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( I N D I A )



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# JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Volume 27

Number 5

Sept./Oct. 1990

## CONTENTS

### Research Papers

- Microbial Profile of Soyslurries During Ripening** 253  
*P. Kumari and S. Singh*
- Modelling of Bran Removal and Whiteness of Milled Rice** 256  
*J. P. Pandey and P. C. Sah*
- Studies on Tribal Foods of South India: Effect of Processing Methods on the Vitamin and *in vitro* Protein Digestibility (IVPD) of Cereals/Millet and Legumes** 260  
*P. Rajyalakshmi and P. Geervani*
- Processing, Evaluation and Storability of Date Jelly** 264  
*Ali K. Yousif, M. Abou Ali and A. Bou Idreese*
- Effect of Additives and *Lactobacillus casei* on Flavour Development in Cheddar Cheese from Buffalo Milk** 268  
*Y.K. Jha and S. Singh*
- Changes in Carbohydrates and Free Amino Acids Caused by the Growth of Pure Cultures of Spoilage Bacteria on Meat Drip** 272  
*R. B. Babiker, J. T. Patterson and A. P. Damoglou*
- Preparation and Properties of Aroma Concentrates from Some Tropical Fruit Juices and Pulps** 277  
*R. S. Ramteke, W. E. Eipeson and M. V. Patwardhan*
- Effect of Different Gumming Agents on the Physico-chemical Characteristics of Rice Bran Oil** 280  
*S.K. Munshi, A. Kakar and P. S. Sukhija*
- Comparitive of Behaviour of Cashew Kernel and Wheat to Phosphine Fumigation** 284  
*J. R. Rangaswamy and V. B. Sasikala*
- Neutral Detergent Fibre from Various Foods and Its Hypocholesterolemic Action in Rats** 290  
*Molly Thomas, S. Leelamma and P. A. Kurup*

### Research Notes

- On the Carotenoids of Ripened Pepper Berries (*Piper Nigrum* L.)** 294  
*Prasad S. Variyar and C. Bandyopadhyay*
- The Stability of Cellulase During Saccharification of Bagasse** 296  
*H. K. Manonmani and K. R. Sreekantiah*
- Shelf Life of Maize (*Zea mays* L.) Flour** 299  
*T. R. Madaan and H. O. Gupta*

<b>Microstructure of Coconut Haustorium</b>	<b>302</b>
<i>Satyavati Krishnankutty, C. K. Mangala Kumari and A. G. Mathew</i>	
<b>Processing of Plums</b>	<b>304</b>
<i>M. A. Wani and S. P. S. Saini</i>	
<b>Effect of Sugar Syrup Concentration and Temperature on the Rate of Osmotic Dehydration of Apples</b>	<b>307</b>
<i>K. Videv, S. Tanchev, R. C. Sharma and V. K. Joshi</i>	
<b>Composition of Some Unconventional Himalayan Wild Fruits</b>	<b>309</b>
<i>S. K. Katiyar, K. Sharma, N. Kumar and A. K. Bhatia</i>	
<b>Functional and Chapati Making Properties of Hull-Less Barley Supplemented Wheat Flour</b>	<b>311</b>
<i>J. S. Sidhu, K. Harinder, A. Kaur and M. B. Ram</i>	
<b>Effect of Addition of Buffalo Milk Protease and Protease-Peptone Components 3, 5, and 8 on the Whippability of Cream</b>	<b>314</b>
<i>Moti Ram and V. K. Joshi.</i>	
<b>Residues of Fenvalerate in Pigeonpea (<i>Cajanus cajan</i> (L.) Millsp.)</b>	<b>317</b>
<i>P. S. Patel, P. G. Shah, B. K. Patel and J. R. Patel.</i>	
<b>Book Reviews</b>	<b>319</b>
<b>AFST(I) News</b>	<b>321</b>

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## Microbial Profile of Soyslurries during Ripening

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The microbial profile of soycheese slurries was followed from 1st to 8th day of ripening at 30°C. Fresh slurries with and without lactic cultures had a total plate count of  $216 \times 10^4$  and  $13 \times 10^4$ /g, respectively. The final total plate count on 8th day was in the order of  $10^8$ /g. Though the coliforms were absent in fresh slurries, they appeared during ripening, the final number being about  $10^3$ /g. The fresh slurries had very low number of yeast and mould (22/g) which increased during ripening upto  $10^5$ /g. In addition, the spore, *Lactobacillus* and *Micrococcus* counts were also determined during storage.

Soybean has tremendous potential to overcome protein and calorie malnutrition of the world population. In order to make the best use of soy-nutrients by application of heat and fermentation Singh and Mittal<sup>1</sup> and Kumari and Singh<sup>2</sup> developed a cheese-spread like product from soybean. Since this is a new product made from non-conventional food solids by an altogether new approach, the information on the subject is lacking. Certain groups of microorganisms, viz. acid and non-acid producers, coliforms, yeasts and moulds, lactobacilli and micrococci are known to affect cheese flavour development. This study was, therefore, undertaken to study the microbial profile of soycheese slurries during ripening.

### Materials and Methods

For each trial 3.5 kg of clean, dehulled soybean was blanched in a double jacketed kettle in ten times water with one per cent sodium bicarbonate (based on soybean wt) at 1.55 kg/cm<sup>2</sup> steam pressure in the jacket for about 60-75 min. The soyslurry was prepared by grinding the blanched soybean with a mixture of fresh cream and spray dried skim milk powder (500 and 120 g/kg soybean, respectively) in 1.5 litres of 12 per cent NaCl solution in a colloidal mill. The adjustment of the initial pH of the slurries to 5.3 was done with 75 per cent lactic acid. The slurry was pasteurized at 75°C for 30 min. The *S. lactis* culture and rennet were added at 5 and 0.01 per cent, respectively. The inoculated slurries were stored in a water bath at 30°C. The slurries were agitated daily with a sterile spatula for proper aeration. During ripening as and when the pH dropped down, it was restored to 5.3 by adding 50 per cent NaOH solution in the case of pH controlled slurries.

The soyslurries were assessed for proximate composition, flavour<sup>2</sup> and microbial changes. Microbial analysis was done at 0, 1, 3, 5 and 8 day of storage. The microbial profile included total yeast, mould, coliform, spore, *Staphylococcus*, *Lactobacillus* and *Micrococcus* counts<sup>3</sup>. The total count was made on tryptone dextrose agar and bromocresol purple agar. The plates were incubated at 37°C for 24-48 hr. The coliform count was determined on violet bile agar. The yeast and mould counts were made using potato dextrose agar. The plates were incubated at 22°C for 3-5 days. The spore count was made on tryptone dextrose agar. The samples after 1:10 dilution were kept in a water bath at 80°C for 10 min. The plates were incubated at 37°C for 48-72 hr. *Lactobacillus* count was made using *Lactobacillus* agar (L.B. agar). The medium was prepared and boiled for 1 min and stored in refrigerator. The plates were incubated at 37°C for 24-48 hr<sup>4</sup>. The *Micrococcus* count was made on tryptone agar. The plates were incubated at 30°C for 24-48 hr<sup>5</sup>.

### Results and Discussion

The soyslurry contained 34 per cent total solids, 12 per cent fat, 10 per cent protein, 2 per cent sodium chloride and 3 per cent ash and its flavour characteristics were quite acceptable.

The total plate count in freshly prepared slurries ranged from  $13 \times 10^4$  to  $216 \times 10^4$ /g where the lower limit represented the slurries without added starter culture and the upper limit represented those with 5 per cent culture (Table 1). The number of bacteria increased sharply during the first 24 hr followed by a relatively slower but steady growth upto the 5th day of ripening. Thereafter, the bacterial growth tended

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TABLE 1. EFFECT OF AGITATION, LACTIC CULTURE, RENNET AND pH CONTROL ON TOTAL PLATE COUNT, COLIFORM COUNT AND YEAST AND MOULD COUNT OF SOY-SLURRIES DURING RIPENING AT 30°C

