulphate[5] and UV spectrophotometry[6] for tannic acid (pentadigalloyl glucose) and UV spectrophotometry[7] and nitrosation[8] for chlorogenic acid (3',4'-dihydroxy cinnamoyl-3-quinic acid). These methods are found to be tedious, time consuming, less sensitive and general for all phenolic compounds. We report here simple, rapid and reproducible methods for the determination of these compounds in microgram quantities. The pairs, metol – Cr(VI)[9] and m-aminophenol – periodate[10] have been found to be selective chromogenic reagents for the determination of 1,3 and 1,2 dihydric phenols respectively.

Spectral and absorbance measurements were made on a Shimadzu UV 140 double beam spectrophotometer with 1 cm quartz cells. The pH measurements were made on an Elico model LI – 120 digital pH meter.

Freshly prepared aqueous solutions of metol (0.2 per cent) and m-aminophenol (mAP, 0.2 per cent), potassium dichromate (Cr(VI), 0.01N) and sodium metaperiodate (IO_4^-, 0.01M) were used. Potassium acid phthalate (0.2M – hydrochloric acid (0.1N) or sodium hydroxide (0.1N) (pH 2.8 – 4.4) buffers were prepared[11].

All the polyphenolic compound solutions (0.1 per cent) were prepared in double distilled water (the compounds insoluble in water being dissolved initially in a minimum volume of alcohol). Lower concentrations were prepared by appropriate dilution of the stock solutions.

To an aliquot of sample solution containing the mentioned compounds in Beer's law range (Table 1), aqueous solutions of 15ml buffer (pH 4.0 for morin, pH 2.5 for chlorogenic acid and quercetin and pH 3.0 for tannic acid), aminophenol and oxidising agent (2 ml of metol and 3 ml of Cr (VI) for morin and tannic acid, 1 ml of mAP and 2 ml of IO_4^- for quercetin and chlorogenic acid) were added and diluted to 25 ml in a volumetric flask. The absorbance was measured during the stability period at appropriate λ max (Table 1) against the corresponding reagent blank. The quantity of each compound was computed from its standard graph.

For the determination of tannins in tea, an aliquot of the tea extract prepared according to AOAC and colour was developed with the metol – Cr (VI) reagent. Tannin content was expressed as tannic acid per 100 g of the sample.

The optimum conditions such as pH, concentration of oxidizing agent and aminophenol necessary for getting maximum absorbance and stability of the coloured species were established through preliminary experiments. The two pairs of reagents (metol – Cr (VI) and mAP – IO_4^-) were found to work well in acidic buffer conditions than in alkaline conditions as polyphenols themselves give colours under alkaline conditions. The stability of the coloured species, optical characteristics such as Beer's law range, molar absorptivity and Sandell's sensitivity values are given in Table 1. The reproducibility of the methods were determined by analysing six replicate samples each containing a final concentration of 10 μg/ml of polyphenol in the assay solution. Table 2 indicates the reproducibility of the proposed method.

Recovery experiments were carried out by adding 5 mg of tannin to a known amount of previously analysed tea dust; the proposed method and Lowenthal – Procter method[12] were followed and the results are given in Table 3.

TABLE 1. OPTICAL CHARACTERISTICS OF POLYPHENOLIC COMPOUND COMPLEXES

Polyphenolic compound	Stability (min)	λ max (nm)	Beer's law range (μg/25ml)	Molar absorptivity (1. $mole^{-1}cm^{-1}$)	Sandell's sensitivity $\mu g/cm^2/0.001$ absorbance unit
Morin	30 – 90	420 – 430*	30 – 600	1.164×10^4	0.026
Tannin	10 – 30	570 – 600*	100 – 450	1.59×10^4	0.110
Quercetin	5 – 60	310	25 – 300	1.43×10^4	0.020
Chlorogenic acid	20 – 50	310	30 – 500	1.06×10^4	0.036

*Used in experiment

TABLE 2. PPRECISION AND ACCURACY OP PROPOSED METHODS

Polyphenolic compound	Polyphenol Taken (μg)	Polyphenol Found* (μg)	% Error	% Recovery	% Relative std. deviation
Morin	500	496.8	−0.64	97.8	0.500
Tannin	300	296.2	−1.27	98.3	1.741
Quercetin	300	297.1	−0.97	98.8	0.947
Chlorogenic acid	200	200.9	+0.45	98.5	0.506

*Each value is the average of six determinations

TABLE 3. ESTIMATION OF TANNIC ACID IN COMMERCIAL TEA SAMPLES

Sample	% of tannin found		% recovery	
	Reported method	Present method	Present method	Reported method
I	1.04	1.02	98.9	98.8
II	0.90	0.89	99.0	99.1*
III	0.86	0.84	98.9	98.7

Each value is the average of three individual determinations

The colour formation in the case of tannic acid and morin with metol and Cr (VI) reagent may be due to the formation of either oxidative coupled product or charge-transfer complex by the involvement of the PMBQMI (p-N-methylbenzoquinone-monoimine, formed *in situ* from metol by the action of Cr (VI)) and polyphenolic compound. We consider that the latter one is more probable[13]. The reagent mAP and IO^-_4 is found to be more specific for catechol units[10] and useful for the estimation of quercetin and chlorogenic acid, which possess free catechol units. The species thus obtained in these cases may be originating through oxidative coupling reaction between polyphenolic compound (involvement of free p-position to one of the phenolic hydroxyls in the catechol unit) and m-aminophenol (involvement of amino group).

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References

1. Leu, R. and Hagedonn, P., Distinction between flavone glycosides and flavones, *Z. analyt. Chem.*, 1953, **139**, 96.
2. Nev, R.A., New organic boron complex as an analytical reagent, *Z. analyt. Chem.*, 1954, **142**, 335.
3. Sakamoto, M. and Takamura, K., Consecutive determination of rutin and quercetin by spectrometry measurements, *Microchem. J.*, 1982, **23**, 374.
4. Dowd, L.R., Spectrophotometric determination of quercetin, *Analyt. Chem.*, 1959, **31**, 1184.
5. Hagerman, A.E. and Butler, L.G., Protein precipitation method for quantitative determination of tannins, *J. agric. Fd Chem.*, 1978, **26**, 809.
6. Sharp, R.N. Sharp, C.Q. and Kattan, A.A., Tannin content of Sorghum grain by UV spectrometry, *Cereal Chem.*, 1978, **55**, 117.
7. Oakley, E.T. and Millham, J.O., Rapid spectrophotometric method for determination of chlorogenic acid in leaf tobacco, *J. ass. off. agric. Chem.*, 1963, **46**, 785.
8. Arai, S., Determination of chlorogenic acid and caffeic acids in coffee, *J. agric. Chem. Soc. Japan*, 1965, **39**, 66.
9. Siraj, P., Ramakrishna, R., Murthy, S.S.N. and Sastry, C.S.P: Colorimetric determination of microgram quantities of meta disubstituted phenols with metol and chromium (VI) *Nat. Acad. Sci. Lett.*, 1979, **2**, 413.
10. Sastry, C.S.P., Rao, K.E. and Prasad, U.V., Spectrophotometric determination of some phenols with sodium metaperiodate and aminophenols. *Talanta*, 1982, **29**, 917.
11. Lurie, Yu., *Hand book of Analytical Chemistry*, MIR, Publishers, Moscow, 1975, 255.
12. Jacobs, M.B., The Chemical Analysis of Foods and Food Products, D. Van Nostrand Company, New York 1958, 632.
13. Sastry, C.S.P., Rao, B.G. and Reddy, B.S., Spectrophotometric determination of pharmaceutically important primary aryl amines using metol and NBS. *J. Indian Chem. Soc.*, 1981, **58**, 655.

AMINO ACID COMPOSITION OF SUBABUL (*LEUCAENA LEUCOCEPHALA*) SEED KERNEL PROTEINS

G. AZEEMODDIN, S. JAGANMOHAN RAO AND S.D. THIRUMALA RAO
Oil Technological Research Institute, Anantapur – 515 001, India

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Subabul seed, its kernels and deoiled kernel flour show crude protein contents of 27.5, 55.0 and 60.0 per cent respectively. The deoiled kernel flour is fairly rich in several essential amino acids.

Subabul (Leucaena leucocephala) belongs to the leguminosae family. It is an important crop encouraged under social forestry schemes in drought prone areas and semi arid tracts in India as it provides, in short time, useful timber and leaves for feed purposes. But for a small quantity of seed which is set apart for regeneration purposes, a large quantity produced in *subabul* plantations is disposed off at uneconomic prices. It is reported that ripe *subabul* seeds are roasted and used as a substitute for coffee[1]. Systematic investigations were taken up on the *subabul* seed both as an oilseed and as a source of protein.

The seeds were freshly procured from the local forestry agency. They were cleaned, decorticated, kernels separated, crushed and extracted with *n*-hexane. The physico-chemical characteristics of the seed and oil were determined by AOCS Methods[2] and the results are given in Tables 1 and 2. The extracted meal (pure kernel powder) was desolventized and ground to a fine, smooth light yellow coloured powder whose characteristics are given in Table 1. The amino acid composition of the extracted meal powder was determined using a LKB 150 Amino Acid Analyser (Biochem) and the results are given in Table 3.

TABLE 1. CHARACTERISTICS OF SUBABUL *(Leucaena leucocephala)* SEED AND KERNEL

Parameters	Seed	Kernel	Extracted kernel
Length (mm)	8.2	—	—
Breadth (mm)	5.7	—	—
Thickness (mm)	1.7	—	—
Wt. of 100 seeds (g)	6.5	—	—
Moisture (%)	6.0	6.3	6.0
Oil (%)	6.0	12.0	0.1
Protein (%)	27.6	55.0	60.0
Ash (%)	3.8	5.4	4.5
Acid insoluble ash (%)	0.22	0.12	0.15
Crude fibre (%)	12.3	4.0	3.5
Hull/Kernel ratio	50.50	—	—

TABLE 2. PHYSICO-CHEMICAL CHARACTERISTICS OF SUBABUL *(Leucaena leucocephala)* SEED OIL

Specific gravity 30/30°C	0.9135
Refractive index at 40°C	1.4662
Iodine value (Wijs)	113.5
Acid value	2.6
Saponification value	188
Unsaponifiable matter (%)	2.5

TABLE 3. AMINO ACID COMPOSITION OF SUBABUL *(Leucaena leucocephala)* SEED FLOUR

Aminoacid	(g/16 gN)
Asp	10.33
Thr	2.38
Ser	5.23
Glu	22.06
Pro	9.90
Gly	6.16
Ala	4.03
Cys	0.96
Val	2.40
Met	1.23
Iso-Leu	12.00
Leu	6.83
Tyr	1.86
Phe	5.70
His	3.90
Lys	2.30
Arg	0.90
Try	1.25

Subabul seeds are ovoid in shape and have hard brown hulls and yellow kernels. The seed is low in oil (6 per cent) and rich in protein (27.5 per cent). Kernels show an oil content of 12 per cent and protein content of 60 per cent. It is significant to note that the proteins of deoiled kernel powder of *subabul* seed are fairly rich in essential amino acids, isoleucine 12.0, leucine 6.83, phenylalanine 5.70 and histidine 3.90 g/16g N. Lysine and methionine are also present in moderate amounts. Eighteen amino acids have been identified in the seed protein. *Subabul* seed is a potentially good source of proteins.