Treatment	Days of storage				
	0	1	3	5	8
<b>Total Plate Count</b>					
A. Control, agitation	$13 \times 10^4$	$145 \times 10^5$	$227 \times 10^6$	$171 \times 10^7$	$47 \times 10^8$
B. <i>S. lactis</i> (5%)	$216 \times 10^4$	$256 \times 10^5$	$247 \times 10^6$	$208 \times 10^7$	$78 \times 10^8$
C. <i>S. lactis</i> (5%), rennet	—	$235 \times 10^5$	$318 \times 10^6$	$347 \times 10^7$	$116 \times 10^8$
D. <i>S. lactis</i> (5%), rennet, pH control	—	$213 \times 10^5$	$279 \times 10^6$	$166 \times 10^7$	$73 \times 10^8$
<b>Coliform Count</b>					
A. Control, agitation	Nil	Nil	$54 \times 10^2$	$69 \times 10^3$	$70 \times 10^3$
B. <i>S. lactis</i> (5%)	Nil	Nil	$114 \times 10^2$	$148 \times 10^3$	$110 \times 10^3$
C. <i>S. lactis</i> (5%), rennet	—	Nil	$112 \times 10^2$	$57 \times 10^3$	$100 \times 10^3$
D. <i>S. lactis</i> (5%), rennet, pH control	—	Nil	$87 \times 10^2$	$166 \times 10^3$	$140 \times 10^3$
<b>Yeast and Mould Count</b>					
A. Control, agitation	$2 \times 10^1$	$19 \times 10^2$	$124 \times 10^3$	$192 \times 10^6$	$103 \times 10^5$
B. <i>S. lactis</i> (5%)	$1 \times 10^1$	$26 \times 10^2$	$229 \times 10^3$	$225 \times 10^6$	$120 \times 10^5$
C. <i>S. lactis</i> (5%), rennet	—	$2 \times 10^3$	$260 \times 10^3$	$186 \times 10^6$	$95 \times 10^5$
D. <i>S. lactis</i> (5%), rennet, pH control	—	$35 \times 10^2$	$118 \times 10^3$	$202 \times 10^6$	$123 \times 10^5$

(Each value is average of three replicates)

to level off in all the samples regardless of treatments used. The rate of bacterial growth was relatively faster in control as compared to the treated sample during the first 24 hr. The total number of bacteria at the end of storage ranged from  $47 \times 10^8$  to  $116 \times 10^8$ /g. Incorporation of rennet showed a stimulatory effect on bacterial growth. The control slurries without added culture contained relatively lower counts. The unopened slurries were analysed for total plate count only on the last day of storage when total plate count was in the order of  $90 \times 10^6$ /g. The daily agitated slurry showed higher counts than those without agitation.

Two types of colonies appeared on bromocresol purple medium. One type was bright yellow, whereas the other was purple, the former representing acid producers whereas the latter non-acid. The number of acid and non-acid producers were  $8 \times 10^5$  to  $101 \times 10^5$ /g and  $102 \times 10^5$  to  $195 \times 10^5$ /g respectively on the 1st day of storage. The number of bacteria increased steadily<sup>6</sup> till the 5th day of ripening reaching acid and non-acid producer counts from  $7 \times 10^7$  to  $42 \times 10^7$ /g and  $118 \times 10^7$  to  $149 \times 10^7$ /g, respectively. Thereafter, the bacterial growth tended to level off.

The present study has unequivocally established that lactic starter cultures are essential for desirable cheese flavour development. Though the control slurries exhibited almost similar plate count, they did not develop desirable cheese

flavour<sup>2</sup>. The total plate count in soyslurries ( $10^8$ /g) was similar to those of regular cheddar cheese ( $10^8 - 10^9$ /g)<sup>5</sup>.

The coliform count was nil in all the slurries during the first 24 hr. Thereafter, the number increased sharply upto 3rd day followed by a slower but steady growth till 5th day of ripening (Table 1). Following this, the bacterial growth levelled off, the final number being from  $70 \times 10^3$  to  $140 \times 10^3$ /g. There was no significant difference in the coliform counts as a result of various treatments of soyslurries. The coliforms were completely absent even at the end of storage in unopened slurries.

It was not possible to obtain slurries free of yeast and mould. Generally, freshly prepared soyslurries contained 10-20/g of yeast and mould (Table 1). They increased rapidly during ripening till 5th day of storage followed by a gradual decline upto the end. The maximum number on 5th day ranged from  $186 \times 10^6$  to  $225 \times 10^6$ /g. The final number was in the order of  $10^5$ /g. Almost all the slurries followed similar trend regardless of treatments used. The yeast and mould count for unagitated slurries was  $116 \times 10^3$ /g. In almost all the slurries, the number of yeast was more as compared to moulds.

The effect of agitation, culture, rennet and pH control on spore count is shown in Table 2. The bacterial spore count ranged from  $76 \times 10^2$  to  $121 \times 10^2$ /g after 24 hr and increased



TABLE 2. EFFECT OF AGITATION, LACTIC CULTURES, RENNET AND pH CONTROL ON SPORE COUNT, LACTOBACILLUS COUNT AND MICROCOCCUS COUNT OF SOY-SLURRIES DURING RIPENING AT 30°C

Treatment	Days of storage			
	1	3	5	8
	<b>Spore Count</b>			
A. Control, agitation	$86 \times 10^2$	$249 \times 10^2$	$232 \times 10^3$	$117 \times 10^4$
B. <i>S. lactis</i> (5%)	$76 \times 10^2$	$298 \times 10^2$	$242 \times 10^2$	$108 \times 10^4$
C. <i>S. lactis</i> (5%), rennet, pH control	$121 \times 10^2$	$229 \times 10^2$	$219 \times 10^3$	$153 \times 10^4$
	<b>Lactobacillus Count</b>			
A. Control, agitation	Nil	$339 \times 10^3$	$37 \times 10^5$	$154 \times 10^6$
B. <i>S. lactis</i> (5%)	Nil	$222 \times 10^3$	$39 \times 10^5$	$183 \times 10^6$
C. <i>S. lactis</i> (5%), rennet, pH control	Nil	$228 \times 10^3$	$33 \times 10^5$	$167 \times 10^6$
	<b>Micrococcus Count</b>			
A. Control, agitation	$204 \times 10^3$	$268 \times 10^4$	$31 \times 10^5$	$123 \times 10^6$
B. <i>S. lactis</i> (5%)	$153 \times 10^3$	$228 \times 10^4$	$34 \times 10^5$	$86 \times 10^6$
C. <i>S. lactis</i> (5%), rennet, pH control	$166 \times 10^3$	$249 \times 10^4$	$36 \times 10^5$	$151 \times 10^6$

Each value is average of three replicates

slowly but steadily during the entire storage period. The final increase in spore count on 8th day was from  $108 \times 10^4$  to  $153 \times 10^4$ /g. There was no specific effect of the treatments given to the slurries on spore count.

The *Lactobacillus* count as affected by agitation, lactic culture, rennet and pH control is presented in Table 2. Lactobacilli were not noticeable upto 24 hr. Thereafter, the number increased logarithmically upto 3 days followed by a relatively slow but steady growth throughout the ripening period. The final count ranged from  $154 \times 10^6$  to  $183 \times 10^6$ /g. The *Lactobacillus* population was not affected by the different treatments used. The number of lactobacilli in soyslurries were similar to those reported by Johns and Cole<sup>7</sup> for one year old cheddar cheese, *L. Casei* has been shown to contribute to cheese flavour development.

The range of micrococcus count on first day was from  $153 \times 10^3$  to  $204 \times 10^3$ /g which increased slowly but steadily from first to 5th day followed by a sharp increase upto the end of storage (Table 2). The final count ranged from  $86 \times 10^6$  to  $151 \times 10^6$ /g. There was no distinct effect of different treatments. Marth<sup>8</sup> reported that certain micrococci were frequently observed in cheddar cheese. A thousand fold increase in number to a maximum of  $10^7$  to  $10^8$ /g was observed in two days of pressing, which was more as compared to the micrococci present in soyslurries ( $10^6$ /g).

In order to improve flavour developments, certain cultures other than *S. lactis* were used either as a complete replacement or *S. lactis* or as a complement of *S. lactis*. The cultures *S. cremoris* and *S. diacetilactis* were used singly

as well as in combination with *S. lactis*. However, *S. thermophilus*, *S. faecalis* and *L. casei* were used as complements replacing 40 per cent of *S. lactis*. It was observed that the profile of total plate, coliform, yeast and mould, and micrococcal counts did not change much as compared to the control sample where only *S. lactis* was used. However, when *L. casei* was added, the lactobacillus count reached upto the level of  $10^2$ /g after one day of storage whereas those without added *L. casei* showed colony development only after 3rd day. Thereafter, there was no difference in slurries with and without *L. casei*.

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## Modelling of Bran Removal and Whiteness of Milled Rice

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Whiteness of rice was found to be strongly influenced by variety and degree of polishing. Regression models relating the process of bran removal and whiteness of rice kernels were developed. Relative increase in whiteness, due to polishing, was found to be dependent on coefficient of whiteness for a variety. Utility of the concept of these parameters for performance predictions of polishers is discussed.

Whiteness of the rice kernels after milling, *inter alia*, is one of the criteria for quality evaluation and acceptance. The whiteness of the kernels can be increased by increasing the degree of polish. However, the process of whitening also causes breakage and reduces head rice yield which in consequence affects the economics of operation and marketability. Acceptable degree of whiteness can be accomplished by polishing the rice by 5 to 10 per cent. The permissible degree of bran removal in some countries is decided by mandatory rules considering the nutritional aspects and net food availability, without much consideration of colour appearance. However, in countries like USA, the official quality gradations<sup>1</sup> are based on comparative evaluation of colour of rice and its categorisation such as white or creamy, dark gray or rosy, well or lightly milled etc. The latter method of evaluation is visual and involves some measures of subjectivity where accuracy depends on skill of the evaluator.