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References

1. The Wealth of India: Raw Materials, Publications and Information Directorate, C.S.I.R., New Delhi, Vol. 6, 1962, 77.
2. *Official and Tentative Methods*, American Oil Chemists' Society Champaign, 1958, 3rd Edn (revised to 1973) 12., Aa 3-38, Aa 4-38, Aa 5-39, Ba 5-49, Ba 6-61, Cc 10a-25, Cc 7-25, Cd 1-25, Cd 3a-63, Cd 3-25, Cd 6a-40

EFFECT OF ADDITIVES ON CITRIC ACID PRODUCTION BY *ASPERGILLUS NIGER*

H.K. MANONMANI AND K.R. SREEKANTIAH*
Discipline of Microbiology and Sanitation,
Central Food Technological Research Institute,
Mysore – 570 013, India.

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Citric acid production by *Aspergillus niger*-16 was studied by solid state fermentation on sugarcane bagasse fortified with sucrose medium. Ethanol and coconut oil at 3% (V/W) level increased the production of citric acid. Fluroacetate at a concentration of 1.0mg/15g bagasse enhanced the yield of citric acid significantly. Addition of ethanol and fluoracetate after 6 hr of growth gave maximum conversion.

Citric acid is one of the most commonly used organic acids in food and pharmaceutical industries. Even though it occurs naturally in high concentration in citrus fruits, the existing demand is almost entirely met by fermentative production[1], using *Aspergillus niger* in submerged or static liquid cultures. Recently, some attempts have been made to produce citric acid by solid state fermentation[2]. Usually, the carbohydrate source is cane sugar or molasses[3]. Although the basic 'know-how' of this process is well known to the industry[4], some problems are still encountered, which result in low yield. Attempts have been made to enhance the yield of citric acid by the addition of stimulants like alcohols, fats[2] and metabolic inhibitors[2].

In this laboratory, a cellulolytic isolate of *Aspergillus niger* was found to secrete citric acid into the medium. Investigations were carried out to cultivate the fungal isolate on bagasse fortified with sucrose. Effect of alcohols, fats and sodium monofluoroacetate, a metabolic inhibitor on citric acid production by this fungus were investigated and the results are presented here.

The fungal isolate, *Aspergillus niger* no. 16 was from the culture collection maintained in this laboratory. Sugarcane bagasse procured from M/s Pandavapura Sahakara Sakkare Kharakhane, Pandavapura, Mandya dist. Karnataka, India was used in the experiments.

Nutrient medium[5] contained (% w/w): sucrose, 7; KH_2PO_4, 0.1; $NaNO_3$, 0.485; $MgSO_4$ $7H_2O$, 0.023 and pH adjusted to 2.8 with 1N HCl. This medium was mixed with bagasse at 3:1 ratio and dispensed into 250 ml Erlenmeyer flasks at 15 g (wet wt.) per flask. Flasks were autoclaved at 1.1 kg/cm^2 for 20 min, cooled and inoculated with 1×10^5 spores of *A. niger* per flask prepared in sterile distilled water containing Tween 80.

Unless otherwise stated, individual alcohols and sodium monofluoroacetate were added before inoculation and fats were added before autoclaving.

After the desired period of growth, the mouldy bagasse was air dried and used for extraction with distilled water (1:10 ratio). Citric acid was estimated by the pyridine – acetic anhydride method[6]. Total acidity was determined according to Chaudhary *et al*[7].

Effect of alcohols: Among four different alcohols tried, ethanol at 3 per cent (v/w) level stimulated citric acid production (Table 1). Methanol at 2 per cent (v/w) level slightly increased citric acid yield, but at higher levels both growth and citric acid production were reduced. There was marked decrease both in growth and citric acid production with iso-propanol, while butanol even at 1 per cent level completely inhibited the growth.

Stimulation of citric acid production increased with alcohol dose reaching a maximum at 3 per cent level, above which there was reduction (Table 1). Growth was very slow at higher levels of ethanol. There was 32 per cent conversion of sugars to citric acid when ethanol was added after 6 hr of incubation (Table 2). Addition at 18 and 24 hr of growth did not increase citric acid yield.

TABLE 1. EFFECT OF ADDITION OF ALCOHOLS ON CITRIC ACID PRODUCTION BY *ASPERGILLUS NIGER*

Alcohol added: Name	Level (%) (v/w)	Final pH	Titrable+ acidity (mg/g)	Citric acid (mg/g)	% Conversion
Control		3.2	12.8	3.1	4.4
Methanol	1	3.6	3.8	0.2	0.3
Methanol	2	3.9	17.9	6.8	9.7
Methanol	3	3.9	3.8	1.2	1.8
Ethanol	1	3.2	21.8	8.3	11.8
Ethanol	2	3.3	25.6	14.5	20.7
Ethanol	3	3.1	38.4	20.0	28.6
Ethanol (6 hr)*	3	3.0	42.9	22.5	32.1
Ethanol (18 hr)*	3	3.2	17.6	7.3	10.7
Ethanol (24 hr)*	3	3.2	14.4	8.5	12.1
Ethanol	4	3.1	20.8	8.2	11.7
Ethanol	5	3.2	16.0	4.3	6.1
Iso-propanol	1	3.3	5.1	2.2	3.2
Iso-propanol	2	3.4	5.1	1.2	1.7
Iso-propanol	3	3.3	4.5	Nil	0.0

+Titrable acidity expressed as citric acid mg/g bagasse.

‡ % conversion based on initial sugar concentration. Incubation period 72 hr. No growth was observed with butanol at 1-3% level.

*Indicates time of addition after incubation.

*To whom correspondence should be addressed.

Ethanol is found to be a stimulant for citric acid production, in contrast to many reports where methanol has been observed to be a stimulant[2,8,10] Methanol has been reported to effect the permeability of cell walls enabling greater excretion of citric acid[2]. Chaudhary *et al*[9] have suggested that methanol helps in conditioning mycelia for citric acid production without impairing their metabolism. Ethanol has been observed to double the activity of citrate synthase[11] while the aconitase activity was reduced by 75 per cent. Bhatt *et al*[12] have stated that ethanol might be converted to acetyl Co-A which acts as a precursor of citric acid. Ethanol also acts as a carbon source. The decrease in growth and citric acid production at higher levels of ethanol may be due to its toxic effects. The yield on addition of iso-propanol is less indicating least stimulation by iso-propanol. Butanol inhibits the growth of the fungus completely by preventing the germination of the spores of the fungus.

Effect of metabolic inhibitor: Effect of sodium monofluoroacetate, a competitive inhibitor of acetate oxidation[13] was tried at different concentrations. At 1.0mg of fluoroacetate per 15g of bagasse stimulated higher conversion of sugars to citric acid (Table 2). There was complete inhibition of growth at higher concentration of fluoroacetate. Addition of fluoroacetate at 6 hr of incubation helped in higher conversion of sugars (Table 2) to citric acid, while additions at 18 and 24 hr did not increase yields. The effect of fluoroacetate in increased citric acid accumulation has been observed to be indirect with the specific inhibition of aconitase activity[14] by the formation of fluorocitrate[15].

Effect of fats: Of the 8 different fats tried (Table 3) coconut oil gave 32 percent conversion followed by oleic acid (26 per cent conversion). There was increase in citric acid yield with increase in coconut oil concentration upto 3 per cent level, above which the yield decreased (Table 3). Fats and oils have been

Table 2. Effect of fluroacetate on citric acid production by *Aspergillus niger*

Fluroacetate conc. (M)	Final pH	Titrable+ acidity (mg/g)	Citric acid (mg/g)	% Conversion
Control	3.20	12.8	3.1	4.4
10^{-5}	4.30	32.0	4.5	6.4
10^{-4}	3.85	19.2	5.6	7.9
10^{-3}	3.30	21.8	17.5	10.6
10^{-2}	2.95	26.9	14.4	20.5
10^{-2} (6 hr)	2.85	25.6	18.3	26.2
10^{-2} (18 hr)	2.70	20.2	10.5	15.0
10^{-2} (24 hr)	3.00	9.9	3.5	5.0

Legend as in Table 1.

Fluroacetate solution was added at the rate of 1 ml/15g (wet wt.) substrate.

No growth was observed at 10^{-1}M. fluroacetate.

Table 3. Effect of different fats on citric acid production by *Aspergillus niger*

Oil/Fat Name	Level added (%) (v/w)	Incubation period (hr)	Final pH	Titrable acidity (mg/g)	Citric acid (mg/g)	% Conversion
Control	-	48	3.10	19.2	4.8	6.9
		72	3.20	12.8	3.1	4.4
Groundnut oil	3	48	2.60	17.9	10.4	14.9
		72	2.80	14.1	8.6	12.4
Mustard oil	3	48	2.70	13.4	8.5	12.1
		72	2.80	9.6	3.2	5.6
Oleic acid	3	48	2.80	25.0	18.6	26.6
		72	2.90	11.5	5.2	7.5
Castor oil	3	48	2.75	13.4	8.4	12.0
		72	2.90	9.0	4.0	5.6
Ghee	3	48	2.80	17.3	11.5	16.5
		72	2.85	9.0	3.1	4.4
Coconut oil	1	48	3.20	19.2	8.9	12.7
		72	3.40	19.2	8.6	12.3
Coconut oil	2	48	3.10	25.6	12.3	17.6
		72	3.10	19.2	9.4	13.4
Coconut oil	3	48	2.70	28.2	22.4	32.0
		72	2.65	14.1	8.5	12.1
Coconut oil	4	48	2.80	27.2	20.1	28.7
		72	2.70	25.6	12.4	17.7
Coconut oil	5	48	3.00	19.2	4.3	6.1
		72	3.10	12.8	3.1	4.4

Legend as in Table 1

reported to be broken down to glycerol and fatty acids[12], glycerol entering the TCA cycle via glycolysis by the formation of glyceraldehyde-3-phosphate and fatty acids entering directly by the formation of acetyl CoA. Unsaturated oils like groundnut oil have been observed to serve as alternate hydrogen acceptors in place of oxygen during fermentation[16]. Since coconut oil is a saturated oil, the hydrogen acceptor theory cannot explain the increase in citric acid yield. It may act as additional source of acetyl CoA, thus improving the citric acid yield. Since the metabolism of these compounds by *Aspergillus niger* is not clear, it is difficult to ascribe their mode of action.

Thus, it is evident that citric acid production by *Aspergillus niger* can be increased significantly by incorporating certain compounds in the medium.