Several methods are available for evaluation of the quality of the milled rice. These can be broadly divided into chemical and optical methods. Detailed review of chemical methods can be found in literature<sup>2-5</sup>. As this work relates with optical method for whiteness evaluation, only a brief review of optical methods follows. Kik<sup>5</sup> made a comprehensive study of several commercially available and specially constructed reflectometers for measuring whiteness of varying degrees of milling. He reported that 18 different shades could be detected between ground brown and white rice samples. Colour filters of different shades were used. It was also reported by him that the last stage of milling makes the rice kernels smoother and less opaque, thereby, resulting in lower reflectance. The work of Borasio<sup>6</sup> on measurement of whiteness of milled rice by reflectance and relative transparency and subsequent improvements lead to commercially usable rice testers. Angladette<sup>7</sup> conducted a test on rice kernels, classified in three lots (completely translucent, white bellies, and completely chalky) for milling

duration between one and three and half minutes. Reflectance tests were conducted on milled samples. Degree of milling was found to be dependent on the translucency of the grain. Stermer<sup>8</sup> constructed a photometer capable of measuring the difference in optical density of rice at two wavelengths (660 and 850 nm). The photometer reading was found to be directly proportional to the per cent surface lipids and provided an accurate determination of the degree of milling.

Comparison of the result of this study with USDA rice inspectors' visual rating showed high correlation. It was concluded that the technique was rapid enough to provide a means of quickly making adjustments during normal milling operations as also suffice for determining grade, based on whiteness. Johnson<sup>1</sup> developed an objective method of evaluation of degree of parboiling and milling of parboiled rice for purpose of routine inspection to determine grades by utilizing a reflectance meter working with light of wave length 546 nm. It was reported that approximately 400 samples of rice could be handled by one operator. Hogan and Deobald<sup>9</sup> has described a Japanese device (KETT Meter) which was used to evaluate the reflectance of light. An extensive review of the past techniques on measurement of degree of milling and whiteness of rice was also done by them. They recommended optical methods over chemical and other methods, as the advantages associated with optical methods are their rapidity and non-destructiveness. Optical methods are advantageous in laboratories for their promptness as well as in commercial rice milling operations where quick adjustments of machines, based on feed back, can be done to regulate the degree of milling. With increasing automation of rice mill machinery, it was suggested that controls based on optical monitoring could be effectively adopted for process optimization. Velupillai and Pandey<sup>10</sup> conducted studies on five Louisiana varieties of rice. They correlated colour values with degree of milling and yield of rice. A linear regression model was proposed to correlate colour value (total colour



difference) of rice with percentage bran removed (in range from zero to fifteen per cent). Total colour difference of milled rice sample was evaluated with a white standard reflectance plate of a colorimeter system.

Review of the past reported work shows that in most of the cases, the evaluation of whiteness/colour values of rice kernels were relative differentials, the wave length of light source used for optical measurements differing by a wide margin. This was probably due to the limitations of photometric devices available for experimentation. Johnson<sup>1</sup> has clearly reported that the refractive index/reflectance of rice samples vary considerably with wave length, therefore, for obvious reasons, the colour values of milled rice reported in literature have restricted local applicability as these are bereft of the ability for universal comparison. Rice whiteness values can have universal applicability and scientific utility for comparison and replication only when an international whiteness standard is followed. International whiteness standard has been defined on a scale 0 to 100. The complete darkness is calibrated as zero and the reflective index (whiteness) of the pure white of a surface produced by layers of magnesium oxide as one hundred. Many models of whiteness meters reporting whiteness of rice on international Whiteness Standard are now available and should be preferred. This study was undertaken to model the process of whitening in a laboratory polisher and to correlate whiteness of seven Indian varieties of rice with degree of bran removal.

### Materials and Methods

Seven varieties of genetically pure rough rice (short grain) were procured from rice breeder of the University for experiments. Shelling of precleaned grains was done in a laboratory Satake Rice Sheller (Model THU-35). After separation of unhulled grains, samples of 100 g of brown rice were milled in a Satake Laboratory Rice Whitener (Model TM 05) for a period of time varying from 0 to 110 sec. with an increment of 10 sec to attain for different percentage of bran removal. The corresponding degree of bran removal (measured as percentage of original weight of brown rice) obtainable were in range from 0 to 22.9 per cent. After allowing a cooling period of 5 min and gentle rubbing with a soft cloth to remove sticking bran from kernels, whiteness of each sample was determined by KETT digital whiteness meter (Model C-300). KETT whiteness meter measures whiteness on International Whiteness Standard scale as mentioned earlier.

### Results and Discussion

The whiteness values of brown rice, obtained from different varieties after shelling, were in the range from 15.7 to 27.9 per cent. Variety 'Manhar' had lowest whiteness of 15.7 per cent. Whereas, 'Jaya' had the highest initial whiteness of 27.9 per cent. Other four varieties namely 'Prasad', 'Sita',

'UPRI-79' and 'Raci' had an initial whiteness in a close range (20.5-25.9 per cent). The whiteness values of brown rice and other characteristic features on different rice varieties used in the experimentation are given in Table 1.

Removal of bran from brown rice in a polisher is an interactive effect of: (i) grain properties (size, shape, moisture content, past hygromechanical history, and other varietal characteristics such as initial whiteness, chalkiness and thickness of bran layers enveloping the pericarp); (ii) operational conditions within polisher (abrasion, speed, clearance between abrasive surfaces, degree of milling, screen perforation, etc.). This is well accepted that polishing is accomplished by abrasion of grain within the polisher by rubbing of grain with metallic surface as well as intra grain rubbing. In the beginning, the rate of polishing is reported to be higher than subsequent times for the reason of higher abrasion rate obtainable. As polishing progresses, the grain surface becomes smoother, thereby, reducing the rate of bran removal<sup>10</sup>. It is also reported previously that the bran for containing relatively higher percentage of fat, may also be a factor for reducing friction, thereby the rate of polishing.<sup>1</sup>

Reported studies on bran removal with respect of time indicate a non-linear behaviour with a decreasing rate of bran removal<sup>10</sup>. No mathematical model has been attempted by previous workers. The utility of a mathematical model need not be over-emphasised for its ability to quantify the rice polishing kinetics, ease in comparison as well as its utility in rice polishing equipment design, operation and control. Various mathematical models, were tested for their suitability (least error prediction criteria) to describe the phenomenon of polishing; the following model was found to correlate the experimental observations satisfactorily:

$$P = a T^b \quad \dots\dots(1)$$

Where  $P$  is per cent degree of polish,  $T$  is the time of polishing(s),  $a$  and  $b$  are coefficients of the equation.

Coefficients of the equation (1) were found by method of least square after linearisation of the equation. The values of regression and correlation coefficients for each variety are

TABLE 1. VARIETAL CHARACTERISTICS OF ROUGH RICE USED IN EXPERIMENTS

Variety	Whiteness of brown rice, % ( $W_0$ )*	Length (mm)	Width (mm)	Thickness (mm)
Prasad	20.5	0.1	2.4	1.7
Manhar	15.7	6.7	2.1	1.7
Sita	22.1	—	—	—
Jaya	27.9	6.5	2.4	1.9
UPRI-79	22.6	5.2	2.3	1.7
Raci	21.5	5.8	2.3	1.7
PD-4	25.9	—	—	—

\*Actually measured

reported in Table 2. Coefficients *a* and *b* were found to range from 0.23 to 2.39 and 0.40 to 1.01, respectively. Incidentally it was also seen that constant of the equation *a* and *b* almost followed a reverse trend as far as their magnitudes are concerned. The values of *b* (for being less than one) are consistent with the earlier hypothesis that the rate of polishing diminishes as it progresses. In the given situation, as obtainable with a polisher, the coefficients of the equation (1) as a preliminary estimate should be dependent on rheological properties of rice grain and also on the machine parameters. However, for establishment of explicit correlation of *a* and *b* with rheological and machine parameters, further extensive studies would be required.

The whiteness and degree of polish relationship, were found to be linear, for all the varieties with the correlation coefficient exceeding 0.95 in all cases:

$$W = C P + W_0 \quad \dots\dots(2)$$

where *W* is per cent whiteness of polished grain, *P* is per cent degree of polish, *W*<sub>0</sub> is the initial per cent whiteness of brown rice, and *C* is model regression coefficient. The values of *W* are based on the presumption that the only change in whiteness occurs during polishing and the kernel whiteness remains unaffected while shelling. Table 3 shows the values of the coefficient *C* and *W*<sub>0</sub> as calculated from equation 2. Comparing the values of *W*<sub>0</sub> as calculated from equation 2 with actually measured values (Table 1) it is seen that deviations are with acceptable limits.