References

1. Prescott, S.C. and Dunn, C.G. Citric acid fermentation, in *Industrial Microbiology*, McGraw Hill Book Inc., New York, 1949. 2nd Edn..
2. Kapoor, K.K.; Chaudhary, K. and Tauro, P., Citric acid fermentation, in *Industrial Microbiology*, Reed, G. (Ed.) AVI Publishing Co., Inc., West Port, Connecticut, 1984. 4th Edn,
3. Roar, M. and Kubicek, C.P., Regulatory aspects of citric acid fermentation by *Aspergillus niger. Process Biochem.*, 1981, **16,** 34.
4. Noyes, R., *Citric Acid Production Process.* Noyes Development Corporation., New Jersey, 1967.
5. Manonmani, H.K. and Sreekantiah, K.R., 1987 Unpublished.
6. Murray, S. and Denstedt, O.F., A rapid method for the determination of citric acid. *J. biol. Chem.*, 1948, **175,** 849.
7. Chaudhary, K., Lakshminarayana, K., Dev, T.K. and Vyas, S.R., Nitrosoguanidine induced mutation of *Aspergillus niger* for obtaining high citric acid producing mutants. *Indian J. Microbiol.*, 1974, **14,** 42.
8. Panda, T., Kundu, S. and Majumdar, S.K. Studies on citric acid production by *Aspergillus niger* using Indian cane molasses. *Process Biochem.*, 1984. **19,** 183.
9. Chaudhary, K., Ethiraj, S., Lakshminarayana, K. and Tauro, P., Citric acid production from Indian cane molasses by *Aspergillus niger* under solid state fermentation conditions., *J. Ferment. Technol.* 1978, **56,** 554.
10. Kimura, K. and Nakanishi, T., Ger. Pat. 2,040,356 – 1971.
11. Manonmani, H.K. and Sreekantiah, K.R., Citric acid production by a selected isolate of *Aspergillus niger* in defined media., 1987 Unpublished.
12. Bhatt, C.S., Agarwal, P.K. and Viswanathan, L., Commercial manufacture of citric acid., VII: Effects of some fats and oils on citric acid fermentation by *Aspergillus niger.* VIII Effects of alcohols and crude extracts on the production of citric acid by *Aspergillus niger., 44th Convention of Sugar Technologists Association of India.* 1980, 1G and 75G.
13. Quastel, J.H., Inhibitors in citric acid cycle., in *Metabolic Inhibitors.*, Rocuster, R.M. and Quastel, J.H. (Eds), Academic Press, New York, 1963, Vol II.
14. Morrison, J.F. and Peters, R.A., Biochemistry of fluroacetate poisoning. Effect of flurocitrate on purified aconitase., *Biochem. J.*, 1954, **58,** 473.
15. Mortins, E., Inhibitors in citric acid cycle., in *Metabolic Inhibitors.*, Rocuster, R.M. and Quastel, J.H. (Eds)., Academic Press., New York., London. 1963. Vol. II
16. Kumar, K. and Ethiraj, S., Influence of methanol and groundnut oil on citric acid production from sugarcane juice by *Aspergillus niger. Int. Sugar J.*, 1976, **78,** 13.

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CHAPATI FROM GERMINATED WHEAT

K. LEELAVATHI AND P. HARIDAS RAO
International School of Milling Technology and Baking Technolog
Central Food Technological Research Institute,
Mysore-570013, India

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Germination of wheat increased the contents of reducing sugars, diastatic acitvity and damaged starch while the gluten content, falling number and chapati water absorption decreased. Chapati made from germinated wheat had better taste (sweetish) but slightly hard texture as compared to chapati prepared from ungerminated wheat (control). Germinated wheat chapati stored for 4 days possessed better texture and overall quality than the stored control wheat chapati.

Germination was reported to adversely affect the functional characteristics of wheat, particularly with respect to its bread making quality[1]. This was also found to be true in case of rain damaged wheat due to unseasonal rains during harvest. The flour obtained from these wheats had excessive alpha amylase activity and hence produced sticky dough and inferior quality bread with poor crumb and crust characteristics[2-4]. Biscuit making quality of such flours, however, was not found to be affected significantly[5]. The information on its suitability for the preparation of chapati – a staple diet of the majority of population in India, is not available. This information is more useful as germination was reported to increase the sugar content and also improve the nutritional characteristics of wheat and hence the chapati[6]. Studies were, therefore, undertaken to determine the suitability of germinated wheat for chapati making and the results are presented in this paper.

Medium hard commercial wheat procured from the local market was used in the study. Part of the wheat was cleaned and ground in a disc mill (chakki) to obtain whole wheat flour. Remaining part of the wheat was germinated by soaking it in water overnight and spreading the soaked wheat for a day and covering with a wet cloth to maintain the humidity. The germinated wheat was sun-dried to about 11-12 per cent moisture and ground in a disc mill. Sieve analysis of both the flour samples was carried out using a Buhler plan sifter by taking 200 g sample and sieving it for 5 min. The overtailings in each sieve were weighed and the percentage calculated.

Chemical characteristics of flour samples such as moisture, gluten, ash, falling number, diastatic activity and damaged starch were determined as per standard AACC procedures[7]. Reducing sugars in the flour as well as in the dough were determined according to standard AOAC procedure[8]. Chapati water absorption of flour was determined using 'Research' water absorption meter' as per the method described earlier[9]. Test baking of Chapati was carried out using the standard procedure[9] and the chapaties were evaluated for different quality characteristics such as hand feel, appearance, eating quality and taste by a panel of six judges[9]. Shear value of chapaties was determined using Warner Bratzler shear press[10]. After storing in polyproplyene pouches for 4 days, the chapaties were evaluated for different quality characteristics. The pliability as well as height on puffing was determined using the pliability tester and a centimetre scale respectively and the values were converted into scores as reported earlier[10]. All the experiments were carried out in quadruplicate and statistical analyses were done using standard methods[11]

Sieve analysis of flour samples (Table 1) indicated that wheat had become more friable on germination as indicated by higher amount of fine flour fractions passing through 10 XX sieve (78.6 per cent) as compared to flour obtained from sound ungerminated wheat (69.4 per cent).

TABLE 1. EFFECT OF GERMINATION ON THE PARTICLE SIZE DISTRIBUTION OF WHOLE WHEAT FOUR (*ATTA*)

Sieve	Sieve opening (μ)	Overtailings – flour: Sound wheat (%)	Germinated wheat (%)
32	670	1.2	2.0
45	480	2.6	4.4
50	355	6.6	3.3
7 XX	193	12.0	5.0
10 XX	129	8.2	6.0
12 XX	112	28.0	26.0
15 XX	85	12.7	16.9
25 P	62	24.7	30.4
Pan	–	4.0	5.3

TABLE 2. EFFECT OF GERMINATION ON SOME QUALITY CHARACTERISTICS OF WHOLE WHEAT FLOUR

Characteristics*	Sound wheat	Germinated wheat
Moisture (%)	8.1	9.3
Ash (%)	1.9	2.1
Protein (%)	9.9	10.3
Gluten (%)	9.4	8.8
Reducing sugars (%)	2.8	4.3
Damaged starch (%)	10.7	14.0
Chapati water absorption (%)	68.6	59.4
Falling number	422	62
Diastatic activity (mg/ 10 g flour)	315.0	475.0

*All values except moisture are expressed on 14% moisture basis.

The chemical characteristics of flour (Table 2) showed that gluten content was reduced on germination from 9.4 to 8.8 per cent possibly due to degradation during sprouting[12]. Falling number which reflects alpha amylase activity decreased, while reducing sugars increased as expected during germination; diastatic activity in germinated wheat was higher than in ungerminated wheat (control).

The flour obtained from germinated wheat required less water (59.4 per cent) than control wheat flour (68.6 per cent) for preparing chapati dough of optimum consistency and this indirectly resulted in lower yield of chapaties. The lower water absorption of germinated wheat flour may be possibly due to degradation of gluten as also the loss of carbohydrate[12]. Though there was no difference in the rolling characteristics of the freshly mixed dough, on resting, the dough made from germinated wheat flour developed slight stickiness.

TABLE 2. EFFECT OF GERMINATION OF WHEAT ON THE QUALITY OF CHAPATI

Quality parameter	Storage period (days)	Sound wheat	Germinated wheat
W.B. Shear value (lb)	0	8.00 ± 0.41	12.00 ± 0.41***
	4	4.00 ± 0.41	7.00 ± 0.91***
Significance+	–	***	***
Height on Puffing (10+)	0	8.80 ± 0.41	6.38 ± 0.25***
	4	–	–
Significance+	–	–	–
Pliability (10+)	0	8.62 ± 0.75	3.98 ± 0.41***
	4	9.85 ± 0.19	7.50 ± 0.58***
Significance+	–	*	***
Appearance of spots (20+)	0	16.25 ± 0.64	12.12 ± 0.85***
	4	11.12 ± 0.48	9.50 ± 0.41**
Significance+	–	***	**
Hand feel (10+)	0	8.00 ± 0.82	6.50 ± 0.41*
	4	4.75 ± 0.64	6.00 ± 0.41*
Significance+	–	***	NS
Eating quality (25+)	0	17.62 ± 0.48	14.00 ± 0.41***
	4	9.62 ± 0.48	12.50 ± 0.41***
Significance+	–	***	**
Taste (25+)	0	14.50 ± 0.58	18.38 ± 0.48***
	4	11.25 ± 0.29	13.62 ± 0.75**
Significance+	–	***	***

+Represents significance between fresh and stored chapaties
Significant at:*** 0.1%, ** 1% and * 5%;
NS: Not significant
+ Maximum score

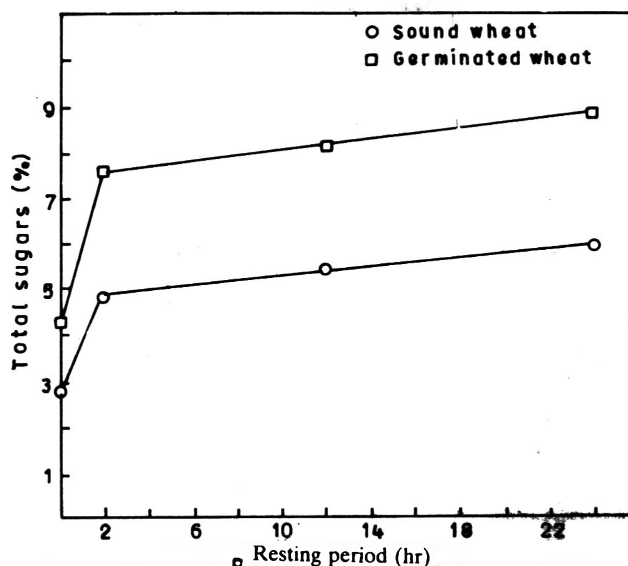

Fig. 1. Changes in sugar content during resting of chapati dough.

Increase in reducing sugars during resting was higher for the dough made from germinated wheat flour (4.3 to 8.5 per cent) than that for control wheat flour (2.8 to 5.3 per cent). This is evidently due to the higher amylase activity in germinated wheat flour. The increase in sugars was steep in the first 2 hr of resting and then it was gradual (Fig.1).

Chapati made from germinated wheat was less pliable and it had darker charred coloured spots as compared to normal chapati. The eating quality of chapati made from germinated wheat flour was poor because of harder texture as indicated by the lower score of 14.0 compared to 17.6 for control chapati as also by its higher W.B. shear value which is related to the sensory texture[10]. However, chapati made from germinated wheat had sweetish taste due to its higher sugar content.

The stored germinated wheat chapati had better eating quality and taste while the control chapati had become dry and brittle on storage. The better eating quality of stored chapati made from germinated wheat is probably due to the high sugar and dextrin contents which confer better water retention capacity. The over-all quality as indicated by the total score was higher for stored chapati made from germinated wheat than that from control wheat.

The above studies indicate that acceptable chapaties can be made from germinated or rain damaged wheat.

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References

1. Selvaraj, A., Leelavathi, K., Haridas Rao, P., and Shurpalekar, S.R., On improving the bread making quality of flour from field sprouted wheat. *J. Fd Sci. Technol.*, 1986, **23**, 24.

2. Huang G. and Varriano – Marston, E., α–amylase activity and pre-harvest sprouting damage in Kansas hard white wheat, *J. agric. Fd Chem.*, 1980, **28**, 509.

3. Tipples, K.W., Kilbern, R.W. and Bushuk, W., Effect of malt and sprouted wheat. *Cereal Sci. Today*, 1966, **11**, 362.

4. Ibrahim, Y. and D'Appolonia, B.A., Sprouting in hard red spring wheat, *Baker's Dig.*, 1979, **53**, 17.

5. Vatsala, C.N., and Shurpalekar, S.R., Suitability of germinated wheat for the preparation of biscuits and cakes. *Indian Miller*, 1985, **15** (4) 27.

6. Lorenz, K., Cereal Sprouts; Composition, nutritive value, Food application. CRC *Crit. Rev. Fd Sci. Nutr.*, 1980, **13**, 353.

7. *Cereal Laboratory Methods*. American Association of Cereal Chemists, St. Paul, Minnesota, USA, 1962, 7th Edn.

8. *Methods of Analysis*. Association of Official Agricultural Chemists, Washington, D.C. 1970.

9. Haridas Rao, P., Leelavathi, K. and Shurpalekar, S.R., Test baking of chapati. Development of a method. *Cereal Chem.*, 1986, **63**, 297.

10. Venkateswara Rao, G., Leelavathi, K., Haridas Rao, P. and Shurpalekar, S.R., Changes in the quality characteristics of chapati during storage. *Cereal Chem.*, 1986, **63**, 131.

11. Snedecor, G.W. and Cochran, W.G., *Statistical Methods*. The Iowa State University Press, Iowa, 1967.

12. Hwang, P., Bushuk, W., Some changes in the endosperm proteins during sprouting of wheat. *Cereal Chem.*, 1973, **50**, 147.

13. Haridas Rao, P., Leelavathi, K. and Shurpalekar, S.R., Objective measurements of the consistency of chapati dough using a Research Water Absorption Meter. *J. Texture Studies*, 1987, **17**, 401.

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LIPID CHARACTERISTICS AND PHOSPHOLIPID COMPOSITION IN SOME BEANS OF KASHMIR VALLEY

S.K. KATIYAR AND A.K. BHATIA
Regional Research Laboratory (CSIR), Canal Road,
Jammu – 180 001. India

Received 20 April 1987; revised 14 August 1987

Commonly consumed legumes viz. *Phaseolus vulgaris, Vicia faba* and *Vigna auriculata* of Kashmir valley have been analysed for their phospholipid composition. The beans contain 1.9-2.5% lipids of which phospholipids constitute 35-55%. Phosphatidyl ethanolamine, the major component, varies from 30-53% of the total phospholipids. The major fatty acids (%) are: 16:0, 16-23; 18:1, 11-18; 18:2, 24-30 and 18:3, 18-35.

Legumes constitute important dietary components in many parts of the world. In Jammu and Kashmir State, *rajmah (Phaseolus vulgaris)* and *rongi (Vigna auriculata)* are the main pulses consumed, while *bogla (Vicia faba)* is not so commonly used pulse. The phospholipids present in the total lipids of these three pulses have been examined.

Three indigenous varieties of rajmah, viz. 'Faram', 'Thool' and 'Ledder' were procured from the growing regions. 'Thool,' *rajmah* is mainly cultivated in Bhadrawah area of Jammu region at an altitude about 1,000 to 1,500m, while 'Ledder' *rajmah* is cultivated in Ledder valley of Kashmir region at an altitude of about 2,000 to 3,000m. Healthy and mature legumes of *rongi* and *bogla* were collected from Srinagar market. The seeds were botanically identified, cleaned, dried, and ground to 100-120 mesh size.

The oil from each seed was extracted by using chloroform; methanol mixture (2:1 v/v), and non-lipid impurities removed[1]. The total content of phospholipid was determined in the lipid by assaying the phosphorus content[2] and multiplying[3] by 25. Crude phospholipids were separated by precipitating with chilled acetone and centrifugation. Phospholipids were resolved by thin layer chromatography on silica gel G coated plates developed in chloroform: methanol:acetic acid:water (50:28:10:5, v/v)[4]. Authentic phospholipids were co-chromatographed in each run, and located by exposure to iodine vapour and spraying with alkaline rhodamine-6G[5]. Amino groups in the phospholipid were further confirmed by spraying with ninhydrin (0.2 per cent in butanol) and heating at 80°C for 15 min, choline-containing phospholipids were detected by the Dragendroff's reagent as modified by Wagner *et al*[6]. Individual silica gel spots were eluted in chloroform – methanol – acetic acid – water (25:10:4:2, v/v). Phosphorus in each constituent was estimated according to Barlett[7].

Fatty acid composition in each phospholipid was assayed by gas liquid chromatography of the methyl esters[8] under the following conditions:
Pye-Unicam GLC instrument, 204 series; Reoplex glass column (6 mm × 2m); FID detector; nitrogen carrier gas, flow rate 35 ml/min; column at 190°C, injection port 270° and detector 300°C.

Table 1 shows the physico-chemical characteristics of the total lipids of the pulses studied. The high

TABLE 1. PHYSICOCHEMICAL CHARACTERISTICS OF SEED OILS

Legume/var	Refr. index $n_d 28°$	Sapon. value	Iodine value	Unsapon. matter (%)
Phaseolus vulgaris				
Faram	1.489	131.2	151.0	6.58
Thool	1.487	132.0	149.6	6.75
Ledder	1.487	131.8	149.8	6.70
Vicia faba	1.485	132.3	147.1	7.00
Vigna auriculata	1.474	133.2	149.4	6.85

Values are means of triplicate determinations

TABLE 2. PHOSPHOLIPID CONSTITUENTS OF LEGUMES

Legume/var	Oil (%)	Total phospholipid (%)	% phos. choline	% phos. ethanolamine	% phos. serine	% phos. inositol
Phaseolus vulgaris						
Faram	2.52	35.8	30.2	32.4	22.8	13.3
Thool	2.54	38.5	33.4	53.6	5.6	7.0
Ledder	1.90	39.5	36.2	30.6	14.1	15.1
Vicia faba	2.00	46.0	40.2	43.1	5.3	11.4
Vigna auriculata	2.51	55.0	40.0	40.0	traces	19.7

Values are means of triplicate determinations

TABLE 3. FATTY ACID COMPOSITION (PER CENT BY WEIGHT) OF TOTAL PHOSPHOLIPIDS

Fatty acids	Rajmah Faram	Rajmah Thool	Rajmah Ledder	*Vicia faba*	*Vigna auriculata*
10:0	–	0.6	0.5	0.8	1.4
12:0	–	–	–	–	1.4
16:1	1.5	3.2	1.2	1.7	5.2
16:0	23.0	19.5	18.7	16.5	18.2
18:0	2.2	5.9	8.5	6.7	9.0
18:1	11.1	18.0	15.2	17.3	15.6
18:2	26.5	25.0	24.4	27.0	30.6
18:3	35.7	27.8	31.5	30.0	18.7

Values are means of triplicate determinations. Fatty acids below 0.5% not included.

iodine values (147.1–151.0) and per cent unsaponifiable matter (6.58–7.00) are note worthy.

The lipid content of the pulses ranged from 1.9 to 2.5 percent (Table 2). The phospholipid content of the total lipid (35.8 – 55.0 per cent) was high, particularly in *rongi*. The major fraction of the phospholipid is phosphatidyl ethanolamine which varies from 30.6 to 53.6 per cent while phosphatidyl choline made up 30-40 per cent. Phosphatidyl serine ranged between 5.3 and 22.8 per cent and phosphatidyl inositol from 7.0 to 19.7 per cent.

The major fatty acids in the total pulse phospholipids are palmitic 16.5 – 23.0 pr cent, oleic 11.1 – 18.0 per cent, linoleic 24.4 – 30.6 per cent and linolenic 18.7 – 35.7 per cent (Table 3). The lower fatty acids noted may be artifacts or oxidation products. The high proportions of the two essential fatty acids, linoleic and linolenic, which cannot be biosynthesised and must come from the diet, show the importance of dietary pulses as source not only of protein, but also of dietary fat.

References

1. Folch, J., Lees, M. and Sloane-Stanley, G.H., A single method for the isolation and purification of total lipids from animal tissues, *J. biol. Chem.*, 1957, **226,** 497.
2. Marinetti, G.V., Chromatographic separation, identification and analysis of phosphatides, *J. Lipid Res.*, 1962, **3,** 1.
3. Colowick, S.P. and Kaplan, N.O., *Methods in Enzymology*, Academic Press, Inc., New York, 1957, Vol. III, 329.
4. Skipski, V.P., Peterson, R.F. and Barelay, M., Quantitative analysis of phospholipids by thin layer chromatography, *Biochem. J.*, 1964, **90,** 374.
5. Rouser, G., Bauman, A.J. and Kritchevsky, G., New methods for the separation and quantitative isolation of lipids-Initial applications to the study of beef brain and spleen lipids in Gaucher's disease, *Am. J. Clin. Nutr.*, 1961, **9,** 112.
6. Wagner, H., Horhammer, L. and Wolf, P., Serum lipids and glycoproteins in atherosclerosis and diabetes, *Biochem. J.*, 1961, **334,** 175.
7. Bartlett, G.R., Phosphorus assay in column chromatography, *J. biol. Chem.*, 1959, **234,** 466.
8. Kates, M., Simplified procedure for hydrolysis or methanolysis of lipids, *J. Lipid Res.*, 1964, **5,** 132.

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HISTOLOGICAL CHANGES IN FRESH WATER FISH MUSCLE STORED IN CHILLED CONDITION

A.V. GIRIJA MENON AND R. BALAKRISHNAN NAIR
Animal Products Technology Discipline,
Central Food Technological Research Institute,
Mysore 570 013, India.

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Structural changes occurring in the skeletal muscle of the fish *Labeo calbasu*, a major carp, during ice storage were studied histologically. The muscle samples were examined in fresh fish and at the end of 7, 14 and 21 days of storage. Fresh muscle tissue showed closely packed uniform bundles of myofibrils, with well-defined striations and clearly visible nuclei. The connective tissues which cemented together the fibres and myotomes were also distinctly discernable. On storage, there was progressive disruption in this compact picture, with deterioration of the organisation of the structure characterized by fissures and breaks in the fibres leading to dislocation and mutilation of myofibrils. Gaps appeared in areas originally occupied by connective tissues, suggesting major alterations or even disintegration of the latter. Such changes were in line with concomitant softening in the texture of the muscle observed in the stored fish.