TABLE 2. VALUES OF COEFFICIENTS OF EQUATION :  $P = aT^b$ \*

Variety	a	b	Correlation coeff. t
Prasad	0.601	0.558	0.992
Manhar	2.395	0.406	0.981
Sita	0.365	0.834	0.988
Jaya	0.235	1.018	0.998
UPRI	0.763	0.669	0.992
Raci	2.080	0.407	0.946
PD-4	0.670	0.692	0.964

\*Values of coefficients were evaluated by linearization of the equation.

TABLE 3. VALUES OF COEFFICIENTS OF EQUATION :  $W = CP + W_0$

Variety	C	W <sub>0</sub> *	Correlation coeff., r
Prasad	1.21	20.7	0.961
Manhar	1.28	13.3	0.977
Sita	1.59	21.7	0.993
Jaya	1.06	31.0	0.963
UPRI-79	1.43	22.4	0.991
Raci	1.49	20.4	0.950
PD-4	1.26	27.3	0.993

\*Computed values from equation (4).

Equation (2) can be rewritten as follows:

$$\frac{W - W_0}{W_0} = W_R = \frac{C}{W_0} P = C_W P \quad \dots\dots(3)$$

The dimension less ratio *W*<sub>R</sub> is defined as relative increase in whiteness of the grain. *W*<sub>R</sub>. In fact it is the real measure of performance of a polisher. As it would be seen from equation (2), the final whiteness of the kernels is also dependent on the initial whiteness of brown rice, a varietal characteristic. Therefore, the final whiteness of kernels after polishing should not be directly attributed to the polisher performance. It is more appropriate to use the term relative increase in whiteness (*W*<sub>R</sub>) accomplished by a polisher. In the past, none reported the performance of polishers. The concept of relative increase in whiteness could be applied in case of multi-stage polishing and design of polishers. The linear plots of *W*<sub>R</sub> versus *P* are shown in Figures 1 and 2.

The term *C*/*W*<sub>0</sub> is referred to as the coefficient of increase in whiteness (*C*<sub>W</sub>) and was found to be a linear function of *W*<sub>0</sub> as shown in Fig. 3 and Eqn. (4).

$$C_W = - 0.2121 W_0 + 10.89 \quad \dots\dots(4)$$

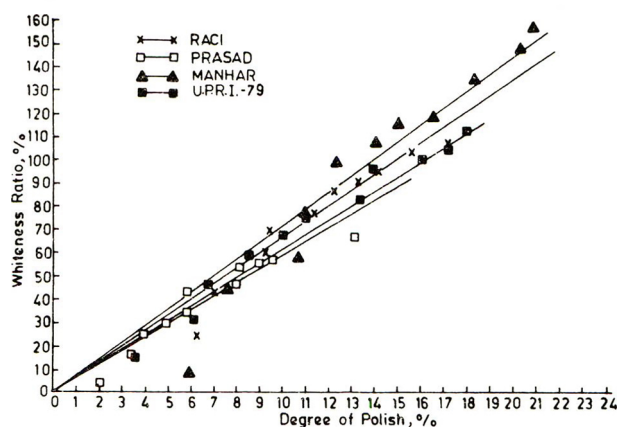


Fig.1 Variation of whiteness ratio with degree of polish for some rice varieties.

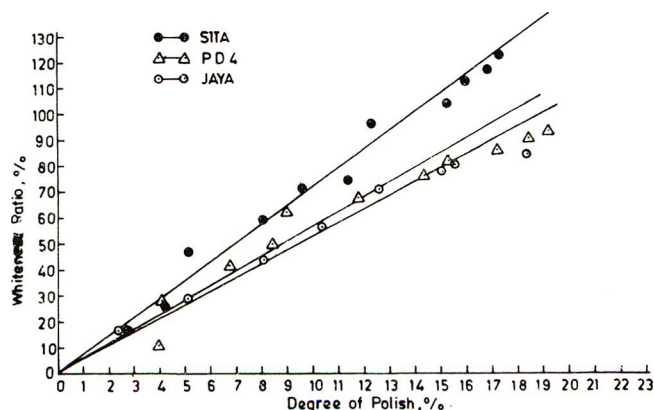


Fig.2. Variation of whiteness ratio with degree of polish for some rice varieties.



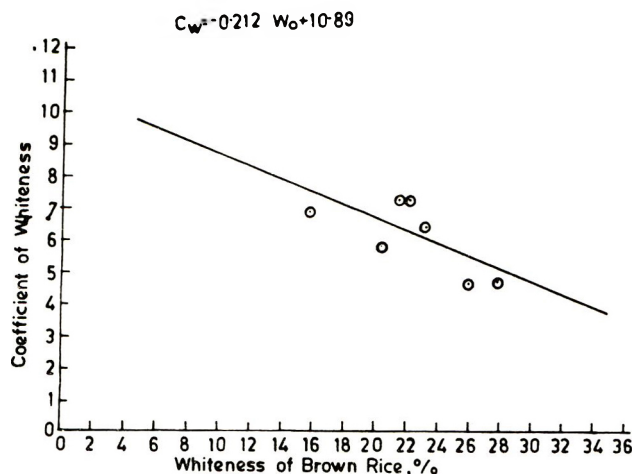


Fig.3. Effect of whiteness of brown rice (W<sub>o</sub>) on coefficient of whiteness.

It was experimentally observed that a variety having relatively higher W<sub>o</sub> would give lesser value of W<sub>r</sub>, thereby showing the effect of W<sub>o</sub>. The above models could be finally combined as:

$$W_R = C_w \times a T^b = C_w \times P \quad \dots\dots(5)$$

The above mathematical expressions describe the kinetics of polishing and its relation with rice kernel whiteness satisfactorily under the conditions obtainable; however for seeking the validity of the proposed mathematical models for other kinds of design of polishers, further test would be required.

It is concluded that the time polish correlations for different varieties of grains were to be a non-linear expression, whereas

whiteness of the milled rice kernels linearly varied with degree of polish. The coefficient of relative increase of whiteness was found to be a linear function of initial whiteness of the kernel. For a given variety, the relative increase in the whiteness linearly varied with the polish.

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# Studies on Tribal Foods of South India : Effect of Processing Methods on the Vitamin and *in Vitro* Protein Digestibility (IVPD) of Cereals/Millet and Legumes

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The cereal/millet preparations traditionally prepared and consumed by the tribals of Vizianagaram district of Andhra Pradesh were prepared in the laboratory by conventional methods. The preparations were analysed for thiamine and niacin contents and for *in vitro* protein digestibility. The tribal cereal/millet preparations prepared by boiling exhibited a marked loss of thiamine (75-100%) and niacin (24-75%). Another traditional millet preparation containing *conjee* (water strained after boiling of rice) and kept overnight showed no loss of the vitamins. Ragi (finger millet) *ambali*, a fermented and cooked product of ragi showed an increase in both thiamine (50%) and niacin (37%) contents. Cereal and millet prepared by boiling showed a decrease of 9-24% in IVPD, whereas legumes showed an increase of 15-32% in IVPD.

Nutrient loss on cooking depends on conditions of cooking, and on the stability of nutrients. Culinary practices vary widely and cooking is supposed to have both beneficial and adverse effects depending on various methods of cooking. The method of cooking of cereals and millets by tribals is somewhat different from that of non-tribals. A major portion of protein and vitamins in the tribal diets is derived from cereals/millets and legumes. The effect of processing methods used by the tribals on the vitamins and IVPD was therefore studied, and the results are reported in this paper.

## Materials and Methods

The cereals/millets and legumes used in this study were collected from the tribals of Vizianagaram district residing in the two agency blocks namely Bhadravari and Pachipenta. In total, 300 tribal families were surveyed from 12 tribal villages. Grains were procured from 5-10 households at random from each of the 12 tribal villages surveyed. Paddy and millets (except bajra and jowar) were processed in usual way by dry abrasive method. Parboiled millets *Ooda* (*Echinochloa* var. *Fruentacia*) and korra (*Setaria italica*) were collected directly from the tribals at random.

The tribals extensively use parboiled grains (also known as *Uppullu*). The freshly harvested grains are parboiled by tribal's method by boiling in a covered pot for about an hour till the water is completely absorbed by the grains (2-3 l of water per 5-6 kg grain). The parboiled grains are spread on a flat bamboo woven, mat-like structure (known as '*Dangari*') and dried in shade prior to sun-drying. They are stored for about 1 to 3 months and the grains are dehusked in small quantities whenever required.

The pooled foodgrains were divided and subdivided to get a fair representative portion for the preparation of tribal food products in the laboratory. The dehusked grain (300 g) was cooked by the method of tribals using large amounts of water (5 volumes). The excess water was drained off and mixed with left over rice and kept overnight. Millets and rice (4:1) combination products commonly consumed by the tribals were prepared in the laboratory by adding rice to half boiled millet before cooking using mud pot and firewood. All the food preparations were standardized prior to analysis.