Degradation of muscle proteins is a major reaction in the spoilage process in fish. This is brought about by endogeneous proteases especially cathepsins or by bacterial activity or both. In chill stored marine fish like cod, bacterial activity is known to start and proceed right from the early stages along with endogenous proteolytic activity. Putrefactive changes are evident from the start, as the activity of spoilage organisms which are predominently psychrophilic is not effectively suppressed by reduction in temperature[1]. But in tropical fresh water fish like carps, a different pattern of spoilage has been observed. They seem to possess a long shelf life in ice[2,3]. Rate of growth of bacteria was sluggish for a considerable period during ice storage and putrefactive changes in terms of sensory and of chemical indices were evident only at later stages of storage. However, there was clear evidence of some proteolysis taking place in the muscle right from the beginning. This would indicate a relatively more important role for autolytic activity and a lesser role for microbial activity in the spoilage process in tropical fresh water fish[4], especially during the early stages of storage.

The gross effect of autolysis and consequent alterations in the structural elements in the muscle viz. connective tissue and the myofibrillar proteins are expected to be reflected in the microstructure of the muscle. The present study is intended to follow such changes histologically in fish stored under chilled conditions.

Labeo calbasu, a major carp was used for the studies. They were procured from nearby landing centres, brought to the laboratory, dressed and filleted. The fillets were packed in polyethylene pouches, chilled quickly by immersing in crushed melting ice and stored at 0-1°C. The analysis was done at specified intervals.

Tissue blocks 0.5 cm in thickness were removed from the fillets and immediately fixed in 10 per cent formalin for 24 hr. They were washed in running water and dehydrated using series of alcohol solutions of gradually increasing strength and cleared in xylene. After clearing, the tissue was infiltrated with and embedded in paraffin (56-58°C) and sectioned with a rotary microtome into 11-12μ thick sections. The slides were prepared by routine histological procedure[5]. The sections were stained with (i) Haematoxylin–Eosin, (ii) Weigerts iron Haematoxylin-Van Gleson stain[6]. The muscle fibres were stained yellow-orange and the collagen tissue bright red. The tissue sections were observed under a Zeiss research microscope.

In longitudinal sections of fresh muscle, the myofibrils were closely packed and arranged as typical bundles of ribbons. The fibres were straight or slightly wavy and slender with narrow spaces in between (Fig. 1A). The observations were similar to those in mammalian muscle[7-9], and in fish[10] by earlier workers. The striations were clear and nuclei distinct. Connective tissue joining two myotomes was discernable distinctly. The cells appear closely packed in the transverse section (Fig. 1B) and connective tissue could be seen clearly. Differentiation between perimysium and endomysium is well marked.

Early signs of disorganization of the muscle structure were characterized by a loss of compactness of the fibres after seven days. Cracks and fissures appeared in the fibres as shown in both longitudinal and transverse sections (Fig. 1C and 1D). These cracks and splits increased in number on further storage, and on the 14th day most of the cells lost their identity, strands being loosened and appeared wavy and intermingled (Fig. 1E and 1F). This is a clear indication of the fibre membrane breaking and myofibrillar contents falling apart. Also linear breakage of myofibrils was also evidences by the fragmentation of the strands. At certain places, linear fissures occurred earlier to breaks in the fibres, suggesting segregation of myofibrils preceding their

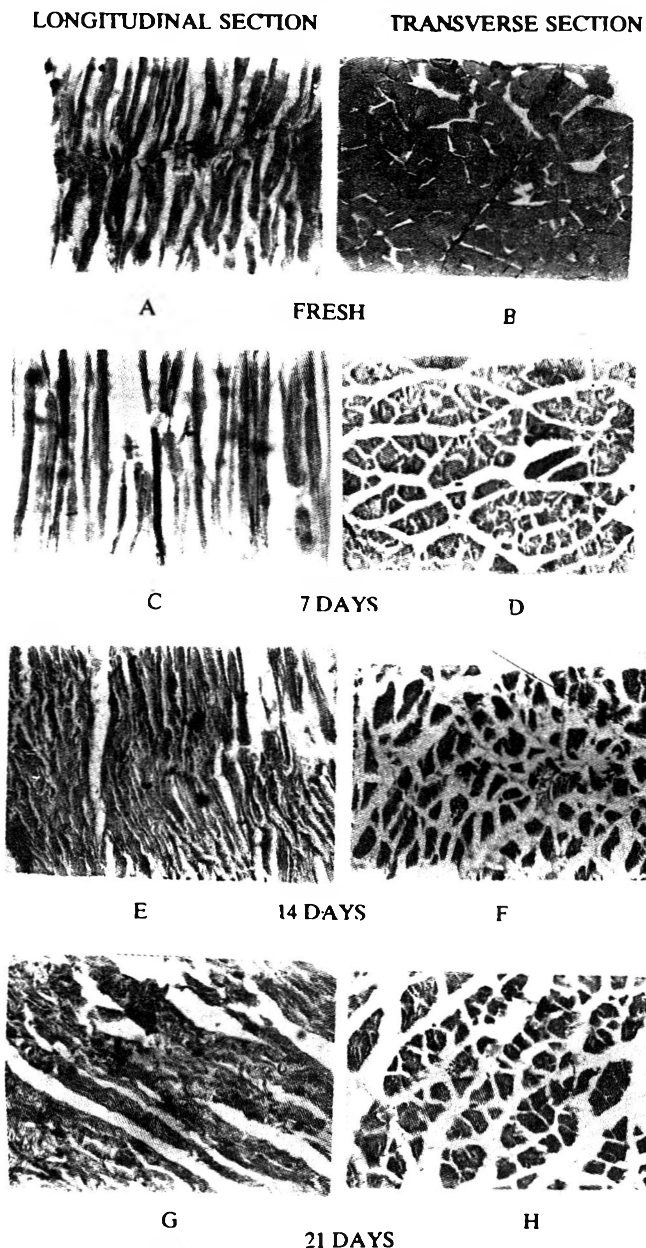

Fig. 1, Histological changes in fresh water fish muscle stored in chilled condition

fragmentation. Extracellular space between fibres and endomysium increased gradually, with partial (Fig. 1F) and later complete disappearance of connective tissues (Fig. 1H). The latter have either disintegrated or lost their staining ability. Consequently gaps or channels appeared at many places. Widening of spaces between the fibres and bundles was previously reported in fish muscle tissue in post-rigor condition[11]. Using scanning electron microscopy, in fish blue grenadier *(Macruronus novaezelandiae)* after 5 days storage in ice, the connections between fibres and connective tissues became less evident and at the fibre surface both the endomysium and plasmalemma layer beneath showed signs of degradation[12]. Towards the end, complete meshing and disintegration were observed (Fig. 1G) with disappearance of longitudinal and cross striations which marked the normal organization of the muscle cell structure. The nuclei also seemed to have disappeared.

From these observations, it may be seen that both the connective tissues and myofibrillar proteins have been affected in equal measure and almost concurrently. The absence of significant putrefactive changes during the storage of carps in ice during early periods as previously reported[2], indicates that cathepsin may be the major contributor to such autolytic changes. This fact, however needs further confirmation through studies under controlled conditions, where bacterial activity is suppressed.

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References

1. Shewan, J.M., The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. *"Proceedings of the Conference on the Handling, Processing and Marketing of Tropical Fish'*, July 1976, TPI, London, 51.

2. Balakrishnan Nair, R., Tharamani, P.K. and Lahiry, N.L., Studies on chilled storage of fresh water fish I. Changes occurring during iced storage. *J. Fd Sci. Technol.*, 1971, **8**, 53.

3. Balakrishnan Nair, R., Tharamani, P.K. and Lahiry, N.L., Studies on chilled storage of fresh water fish-II. Factors affecting quality. *J. Fd Sci. Technol.* 1974, **11**, 118.

4. Disney, J.G., Cole, R.C. and Jones, N.R., Considerations in the use of tropical fish species. *Fishery Products*, by Rudolf Kreuzer, (Edn) 1974, 329.

5. Gray, P., *The Microtomist's Formulary and Guide.* The Blakiston Company, Inc., New York, 1954, 94.

6. Clayden, E.C., *Practical Section Cutting and Staining*, J and A Churchill Ltd., London, 1951, 58, 2nd Edn.

7. Paul, R.C., Lowe, B., McClurg, B.R., Changes in histological structure and palatability of beef during storage. *Fd Res.*, 1944, **9**, 221.

8. Dorothy, L.H., Lowe, B., McClurg, B.R. and Shearer, P.S., Physical, organoleptic and histological changes in three grades of beef during aging. *Fd Technol.*, 1949, **3**, 284.

9. El Dashlouty, M.S. and El-Ashry, M.A., Studies of some histological changes in buffalo meat during autolysis. *16th European Meeting of Meat Research Workers*, 1970, I, 92.

10. Murray, C.K., and Gibson, D.M., The histology of blue whiting. *Advances in Fish Science and Technology*. Connel J.J. (Ed.), Fishing News (Books) Ltd.. England. 1980, 503.

11. Bello, R.A., Luft, J.H. and Pigott, G.M., Improved histological procedure for microscopic demonstration of related changes in fish muscle tissue structure during holding and freezing. *J. Fd Sci.,* 1981, **46,** 733.

12. Bremmer, H.A., and Hallett, I.C., Muscle fibre connective tissue junctions in the fish blue grenadier *(Macruronus novaezelandiae)*. A scanning electron microscope study. *J. Fd Sci.*, 1985, **50**, 975.

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HIGH PRESSURE LIQUID CHROMATOGRAPHIC DETERMINATION OF ARGEMONE OIL IN EDIBLE OILS

T.N. MURTHI, (Mrs) M. SHARMA AND V.D. DEVDHARA
National Dairy Development Board, Anand - 388001, India

Received 3 July 1987; revised 26 September 1987

A rapid liquid chromatographic method has been developed for the determination of argemone oil in groundnut and mustard oils. This is based on the appearance of a peak with 3 min R_T for sanguinarine using a Zorbax silica column and a fluoresence detector. Argemone oil in edible oils at as low as 20 p.p.m can be determined.

Argemone mexicana plant which grows abundantly in waste lands all over the country mainly during March to May produces numerous seeds resembling mustard seeds. Argemone oil obtained from these seeds is known to contain toxic alkaloids namely sanguinarine and dihydrosanguinarine which cause acute and toxicological symptoms such as epidemic dropsy, necrosis, etc., in humans even at low levels of contamination in edible oils[1-3]. Based on results of animal studies, 0.01 per cent argemone oil in edible oils has been suggested as a 'safe limit'[4]. A few methods based on colorimetry,[5-7] thin layer chromatography[8] and paper chromatography[9,10] are in use for the determination of argemone oil in other oils. In general, these methods, though claimed to be simple, rapid and also capable of determining its presence at low levels, require about three hours and further confirmation of the fluorescence spots. Hence, there is a need to develop a simple, fast and accurate method for the determination of argemone oil in other oils. The high pressure liquid chromatography (HPLC) method described here involves extraction of alkaloids from a few milligrams of argemone oil/suspected oil sample with HPLC grade methanol, injection of the methanol extract directly onto a Zorbax silica column without any further clean up procedures and detection using a fluorescence detector.

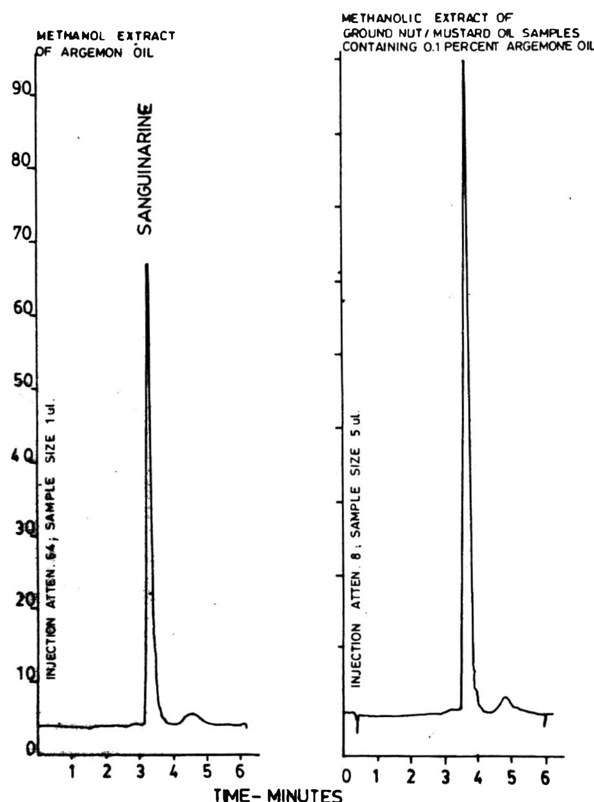

Fig. 1. Fluorescence detection of Argemone oil; Methanolic Extract injected on Zorbax Silica Column; conditions;- Flow Rate 1 ml/min; Mobile Solvent-Methanol; Excitation at 335 nm and emission at 490 nm.

Pure argemone oil was obtained by pressing the argemone seed in a laboratory hydraulic press. Other oils used in this study were refined groundnut oil, expeller groundnut oil and ghani mustard oil. A stock solution containing 0.07 g of argemone oil in 90 g of refined groundnut oil was prepared. From this stock solution, 10g were taken and diluted to 100g with refined groundnut oil to give a standard for argemone oil (80 p.p.m). This standard solution was used in preparing groundnut oil/mustard oil samples containing argemone oil levels of 19, 41, 57, 76 p.p.m. Higher concentrations of argemone oil in these oils at 0.08, 0.18, 0.40, 0.70 and 1.0 per cent levels were also prepared separately and all the samples were thoroughly shaken for a few minutes for uniform mixing.

A Shimadzu HPLC LC-4A unit equipped with a RF-530 fluroscene detector was used for all analyses. Its sensitivity is 0.45 ng/12μl quinine sulphate, 12μl being the volume of the square flow cell made of quartz, on a stationary phase of Zorbax silica column (4.6mm × 25 cm). In a series of 5 ml glass stoppered vials about 0.2g of each oil sample containing argemone oil at different concentrations was shaken vigorously with 4 ml of HPLC grade methanol for 2 min. Pure argemone oil (0.1g) was also shaken with 50 ml of methanol in a volumetric flask as a control sample. All these samples were allowed to stand for about 10 min to separate the phases. After stabilising the HPLC at a flow rate of 1 ml/min, the excitation at

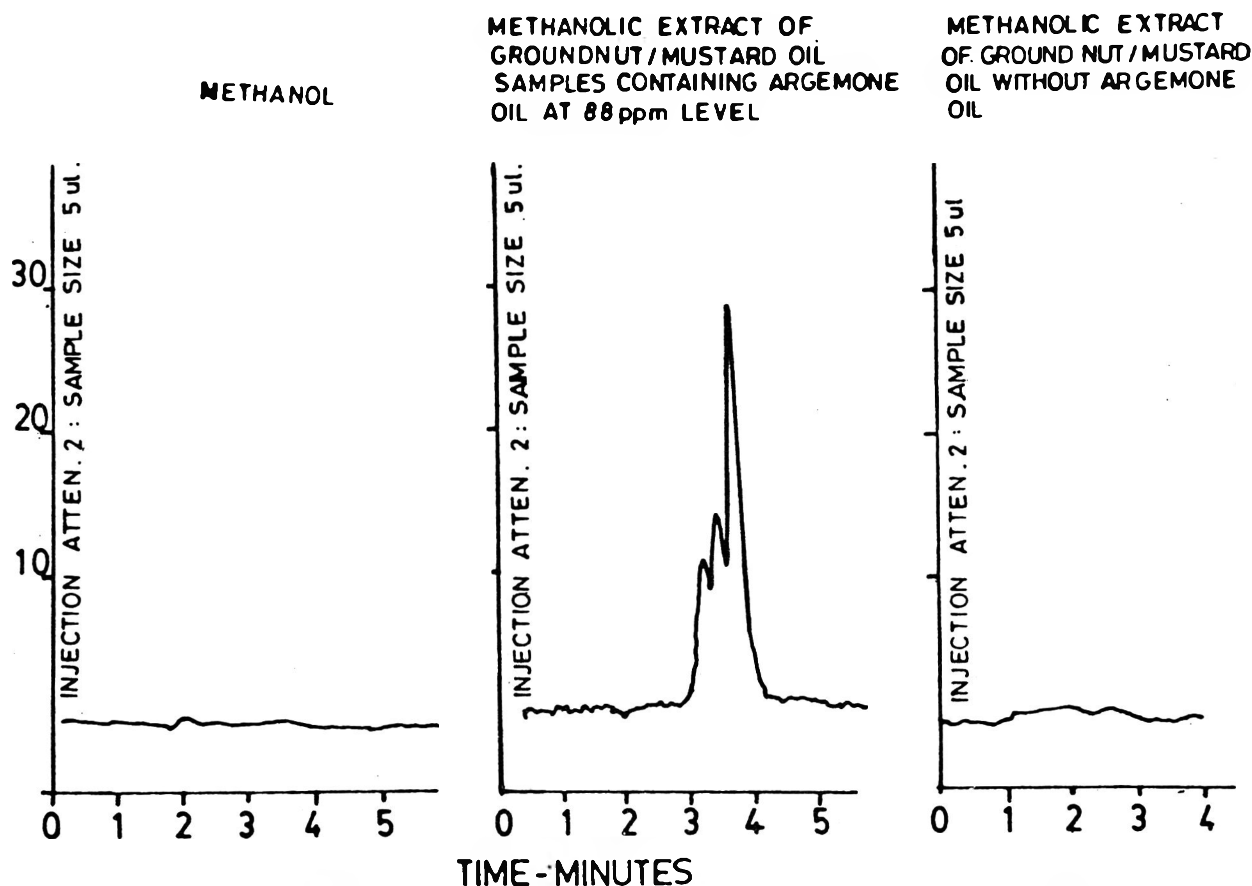

Fig. 2. Fluorescence detection of argemone oil; methanolic extract injected on Zorbax silica column; conditions;- Flow rate 1 ml/min; Mobile solvent-Methanol; Excitation at 335 nm and emission at 490 nm.

335 nm and emission at 490 nm was set on the fluorescence detector and, without any further clean-up procedure. the methanolic laver (1-5 μl) was injected. Aliquots of the acid extract of argemone oil, was also spotted on to a TLC[8] silica gel 'G' plate and developed in butanol – acetic acid – water (63:10:27). After drying the plate first to room temperature, an orange or golden spot was located at Rf 0.45 (sanguinarine) under UV light. The silica gel at this spot was carefully collected in a vial and shaken with known volume of methanol.

In about 3 min of injection of the methanolic extract of pure argemone oil, a single peak appeared (Fig. 1). Also, injection of 1 to 5 μl of methanolic extract from the samples containing argemone oil at different levels gave a similar peak at the same retention time (Fig. 1). As the concentration of the argemone oil decreased to p.p.m. levels, the sensitivity of the detector was increased to give peaks of about 50 per cent full scale recorder deflection (Fig. 2). However, it is desirable to use the same detector sensitivity for both extracts of pure argemone oil and samples.

Identification of this peak as the alkaloid sanguinarine, present in argemone oil was also confirmed since the methanolic extract of the orange spot collected from the TLC (located at Rf 0.45) plate gave a single peak in about 3 min while the methanol solvent (blank) and methanolic extracts from pure groundnut oil and mustard oil did not give any peak.

The concentration of alkaloid in the methanolic extract of pure argemone oil was determined from the UV absorbance measurements of this extract using a Shimadzu UV/visible 240 spectrophotometer equipped with a double beam ratio recorder (Fig.3). The mM concentration of sanguinarine in this extract was calculated using E^{1cm} = 33110.

The calibration curve of known weight of argemone oil present in different samples against their peak heights as determined by HPLC is shown in Fig. 4 from which the level of argemone oil in unknown samples can also be estimated. It is essential to limit the size of oil sample to a few mg (200 to 300 mg) in 4 ml methanol for the complete extraction of the alkaloid. If the sample size is increased, the ratio between sample (g) and methanol should be 1:25. Thus, the detection limit of argemone oil by this

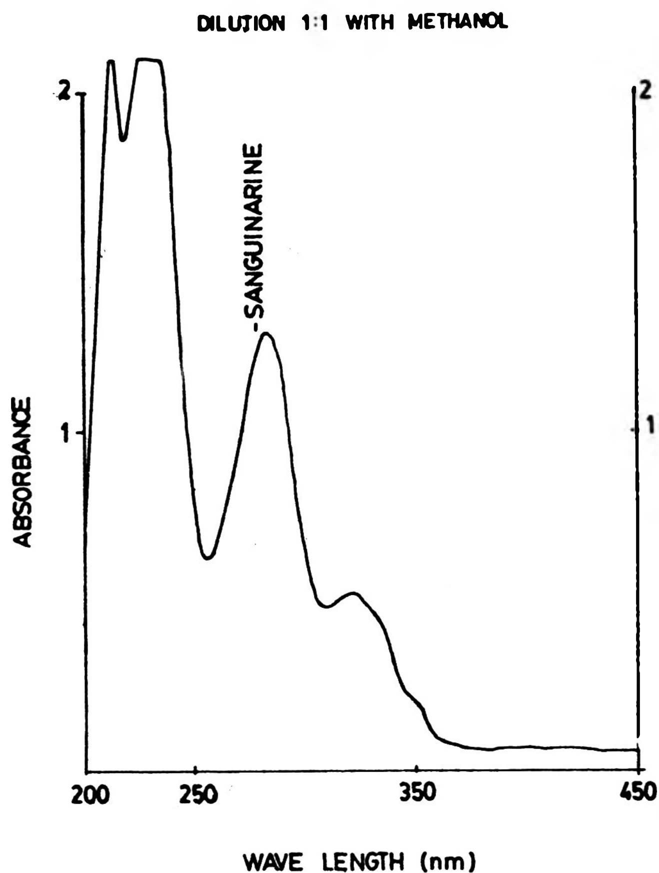

Fig. 3. Absorption spectra of methanolic extract of argemone oil.