Initially, the food material was divided into 4 equal portions. One portion was kept aside for estimation of nutrients in the raw sample, the remaining three parts were cooked separately. Each of the cooked product was further divided into two equal parts. One part was mixed with the left over *conjee* and kept overnight. The raw and cooked sample (in triplicate) were pooled and mashed separately in a stainless steel grinder. An aliquot of this was immediately taken for the estimation of thiamine and niacin. Rest of the material was dried at 50 to 60°C overnight, powdered. Protein digestibility *in vitro* was determined in the powdered samples.

*Cooking of legumes:* The boiled legumes were drained, dried overnight in an oven at 50 to 60°C and powdered in a stainless steel grinder for estimation of protein digestibility *in vitro*.

Three lots of each of the nine pulses collected from the tribals were individually divided into three-60 g portions. One portion was used for analysis of nutrients in the raw state. The other two portions (60 g each) were cooked separately with adequate amounts (10 volumes) of water. No prior soaking was done. The legumes were cooked until they were



soft when pressed between fingers. In the case of *Dukka chikkudu*, a toxic legume was specially processed by boiling and draining off the blackish water several times till the liquid appeared milky white.

**Nutrient analysis in tribal foods:** Thiamine was estimated using modified thiochrome method of Leveille<sup>1</sup> as modified by Ramasastry<sup>2</sup>. Niacin was determined microbiologically using *L. arabinosis* following AOAC procedures<sup>3</sup>. *In vitro* digestibility of protein (IVPD) was determined using pepsin (Sigma chemical Co, USA) and pancreatin (Loba chemical Bombay) as described by Akeson and Stahmann<sup>4</sup>. Trypsin inhibitor content of the pulses was estimated by using the method described by Kakada *et al.*<sup>5</sup>.

**Results and Discussion**

The cereal/millet products prepared in the laboratory are listed in Table 1. The data on processed legume are shown in Table 2. The effect of boiling and fermentation on the thiamine and niacin contents of cereals and millets (Table 3) indicates a loss of 75-100 per cent in thiamine and 24-75 per cent in niacin on boiling and draining. Similar trend has been

TABLE 1. CEREAL AND MILLET PRODUCTS PREPARED BY TRIBAL COOKING METHODS

Product	Wt of raw food (g)	Wt of cooked food (g)	Cooking time (min.)	Conjee left over (ml)
Bajra and rice	300	660	16	170
Korra and rice	300	750	20	250
* Korra (raw)	300	660	18	160
**Korra (parboiled)	300	750	18	350
**Ooda (parboiled)	300	720	27	300
Jowar	300	1080	20	150
Rice	300	1065	15	150
Ragi gruel	100	750	15	—
Bajra gruel	100	250	12	—

Values are means of three samples

\*Locally known as *Veldulu*; \*\*Locally known as *Uppullu*

TABLE 2. DATA ON PROCESSED PULSES

Legume	Initial wt of raw food taken (g)	Cooked wt of food (g)	Cooking time of the unsoaked food (hr.-min.)
Horsegram (white var)	60	148	2-05
Horsegram (black var)	60	138	2-20
Judumulu	60	170	0-50
Redgram (podu cultivated)	60	158	2-05
<i>Dukka chikkudu</i>	60	106	8-00
Field bean (white var)	60	142	1-10
Field bean (red var)	60	152	1-30
Cowpea (green var)	60	142	0-55

Average of duplicate determinations

reported by Aliya and Geervani<sup>6</sup> in rice and millet products subjected to similar processing. In case of prefermented finger millet (ragi) product, increase in thiamine (50 per cent) and niacin (36 per cent) was observed. Hence, fermentation before cooking is advantageous in terms of improving the B-vitamin contents of the product. Tribals have a unique practice of leaving the left over rice and millets in drained *conjee*. Overnight soaking did not increase the thiamine and niacin contents.

The effect of processing on the IVPD of cereals/millet is presented in Table 4. The IVPD of cereals/millets was in the range of 43-97 per cent in uncooked samples and 19-85 per cent in cooked samples. The reduction in IVPD upon processing of cereals and millets was 9-24 per cent. The loss in IVPD was minimum (9 per cent) in *korra* and rice combination product and maximum in ragi gruel (24 per cent). Similar trends in processing of millets have been reported by Axtell *et al.*<sup>7</sup> and Bradbury *et al.*<sup>8</sup>. Maximum loss in IVPD (22 per cent) was noticed in combination product of bajra and rice followed by bajra gruel (20 per cent). Variations in IVPD of cereals/millets due to processing may be attributed to the differences in solubilities of proteins as reported by Loranz *et al.*<sup>9</sup>.

TABLE 3. EFFECT OF PROCESSING OF CEREALS AND MILLETS BY TRIBAL METHODS ON THIAMINE AND NIACIN (mg/100 g) CONTENTS

Cereals/millets	Before cooking		After cooking		The product with <i>conjee</i> kept overnight	
	Thiamine	Niacin	Thiamine	Niacin	Thiamine	Niacin
Rice	0.15 ± 0.005	1.2 ± 0.005	0 ± 0.0	0.6 ± 0.0	0.25 ± 0.0	0.5 ± 0.0
Bajra and rice combined	0.22 ± 0.005	2.1 ± 0.0	0.01 ± 0.0	0.6 ± 0.01249	0.30 ± 0.0	0.9 ± 0.0
<i>Korra</i> and rice combined	0.41 ± 0.00248	2.0 ± 0.0	0.02 ± 0.0	1.4 ± 0.0	0.35 ± 0.0	1.4 ± 0.0
<i>Korra</i> rice	0.33 ± 0.0	1.5 ± 0.0024	0.01 ± 0.0	0.4 ± 0.0	0.25 ± 0.0	1.1 ± 0.0
<i>Korra</i> rice (parboiled)	0.35 ± 0.0005	2.6 ± 0.1	0.05 ± 0.0	1.9 ± 0.01249	0.26 ± 0.0	2.2 ± 0.0124
<i>Ooda</i> rice (parboiled)	0.40 ± 0.005	1.5 ± 0.111	0.10 ± 0.0	0.9 ± 0.0	0.20 ± 0.0	0.7 ± 0.0
Jowar rice	0.21 ± 0.0024	2.5 ± 0.0	0.02 ± 0.0	0.9 ± 0.0	0.30 ± 0.0	2.2 ± 0.01249
Ragi gruel (fermented)	0.40 ± 0.0024	1.1 ± 0.01249	0.60 ± 0.0	1.5 ± 0.0		
Bajra gruel (fermented)	0.30 ± 0.0	3.7 ± 0.05	0.05 ± 0.0	2.8 ± 0.025		

Values are the average of four independent determinations

TABLE 4. EFFECT OF PROCESSING BY TRIBAL METHODS ON *IN VITRO* PROTEIN DIGESTIBILITY OF CEREALS AND MILLETS

Cereal and millet	%IVPD		% loss
	Before cooking	After cooking	
Rice	97 ± 0.50	85 ± 0.50	12
Bajra and rice combined	97 ± 0.00	75 ± 0.50	22
Korra and rice combined	87 ± 0.707	78 ± 0.707	9
Korra rice (unparboiled)	78 ± 0.86	67 ± 0.0	11
Korra rice (parboiled)	77 ± 0.00	61 ± 0.50	16
Ooda rice (parboiled)	73 ± 0.3125	59 ± 0.707	14
Jowar rice	81 ± 0.50	71 ± 0.707	10
Ragi gruel (fermented)	43 ± 0.50	19 ± 0.10	24
Bajra gruel (unfermented)	96 ± 0.707	76 ± 0.50	20

Values are the average of four independent determinations

TABLE 5. EFFECT OF PROCESSING BY TRIBAL METHODS ON *IN VITRO* PROTEIN DIGESTIBILITY OF LEGUMES

Legume	% IVPD		% improvement
	Before cooking	After cooking	
Cowpea (green)	69 ± 0.50	88 ± 0.0	19
Field bean (dry)			
Black	40 ± 0.707	70 ± 0.50	30
White	72 ± 0.00	98 ± 0.50	26
Red	67 ± 0.50	99 ± 0.707	32
Judumulu	65 ± 0.707	80 ± 0.00	15
Horsegram			
White	65 ± 0.00	80 ± 0.50	15
Black	62 ± 0.50	83 ± 0.50	21
Redgram			
Dryland cultivated	71 ± 0.00	100 ± 0.707	29
Hill cultivated	70 ± 0.00	99 ± 0.00	29
Dukka chikkudu (dehusked)	40 ± 0.50	70 ± 0.50	30