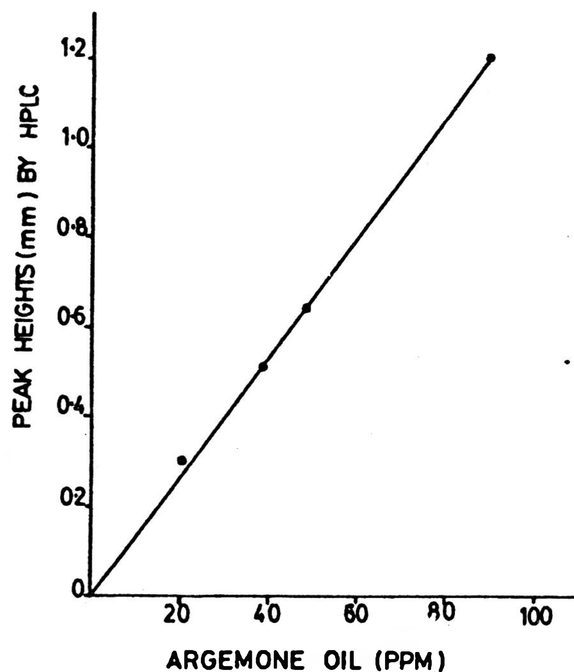

Fig. 4 Relationship between peak heights and ppm levels of argemone oil in other oils (Musstard & Groundnut oils)

method in other oils is as low as 20 p.p.m. However, the content of the alkaloid, sanguinarine, in argemone oil obtained from different sources will not be the same, which may influence the accuracy of the results.

References

1. Krishnamachari, K.A.V.R. and Satyanarayana, K., Epidemic dropsy in Andhra Pradesh. *Indian J. med. Res.*, 1972, **60**, 741.
2. Rukmini, C., Sanguinarine potentiating factor in argemone oil. *Indian J. med. Res.*, 1971, **59**, 1976.
3. Shenolikar, I.S., Rukmini, C., Krishnamachari, K.A.V.R. and Satyanarayana, K., Sanguinarine in the blood and urine of epidemic dropsy. *Fd Cosmet. Toxicol.*, 1974, **12**, 699.
4. *Annual Report, 1974,* National Institute of Nutrition, Hyderabad, 1975, 47.
5. Bose, P.K., A New colorimetric method for estimation of argemone oil, *J. Am. Oil Chem. Soc.*, 1972, **49**, 201.
6. Pundlik, M.D, and Meghal, S.K., Spectrophotometric identification and estimation of argemone oil. *Res. Ind.*, 1977, **22**, 182.
7. Mohiuddin, M.M. and Zaidi, H.R., Spot and colour tests for detection of *argemone mexicana* seed oil. *J. Am. Oil Chem. Soc.*, 1973, **50**, 543.
8. Shenolikar, I.S., Babu, S., Thapar, G.S. and Ramasastri, B.V., Quantitative determination of argemone oil in edible oils by thin layer chromatography. *Indian J. med. Res.*, 1981, **73**, 204.
9. Kasbekar, M.G., Miss Kanga, K.F., Patel, S.M. and Bringi, N.V., Detection of argemone oil in edible oils. *J. Oil Technol. Ass. India,* 1973, **5**, 1.
10. Hartman, C.P., Nagaraja Rao, U.N. and Divakar, N.G., Paper chromatographic method of the detection of argemone seeds in mustard seeds. *Curr. Sci.* 1972, **42**, 465.

BOOK REVIEWS

Developments in Food Preservation: Vol. 4, Stuart Thorne, (Ed) Elsevier Applied Science Publishers, Crown House, Linton Road, Barking Essex – IG 11 8 JU, England; 1987; pp:278; Price $ 39.

The book under review has as its theme the quality of preserved foods. In a timely manner, the contents of the book sheds more light on the controversial question as to which is superior, whether the home cooked food or the preserved food. The book itself contains seven chapters ranging from the nutritional changes in food processing, packaging of thermally stabilised foods, effect of refrigeration on quality, the blanding process and to engineering aspects such as microcomputer based process control, food freezing aspects and finally the developments in the production of fermented and pickled vegetables.

In the first chapter dealing with nutritional changes in food processing, the losses due to processing of the various components of food ranging from vitamins, proteins down to lipids and minerals are given. Surprisingly no definite conclusions are drawn. The second chapter gives an overview of the growth of microcomputer applications with special reference to process control in food industry. Important topics such as the choice of the control systems architecture and choice of appropriate systems for a given applications are outlined.

The next chapter deals with the recent advances in heat sterilisation both from the view point of packaging materials and energy efficiency. Food preservation by the use of aseptic packaging techniques is discussed indicating the possible future developments in these areas. The use of low temperatures in food preservation is well known but less well known in the losses in nutrition and sensory values. The chapter on refrigeration and quality of prepared foods describes the various physico-chemical aspects and concludes that the freezing process causes the greater damage in texture of some foods than the chilling process. The next two chapters mainly deal with engineering aspects of food freezing and the blanching process – essentially a review of the physical mechanisms, heat transfer rates and the different variations of the same process. The effect of the blanching process on the changes in food quality has been well described. The last chapter addresses itself to the problem of preservation in vegetable processing industry in the form of fermented and pickled vegetables.

The book has a fairly high rating in terms of its reference value but does not provide much critical analysis of the subject. Perhaps this shortcoming is due to the nature of the subject itself.

G.D. SURENDER
REGIONAL RESEARCH LABORATORY,
TRIVANDRUM.

Seafoods and Fish Oils in Human Health and Disease: Series 23: by John E. Kinsella, Marcel Dekker, Inc, 270, Madison Avenue, New York, N.Y. 10016; 1987; pp:336; Price: $ 99.75 (US and Canada), $ 119.50 (All other countries)

At this time of increased public awareness about everything natural such as natural food, natural food additives, natural medicine, etc., the observation that dietary fish offers protection against certain diseases has attracted a lot of attention from scientists and the general public as well. Fish lipids due to their high content of polyunsaturated fatty acids (PUFA) of ω-3 series are mainly responsible for the beneficial effects. The author of this book Dr. J.E. Kinsella, a well known scientist in the field of PUFA metabolism has done a commendable job by covering all aspects of fish and fish oils connected with diet. This is the 23rd book published as a series on Food Science and Technology.

This slim book is divided into 11 Chapters, some providing a broad background information and the rest dealing with specific indepth studies. The first four chapters which form a major part of the book discuss in detail the role of PUFA in cardiovascular diseases, formation of eicosanoids and feeding trials in human volunteers and laboratory animals. A link between dietary PUFA of ω-6 series with cancer is briefly discussed in a separate but small chapter (No. 5) and a possible beneficial role of ω-3 PUFA is suggested though not tested so far. Practical difficulties of getting enough ω-3 PUFA comparable to those of feeding trials or consumption of Eskimos in a common man's diet was appropriately discussed in Chapter 6. Fish oil is not only a rich source of fat soluble vitamins A & D, but also contains cholesterol which is an undesirable component as far as the

therapeutic value is concerned. These aspects are dealt with in Chapter 7. Due to the high PUFA content, fish oils are easily oxidized and the oxidized products are more cytotoxic. In addition, fish oil may be contaminated with toxic pollutants such as DDT, PCB etc. These safety aspects of fish oil as discussed in Chapter 8 could be very informative to many readers, whereas the technological aspects of fish oil processing dealt in Chapter 9 is of limited value, but makes the story on fish oil complete. Chapter 10 is nothing but an overview of the book. Lot of information about the types of edible fishes of U.S. origin with their lipid content and fatty acid composition are presented in Chapter 11. This would be very useful to dietitians.

The role of PUFA in biomembranes especially their effect on membrane fluidity has been totally ignored in this book. This aspect should have been discussed at least as a small section in a Chapter. Structures of eicosanoids are totally missing in the book (except as decoration on the front cover). I think structures help the reader to understand the text better. Typical structures of important metabolites at least in the form of a single figure should have been given in Chapter 2. There are some typographical errors, though not of a serious nature. In general, this book is well written, covers both clinical and basic aspects in most instances. Therefore, it could serve as a good source book for food scientists, nutritionists, dietitians and other biomedical researchers interested in PUFAs in health and disease. Finally, the title of this book is bound to attract many non-scientific/non-lipid scientific public readers, eventhough it is heavily dosed with scientific data inside. However, a casual reading especially of Chapters 1, 5 and 10 even by these group of readers might be worth the time spent. For those who are in the field, it could be a source of information and collection of valuable data.

S.G. BHAT
C.F.T.R.I. MYSORE

Coming Full Circle: Farmers' participation in the development of technology: Edited by Peter Matlon, Ronald Cantrell, David King and Michel Benoit-Cattin, published by International Development Research Centre, Ottawa, Canada; pp:176, 1984.

This publication is based on the papers presented in a workshop on farmers' participation in the development and evaluation of technology, which was organized by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the International Development Research Council (IDRC), together with the Semi-Arid Food Grains Research and Development (SAFGRAD) Project of the Organisation of African University and the Institute de recherches agronomiques tropicales et des cultures vivrieres (IRAT) and held in Ouagadougou, Upper Volta, 20-25 September 1983.

Involving farmers in identifying the constraints to rural agriculture and in designing measures to alleviate them is the subject of the book. Why, how, where and when to get farmers involved in research are the focus of this publication.

The book has been divided into 4 main sections excluding references and appendix: Introduction, Diagnosis and Description, Design and Evaluation, and Conclusions. T. Tourte points out in Introduction "That farmers should participate in developing and evaluating technology for their own use is so evident that it has generally been ignored. This book aims to help correct this error-once again, unfortunately, without the farmers". The section on Diagnosis and Description has 7 papers with a commentary by eminent workers in the field. There are 5 additional papers under Design and Evaluation. Roger Kirkby and Peter Matlon have presented conclusions of the workshop.

The ideas and experiences presented in the book are worth serious experimentation in different parts of the world.

AMERIKA SINGH
GOVIND BALLABH PANT
UNIVERSITY OF AGRIC. & TECHNOL
PANTNAGAR

Grazing Research at Northern Latitudes Vol. 108: Edited by Olufur Gudmundsson, Plenum Publishing Corporation, 233 Spring St., New York, N.Y. 10013, 1986; pp:374; Price $ 62.50

The volume contains the papers presented at the workshop on Grazing Research at Northern Latitudes held at Hrvanneyriin, Iceland in August 1985. The increasing grazing pressure on the fragile nature of the ecosystem has in many areas led to irreversible degradation of soil and dependent plant life. A thorough understanding of this ecosystem is needed for sustained yield of forage crops for the grazing

animals which are an important source of meat for the population. The theme of the workshop has been to discuss the interaction between plants and livestock and problems of herbivore grazing under northern conditions with the objective of identifying gaps in the knowledge relating to the management of grasslands in the area.

The papers presented are grouped under:
1. Northern ecosystem and their response to grazing; 2. Primary productivity of northern range lands; 3. Grazing in the North; 4. Pasture utilization; 5. Factors affecting dry matter intake and utilization under northern conditions; 6. Stocking rate theory and its application.

In the final chapter, conclusions and recommendations of the workshop are given. The geographical area encompasses landmasses lying between the barrenness of the circumpolar Arctic region and the north of coniferous forests and stretches across the vast regions of North America and Eurasia.

In the far off northern regions which cover large areas of sub-Arctic, low temperature and short growing seasons are the chief limiting factors. The primary productivity is very much affected by fluctuating climate. The vegetation is characterised by patchy mantles of grasses, sedges and dwarf shrubs and is known as Tundra. There is less variety of animal species but surprisingly large in number. The straight and simple food chains and small number of plant and animal species are upset by any change in the critical species by climatic factors and human intervention.

The first set of papers dwell on the fragile nature of the ecosystem, the factors affecting its productivity and stability, changes in the rangelands due to overgrazing and soil erosion, the impact of grazing on plant species and their responses to population and community level. Fridriksson has traced the deteriorating influence of human intervention and points to the drop in productivity to half during the 15th century.

The section dealing with primary productivity of rangelands will be of great interest as the data presented will enable us to compare the productivity of marginal lands under different geographical regions.

That the varied kinds of pastures like coniferous woodlands, bogs, low and highlands need different management considerations has been indicated in the papers on 'Grazing in North'. In the semiarid regions prevailing in large parts of India as well as in temperate pastures, preservation of resource appears to be a more important aspect of management than measures for improving productivity.

In a paper by Russian authors dealing with the use of concentrates, in dairy cattle in the extreme north region, it is interesting to note that modern feeding practice dose not favour increased milk yield, the use of pasture being the most important factor in normalisation of metabolism. One will have to closely follow the argument to understand its fuller implications. It has been emphasised that the value of natural pasture on marginal lands is dependent on composition and nutritive value of individual plants as well as period of growth, maturity and availability of moisture. Restricted grazing in natural pastures and in forested areas in certain parts of Lappland and Finland have given better results than unrestricted grazing throughout the season.

As Martın Varvice points out in the paper on manipulative grazing, scientific management of resources would much depend on total inventory of grazing resources available. It is interesting to note that domestic animals have developed a behaviour pattern which follows the law of least effort necessitating livestock movements to be also a factor in improving efficiency of production. The potential usefulness of grazing management by reducing plant chemical defenses by manipulating environmental conditions and altering physical structure of grass species appear to be very novel concepts suggested by Maccheck *et al.* in their paper on 'Plant-Defense and Herbivore Learning'.

In the section "Factors affecting dry matter intake and utilization", the authors discuss plant animal relationship in herbage grown in different types of rangelands like hill slopes, sward etc. The role of internal parasites and insects nuisance in cold climatic conditions on productive efficiency of domestic animals is not left untouched.

While discussing stocking rates, Rices uses computer simulation models and stresses that although at the ecosystem level the model has limitations, the perspective presented will greatly assist in scientific research and management. Hurt has proposed a model broadly based on herbage growth rate and animal weight gain and puts forth a few simple concepts to determine the stocking rate. The relationship between grazing pressure and stocking rate is illustrated by a number of figures in the paper by Gudmundsson and Bemet. It has been indicated that the impact of overgrazing on land could be avoided by incorporating sound ecological principles in the management. To some extent, reconciling ecological imperatives and human goal becomes a difficult problem when productive efficiency alone is the criterion.

The consensus that emerged from the discussion emphasised the need for understanding processes in grazing systems for wider application of the results. Descriptive approach is deemphasised in favour of integrated and multi-disciplinary studies to test specific hypotheses relating to productivity of range lands. The research problems are identified in the categories of ecology, production and socio-economic. The research papers presented discuss a wide range of situations in the Northern region. The results and the models developed based on the data collected would be useful in deriving general hypotheses for overgrazing problems of marginal lands in tropical regions.

N.P. Dani
C.F.T.R.I. MYSORE

Bibliography on Ethanol Production.

The National Information Centre for Food Science and Technology at the Central Food Technological Research Institute, Mysore, has recently compiled a "Bibliography on Ethanol Production" covering the period 1976 to 1986. This bibliography lists 582 references collected from diverse sources. Entries have been arranged under two broad groups comprising (I) *Raw Materials* (Cereals, Millets, Beets, Tubers, Sugarcane, Molasses and Bagasses, Agricultural wastes, Carbohydrates), used for ethanol production and (II) *Microorganisms* (Yeasts, Bacteria) used for ethanol production. A limited number of patents have also been covered. For copies, please write to: Head, FOSTIS, CFTRI, Mysore-570 013. Price Rs. 50 + mailing charges Rs. 6/-).

Dairy India 1987: Published by P.R. Gupta, A-25, Priyadarshini Vihar, Delhi-110 092; 3rd Edition; 1987, pp.738 + xvi; Price: Rs. 200 + Postage and Packing Rs. 18.

In this issue specially included are various upcoming trends including those of packaging, biotechnology, fodder production, economic and technical feasibility of dairy farming, cheese and ice cream. More than 7000 organizations and specialists, engaged in dairying are also identified.

JUST PUBLISHED

'Production and Processing of Meat and Poultry Products'

This is the Proceedings of the Symposium on Production and Processing of Meat and Poultry Products held in 17-18 January 1986 at CFTRI, Mysore. This publication brought out by AFST(I) comprises 39 papers arranged under productive Systems/Resources (5); management and fresh meat production (9); Processing and Packaging of Meat and Poultry Products (8); Quality Control (11); and Marketing of Meat and Poultry Products (7). Also included are the inaugural and keynote addresses by experts besides presidential address and the recommendations of the symposium. Size: A 4; Price: Rs.100/-+13 (Postage); Pages 138 + IV; 1987.

For copies, write to:
Hon.Exec.Secretary
AFST (I),
CFTRI Campus,
Mysore-570 013

AFST (I) News

Karnal Chapter

The Annual General Body Meeting was held on 8th January 1988. The following office bearers were elected for 1988-89.

President	:	Dr. G.S. Rajorhia
Vice-President	:	Dr. D.K. Mathur
Hony. Secretary	:	Dr. H. Abichandani
Hony. Treasurer	:	Dr. Des Raj

Statement about ownership and other particulars about the periodical entitled JOURNAL OF FOOD SCIENCE AND TECHNOLOGY as required to be published under Rule 8 of the Registration of Newspapers (Central) Rules 1956.

FORM IV

1.	Place of Publication	...	Mysore City
2.	Periodicity of the Publication	...	Bimonthly
3.	Printer's Name	...	Dr. T.S.S. Rao (For and on behalf of AFST(I))
	Nationality	...	Indian
	Address	...	CFTRI, Mysore-570 013
4.	Publisher's Name	...	Dr. T.S.S. Rao (For and on behalf of AFST(I))
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	Address	...	CFTRI, Mysore–570 013
5.	Editor's Name	...	Dr. N. Chandrasekhara
	Nationality	...	Indian
	Address	...	CFTRI, Mysore–570 013

1, Dr. T.S.S. Rao hereby declare that the particulars given above are true to the best of my knowledge and belief.

Dr. T.S.S. Rao,
Signature of the publisher

CIRCULAR

Late Prof. D.V. Tamhane Memorial Endowment Fund Essay Competition

Under the Late Prof. D.V. Tamhane Memorial Endowment Fund, students registered for graduate/post-graduate degree course in Food Technology, Fermentation Technology and allied fields like Biochemistry and Microbiology, are inivted to submit essays on any one of the following topics:

1) New food and food adjuncts through biotechnology,
2) Convenience food industry in India – Prospect and retrospect,
3) Newer concepts in human nutrition

The essays should not exceed 3000 words. Only those students below the age of 30 years before June 1, 1988 are eligible to participate. Essays will be judged by a committee and the decision will be final. Best two essays will be awarded prizes of Rs. 500/- each.

Three copies of the manuscript, typed in double space on good quality paper (size·9"× 11") should reach the following address, not later than August 15, 1988. The manuscript, must carry an abstract of not more than 100 words and should be accompanied by certificates about date of birth and degree for which registered, from the Principal/Director of the institute.

Address:

Prof. (Ms) P.R. Kulkarni
Food and Fermentation Technology Division
Department of Chemical Technology
University of Bombay
BOMBAY-400 019.

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (INDIA)

CFTRI Campus, Mysore-570 013, India

NOMINATIONS FOR AFST (I) AWARDS FOR 1988

Nominations for the following awards of the AFST (I) for the year 1988 are invited. All nominations should be sent by Registered post, so as to reach Honorary Executive Secretary, Association of Food Scientists and Technologists (India), CFTRI Campus, Mysore-570 013, before 31 October 1988.

PROF. V. SUBRAHMANYAN INDUSTRIAL ACHIEVEMENT AWARD

The guidelines for the award are:

(i) Only Indian nationals with outstanding achievement in the field of Food Science and Technology will be considered for the award.

(ii) The nominee should have contributed significantly to the enrichment of Food Science and Technology, and the development of agro-based food and allied industries in India.

(iii) The nomination duly proposed by a member of the Association must be accompanied by the biodata of the candidate highlighting the work done by him for which he is to be considered for the award.

(iv) The awardee will be selected by an expert panel constituted by the Central Executive Committee of the Association.

The envelope containing the nominations along with biodata and contributions (five copies) should be superscribed "Nomination for Prof. V. Subrahmanyan Industrial Achievement Award – 1988".

LALJEE GODHOO SMARAK NIDHI AWARD

The guidelines for the award are:

(i) The R & D group/person eligible for the award should have contributed significantly in the area of Food Science and Technology in recent years with a good standing in his/her field of specialisation.

(ii) The nominee(s) should be duly sponsored by the Head of the respective Scientific Institution and the application for this award should highlight complete details of the contributions made by the candidates and their significance.

(iii) The awardee(s) will be selected by an expert panel constituted by the Central Executive Committee of the Association.

The envelope containing the nominations (five copies) should be superscribed "Nomination for Laljee Godhoo Smarak Nidhi Award 1988".

SUMAN FOOD CONSULTANTS TRAVEL AWARD

This award is instituted in the name of M/s Suman Food Consultants, New Delhi, to be awarded to a student pursuing Post Graduate Degree/Diploma courses in Food Science/Technology in any recognised University in India.

The Award will be decided based on the best essay to be submitted by the applicant on a topic to be announced later. Five copies of the essay containing 15-20 pages (A4 size) of typed matter with appropriate bibliography and a certificate from the head of the department under whom the student is working should be enclosed along with the application. The envelope containing the above documents should be superscribed "Suman Food Consultants Travel Award – 1988".

BEST STUDENT AWARD

The award is to be given to a student having a distinguished academic record and undergoing the final year course in Food Science and Technology in any recognised University in India. The aim of the award is to recognise the best talent in the field and to encourage excellence amongst the student community.

The guidelines for the Award are:

(i) The applicant must be an Indian national

(ii) He/she must be a student of one of the following courses:

(a) M.Sc. (Food Science)/(Food Technology)

(b) B.Tech., B.Sc. (Tech), B.Sc. (Chem. Tech) with Food Technology specialisation.

(iii) He/she should not have completed 25 years of age on 31st December 1988.

Heads of the Department of Food Science and Technology in various Universities may sponsor the name of one student from each institution supported by the candidate's biodata, details starting from high school onwards, including date of birth and post-graduate performance to date (five copies).

The envelope containing the nomination should be superscribed "Nomination for Best Student Award – 1988".

YOUNG SCIENTISTS AWARD

This award is aimed at stimulating distinguished scientific and technological research in the field of Food Science and Technology amongst young scientists in their early life.

The guidelines for the Award are:

1. The candidate should be an Indian national below the age of 35 years on 31st December 1988, working in the area of Food Science and Technology.

   (i) The Candidate should furnish evidence of either:

   (a) Original scientific research of high quality, primarily by way of published research papers and (especially if the papers are under joint authorship) the candidate's own contribution to the work.

   OR

   (b) Technological contributions of a high order, as reflected by accomplishments in process design etc., substantiated with documentary evidence.

The application along with details of contributions of biodata (five copies) may be sent by registered post with the envelope superscribed: "Nomination for Young Scientists Award 1988".

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) × 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:* Josbi, 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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