Values are the average of four independent determinations

TABLE 6. EFFECT OF PROCESSING BY TRIBAL METHODS ON THE TRYPSIN INHIBITOR CONTENT OF LEGUMES

Legume	*TIU/ml of extract		% loss of TIU
	Before cooking	After cooking	
Cowpea (green)	51 ± 0.00	11 ± 0.4082	78.0
Field bean (dry)			
Black var	50 ± 0.00	14 ± 0.577	72.0
White var	52 ± 0.577	9 ± 0.2886	82.7
Red var	53 ± 0.577	7 ± 0.00	86.8
Judumulu	50 ± 0.00	11 ± 0.2886	78.0
Horsegram			
White var	49 ± 0.816	8 ± 0.00	83.7
Black var	50 ± 0.577	10 ± 0.00	80.0
Redgram			
Dryland cultivated	49 ± 0.00	8 ± 0.00	83.7
Hill cultivated	51 ± 0.25	8 ± 0.00	84.3
Dukka chikkudu	39 ± 0.00	6 ± 0.2886	84.6

\*TIU = Number of Trypsin Units Inhibited (TUI)

Values are the average of four independent determinations



Parboiling of rice is a traditional practice among rural and urban rice eating population in some parts of South India. Tribals, however, parboil not only paddy but also millets. Parboiling resulted in reduction in IVPD of cereals/millets due to an increase in lipid bound to starch and protein and free phenolic acid resulting in reduced extractability of protein<sup>10</sup>. The reasons for decrease in IVPD due to fermentation of ragi batter observed in cereals and millets in the present study are not known. Contrary to the observations in cereals and millets, boiling of legumes improved the IVPD by 15-32 per cent (Table 5). This may be due to destruction of inhibitors present<sup>11,12</sup>. All the varieties of legumes tested contained trypsin inhibitor. However, most of it is destroyed upon cooking (Table 6). The results indicate 87 per cent destruction of trypsin inhibitor upon boiling. Maximum destruction of trypsin inhibitor content is observed in field bean-red variety (87 per cent) and minimum destruction with field bean-black variety (72 per cent).

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## Processing, Evaluation and Storability of Date Jelly

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Results of the organoleptic and chemical evaluation revealed the possibility of processing date jelly of good quality. The pH of the fresh jelly was 3.57 whereas its Brix and  $a_w$  were 73.3 and 0.75 respectively. Storage up to 6 months had a slight effect on the moisture and  $a_w$  and a moderate effect on the pH, colour and sugar content of the date jelly. No significant differences were found between the scores of the date jelly stored for 0, 2, 4, and 6 months when compared with the imported jelly. These results highlight the possibility of storing date jelly up to 6 months without affecting their good quality attributes.

Dates (*Phoenix doctylifera* L.) are considered a major fruit crop in the Middle East Countries as 75 to 80 per cent of the world production is produced in this region. Dates consist mainly of sugars (75–85 per cent on dry wt basis) with small amounts of other components. Sucrose, fructose and glucose are the simple sugars found in most date cultivars. The ratio of fructose to glucose in ripe soft dates is in the range of 1:1. Besides sugars, dates are also considered rich in fibre, some vitamins and minerals and trace elements<sup>1</sup>.

The annual production of date crop has doubled in the Kingdom of Saudi Arabia in the last few years. On the other hand, less than 10 per cent of the produce (50,000 tons) is processed and as a result, substantial quantities of this important crop are lost annually due to lack of efficiency in the marketing and pest infestation. The substantial quantities of dates available in the Kingdom, their high nutritive value, and their high level of invert sugar, justify processing them to jelly to replace the imported jelly which accounted for \$3.5 millions for the year 1987<sup>2</sup>.

Several studies have been carried out to incorporate dates in different food products such as bakery items, beverages, jam and other confections<sup>3,4,5</sup>. Recently, promising results were obtained by using date paste to replace caramel in candy and fruit bars<sup>6</sup>. The possibility of using second quality date in date jelly, jam and date kutter making was also studied<sup>7</sup>. The results indicated that the processed date by-products possessed high quality attributes and were well accepted by the panel members. Recipes and methods of preparation for jellies of different toughness are well documented<sup>8,9</sup>.

The present work deals with the production of different types of date jelly, their chemical composition, organoleptic properties and storability.

### Materials and Methods

Date fruits of the 'Ruzeis' variety, of the 1987 season, were obtained from the Nadek Date Factory at Al-Hassa, Saudi Arabia. The date fruits were cleaned, pitted and soaked in

sufficient water (1:2 portion) at 25°C for 5 min, drained for 10 min and ground in a Kraft meat grinder, model A2-3, to produce date paste. Date juice was prepared by adding water to date paste in the ratio of 3:1 (w/w). The mix was boiled gently with continuous stirring for 5 min, filtered through cheese cloth, to remove the fibre and other impurities.

Sucrose and citric acid were procured from the local market whereas commercial pectin was of BDH grade.

Date jelly was prepared on laboratory scale by boiling date juice and addition of sugar at the ratio 50/50 and 50/10 (w/v). Boiling was continued until a Brix of 65 or a temperature of 105°C was reached. Citric acid and pectin were added as percentages of date juice used and were dissolved separately in small quantities of the hot syrup and were added to the boiling mix. Boiling was continued for 5 min or until a temperature of 108°C was reached. Ascorbic acid (0.1 per cent) and benzoic acid (0.01 per cent) of date juice were dissolved in small quantities of the hot syrup and were then added to the boiling mix. The produced jelly was filled in glass jars and cooled immediately. Four samples of date jelly were prepared with a code no. of 70, 110, 130 and 160. The prepared samples were subjected to sensory evaluation. Large quantities of date jelly no. 70 were produced and filled in jars which were stored for 0, 2, 4 and 6 months at 25°C for storage stability studies. The effect of storage time on the chemical (moisture, pH,  $a_w$ , colour and sugar content) and sensory qualities (colour, flavour and texture) of the date jelly were studied.

Moisture, pH, and total soluble solids (Brix values) were determined according to the methods outlined in AOAC<sup>10</sup>. Colour was measured using an extraction procedure as described by Maier and Schiller<sup>11</sup>. The sugar monomers were determined by high pressure liquid chromatography (HPLC) as described by Yousif<sup>12</sup>. Water activity was determined using standard salt solutions as described by D,Alton<sup>13</sup>.

The date jelly samples were evaluated for sensory qualities at the time of preparation using the multiple comparison



difference test as recommended by Lrmond<sup>14</sup>. Coded samples of date jelly were given to a panel of 10 judges. An imported fruit jelly sample was selected as the reference and was labelled R. The judges were asked to test the date jelly samples and to show whether they are better than, equal to or inferior to the reference. The scoring sheet also included the extent of difference. Numerical scores were assigned to the ratings with "no difference" equaling five, "extremely better than R" equaling one and "extremely inferior than R" equaling nine. Analysis of variance of the scores was then conducted. The scores were also rated as "excellent" for 1-2, "V. good" for 2.1-3, "good" for 3.1-4, "acceptable" for 4.1-5 and poor for 5.1 or more. Data were analysed using SAS computing system<sup>15</sup>. The analysis of variance procedure was used and the parameters which were computed included: means, coefficient of variance (C.V.), standard deviation, standard error and least significant difference (LSD).

### Results and Discussion

Table 1 shows the chemical and sensory evaluation of fresh date jelly. These results show that the prepared date jelly samples have Brix values of 65.8 to 76.6, whereas their pH values ranged between 3.19 and 3.69. The sensory evaluation results (Table 1) reveal that jelly sample no. 70 achieved the best scores (2.5 which is equivalent to very good) followed by jelly sample no. 160. An attractive colour and good texture and flavour were characteristic of these two jelly samples. On the other hand, jelly samples no. 110 and 130 were of good colour and acceptable flavour but had a tough or harsh texture.

Large quantities of the jelly samples were produced and used for storage studies. The effect of storage time (0, 2, 4 and 6 months) on the moisture,  $a_w$ , pH, sugar content and the colour of the date jelly was studied. The results obtained are presented in Fig. 1-8. The sensory evaluation mean scores for fresh and stored samples are also included in Table 2.

It is clear from Fig.1 that the moisture content was affected significantly after 4 and 6 months storage time. This decrease in the moisture content of date jelly might be partly attributed to the change from the crystalline to the amorphous form for components in date jelly which permits the binding of water.

Both chemical reaction rates and microbial activity are directly controlled by water activity ( $a_w$ ). The processed date jelly is of high stability since it has a  $a_w$  value of 0.75

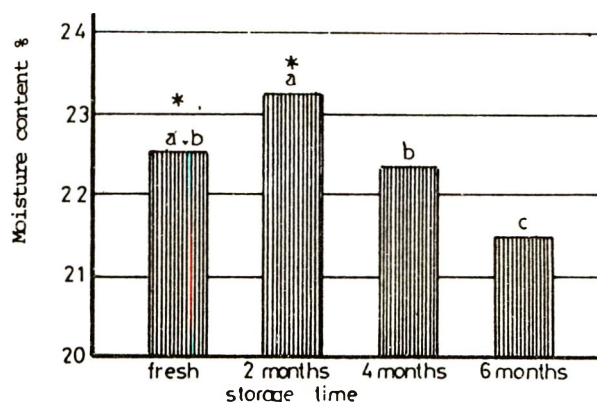


Fig.1. Effect of storage on the moisture of date jelly. \*Bars with different letters are significantly different.

(Fig. 2) which is within the safe level<sup>16</sup>. After 6 months storage, a slight, although statistically significant ( $p < 0.05$ ), decrease can be seen in the  $a_w$  value of date jelly.

The pH of the date jelly is slightly influenced by storage time (Fig. 3). A significant decrease in the pH of date jelly can be observed after 4 and 6 months storage time.

The colour of date jelly is one of its most important quality attributes. Results in Fig. 4 show that storage for 2 months at 25°C has no effect on the colour of date jelly. Increasing storage time to 4 and 6 months causes a considerable increase in the darkening of date jelly. Nevertheless, the colour at the end of 4 and 6 months was still acceptable as had been judged

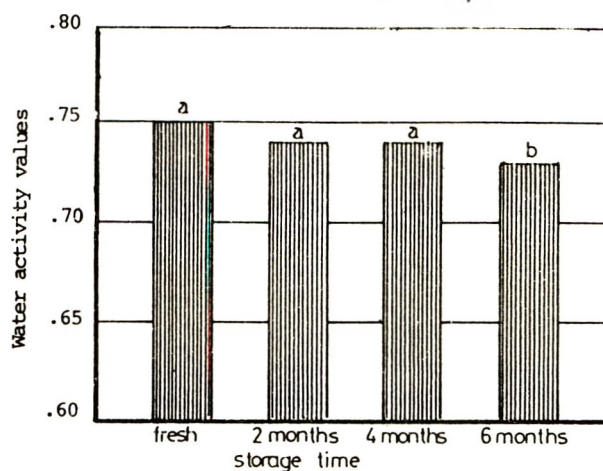


Fig.2. Effect of storage on water activity of date jelly.

TABLE 1. RECIPES FOR DATE JELLY

Sample Code No.	Date juice (l)	Sugar date juice	Ingredients				Final product pH	Final product Brix	Sensory evaluation scores
			Pectin (%)	Citric acid (%)					
70	2	50/50	1.5	0.8		3.24	76.60	2.50	
110	2	50/50	1.5	0.8		3.38	68.70	3.00	
130	2	50/10	1.5	0.8		3.19	65.80	3.50	
160	2	50/50	1.0	0.8		3.69	69.50	2.75	

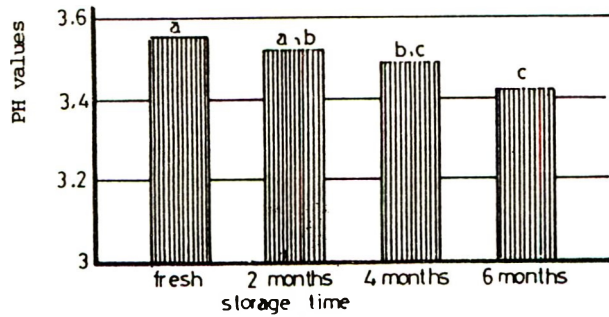


Fig.3. Effect of storage on the pH of date jelly.

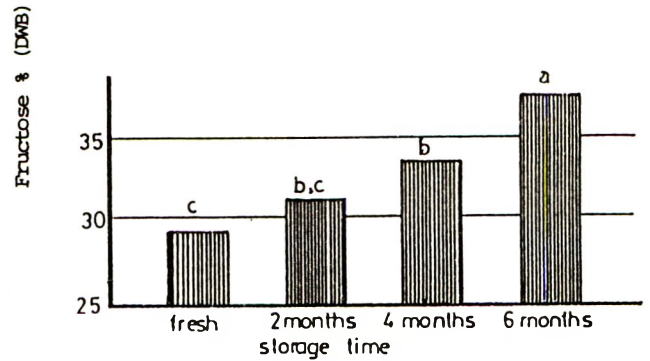


Fig.6. Effect of storage on fructose of date jelly.

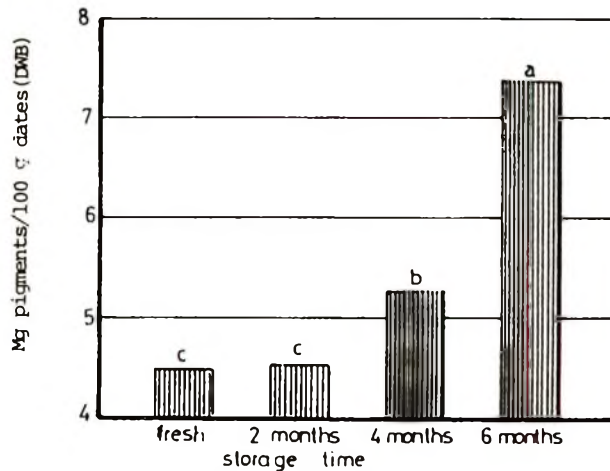


Fig.4. Effect of storage on the colour of date jelly.

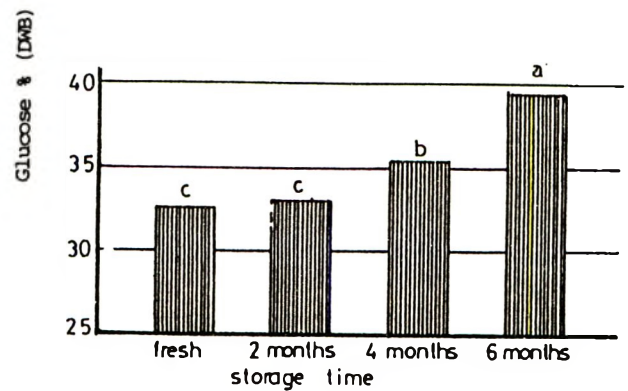


Fig.7. Effect of storage on the glucose of date jelly.

by the panelists. The changes in the colour of date jelly during storage is due to non-enzymatic browning reactions.

The present results (Fig. 5-8) show although the total sugar content was not significantly affected during storage, fructose, glucose and sucrose individually showed great variations. The increase in fructose and glucose (Fig. 6 and 7) is more pronounced which is statistically significant after 4 and 6 months. On the contrary, sucrose shows a pronounced decrease after 4 and 6 months storage. These changes may be due to sucrose inversion due to the acid environment.

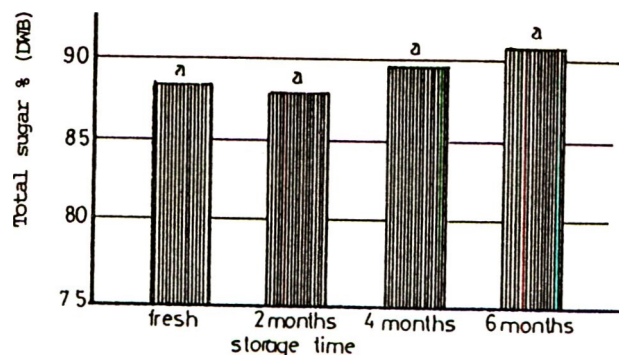


Fig.5. Effect of storage on the total sugar of date jelly.

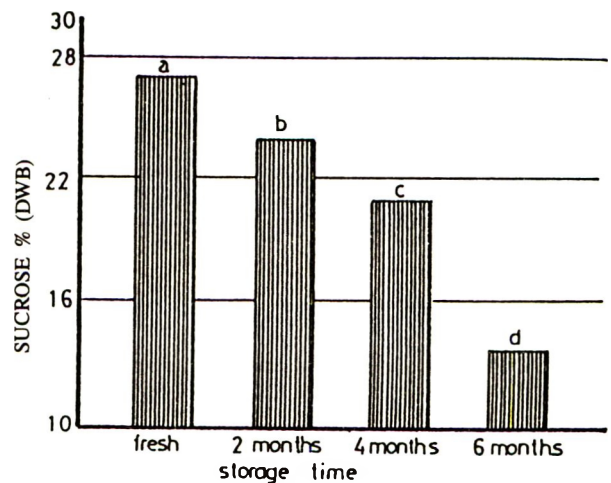


Fig.8. Effect of storage on the sucrose of date jelly.

The effect of storage at 25°C for 2, 4 and 6 months on the organoleptic properties of date jelly is presented in Table 2. It is apparent from the results of the analysis of variance that storage time up to 6 months did not significantly affect the organoleptic properties of the date jelly since the calculated F value (2.15) was less than the tabulated one (2.95). This indicates the possibility of storing the date jelly up to 6 months

TABLE 2. ORGANOLEPTIC EVALUATION OF DATE JELLY

Panelist	Fresh	Storage period			Total
		2 months	4 months	6 months	
1	1	2	2	7	12
2	2	2	3	4	11
3	2	3	3	4	12
4	3	3	4	4	14
5	3	3	4	4	14
6	3	3	4	4	14
7	3	4	4	3	14
8	3	4	4	3	14
9	5	5	5	2	17
10	5	5	5	7	22
Total	30	34	38	42	144
Mean	3.0	3.4	3.8	4.2	

ANOVA (Analysis of variance)					
Source of difference	DF	SS	MS	F (Cal)	F (Tab)
Samples	3	8	2.67	2.15	2.95
Panelist	9	22.1	2.46	1.98	
Error	27	33.5	1.24		
Total	39	63.6	1.63		

without affecting its organoleptic properties. However, the mean sensory scores ranged between 3 (very good) for the fresh date jelly and 4.2 (acceptable) for 6 months stored date jelly.

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## Effect of Additives and *Lactobacillus casei* on Flavour Development in Cheddar Cheese from Buffalo Milk

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Acceleration of flavour development during ripening process of Cheddar cheese was studied. The addition of sodium citrate and sodium bicarbonate to milk failed to accelerate the flavour development. Supplementation of milk with *Lactobacillus casei* at 0.5% level along with the normal starter bacteria culture accelerated flavour development which was correlated with glycolysis, proteolysis and lipolysis during ripening.

Cheese is an important dairy product with a range of varieties, of which cheddar cheese is the foremost. The consumption of cheddar cheese has increased steadily during the past years. The present estimate of total cheese production in India is 2000 tonnes. Cheddar cheese prepared from cow's milk has the characteristic flavour and texture. However in India, buffalo milk constitutes 66 per cent of total milk production<sup>1</sup> and cheese prepared from buffalo milk lacks the characteristic cheesy flavour in spite of prolonged ripening for 8-12 months. Acceleration of ripening process has been attempted through the use of certain chemical additives and bacterial cultures<sup>2</sup>. Among the bacterial cultures, *Lactobacillus casei* has been shown to have a positive role of flavour development due to its proteolytic and lipolytic activities<sup>3,6</sup>. Addition of sodium bicarbonate, calcium carbonate, sodium citrate or sodium pyrophosphate has resulted in improvement of cheese quality prepared from buffalo milk as well as reduction in the ripening period<sup>7,9</sup>. In this study, an attempt has been made to accelerate the flavour development in cheddar cheese prepared from buffalo milk through the use of certain chemical additives and *L. casei*.

### Materials and Methods

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

**Manufacture of cheddar cheese:** Buffalo milk was procured from the Experimental Dairy of this Institute. Cheddar cheese was manufactured from 90 l of buffalo milk

standardized (casein/fat ratio of 0.70), pasteurized (63°C/30 min), cooled (28°C) and to which sodium citrate (0.015 per cent) and sodium bicarbonate (0.05 and 0.01 per cent) and *L. casei* cells (0.5 per cent) were added. The rest of the procedure was as suggested by Jha and Singh<sup>10</sup>.

**Sensory evaluation:** The sensory characteristics of cheddar cheese in terms of flavour, body and texture and colour were evaluated by a panel of 5 judges using cheddar cheese score card<sup>11</sup>, at 2 months interval upto 10 months of ripening at  $8 \pm 1^\circ\text{C}$ .

**Chemical analysis:** Cheddar cheese was analysed for glycolysis, proteolysis and lipolysis. The fat in milk was determined by Mojonnier method<sup>12</sup>. The pH of the cheese was measured by Digital pH meter. The soluble protein content in cheese was analysed by the method described by Kosikowski<sup>13</sup>. Titrable acidity was determined by A.O.A.C. method<sup>14</sup>. The total free fatty acids were determined by the method recommended by Ramamurthy and Narayanan<sup>15</sup>.

### Results and Discussion

**Sensory characteristics:** The effects of additives (sodium bicarbonate and sodium citrate) and *L. casei* on sensory characteristics of cheese are presented in Table 1. Cheese samples made with sodium bicarbonate (0.005) and sodium citrate (0.01 and 0.015 per cent) were rated subnormal throughout the ripening period, whereas those prepared with *L. casei* were normal after 2 months and remained highest (37.5) upto 8 months followed by a decline (36.5) after 10 months of ripening. These additives failed to have any beneficial effect on the flavour development in cheese. On

TABLE 1. EFFECT OF ADDITIVES ON SENSORY CHARACTERISTICS OF CHEDDAR CHEESE DURING RIPENING AT  $8 \pm 1^\circ\text{C}$ .

Additives	Flavour at indicated ripening periods (months)					Body and texture at indicated ripening period (months)				
	2	4	6	8	10	2	4	6	8	10
Control	34	35	35	36	36.0	25.0	26.0	26	26.0	24.0 Gr
Sodium bicarbonate(0.005%)	34 He	35 He	35 He	35 He	34.0 Bi	25.0	25.0	26	25.0	24.0 Gr
Sodium citrate (0.01%)	34 Fl	35 Fl	35 Fl	35 Fl	35.5 Fl	25.0	25.5	26	25.5 Gr	24.0 Gr
Sodium citrate (0.015%)	34	35	35	34	34.0 Mo	25.0	25.5	26	24.5	23.5 Gr
<i>L. casei</i> (0.5%)	36	37	37	37	36.5	26.5	28.0	28	27.0	27.0

Fl-Flat; Bi-Bitter; He-Heated; Mo-Mouldy; Gr-Granules;  
Average of 3 replicates

the contrary, they appeared to be detrimental to flavour, body and texture. Cheese sample treated with sodium bicarbonate tended to show a heated kind of flavour which became bitter towards the end of ripening. The cheese with sodium citrate showed a flat taste. It appeared that the white granulation which is often a persistent problem in cheddar cheese is mainly due to the precipitation of calcium citrate or lactate because the intensity of granulation increased markedly in those samples wherein sodium citrate was added. This is in contrast to the reports of El-Safty *et al*<sup>0</sup> who reported that addition of sodium citrate to milk improved the cheese quality.

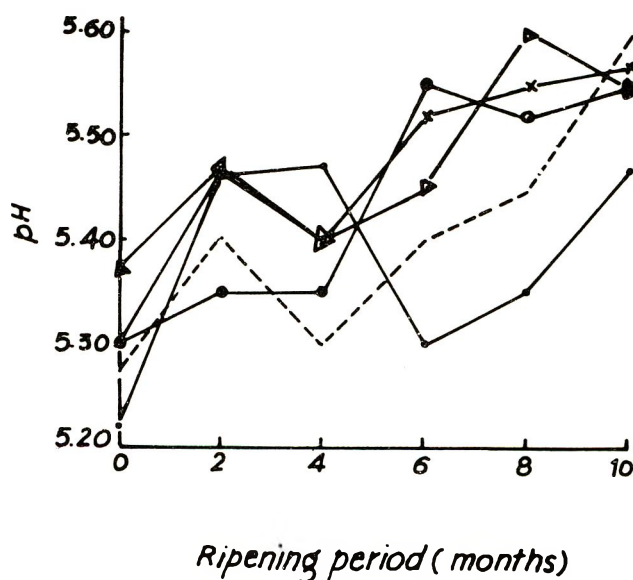
Supplementation of milk with *L. casei* along with the normal starter showed definite improvement in flavour quality of cheese. The results of our study also showed that supplementation of *L. casei* resulted in higher proteolysis<sup>3,16</sup>. The positive contribution of *L. casei* to cheese flavour development in cheese made from buffalo milk may be due to its relatively higher proteolytic activities<sup>5,6,16</sup>.

Addition of additives to milk did not improve the body and texture of cheese in comparison to control. *L. casei* cheese samples improved the body and texture significantly and scored maximum (28) after 4 months and declined to 27 after 6 months of ripening.

**Biochemical changes:** The effects of additives and *L. casei* on the biochemical changes are shown in Fig. 1, 2, 3 and 4. The initial pH of cheese ranged from 5.22 to 5.37, being minimal and maximal in samples having *L. casei* and sodium citrate (0.015 per cent). The pH values in cheese samples having additives followed more or less same pattern during ripening. Addition of *L. casei* brought down the initial pH to 5.22 which is supposed to be normal pH for cheddar cheese at the end of pressing. The pH increased sharply upto 2 months followed by stabilization at 4 months and rapid decrease thereafter. After 6 months of storage, the pH showed a steady increase<sup>17</sup>. The higher levels of pH in the cheese

samples may be attributed to the release of ammonia and their compounds after deamination of amino acids<sup>16</sup>. (Fig.1.)

The 0-day titratable acidity of cheese treated with additives and *L. casei* ranged from 0.58 to 0.66 per cent, being lowest and highest in control and sodium citrate (0.015 per cent) cheese sample. Additives did not have any specific effect on titratable acidity. Supplementation of milk with *L. casei* maintained a consistently higher level of titratable acidity which increased sharply after 8 months of ripening, reaching a final value upto 1.27 per cent. (Fig. 2.) This observation

Fig.1. Effect of additives and *L. casei* on pH in cheddar cheese during ripening at  $8 \pm 1^\circ\text{C}$ .

— Control  
 X---X Sod. bicarbonate (0.005%)  
 o----o Sod. citrate (0.01%)  
 Δ----Δ Sod. citrate (0.015%)  
 ●----● *L. casei*

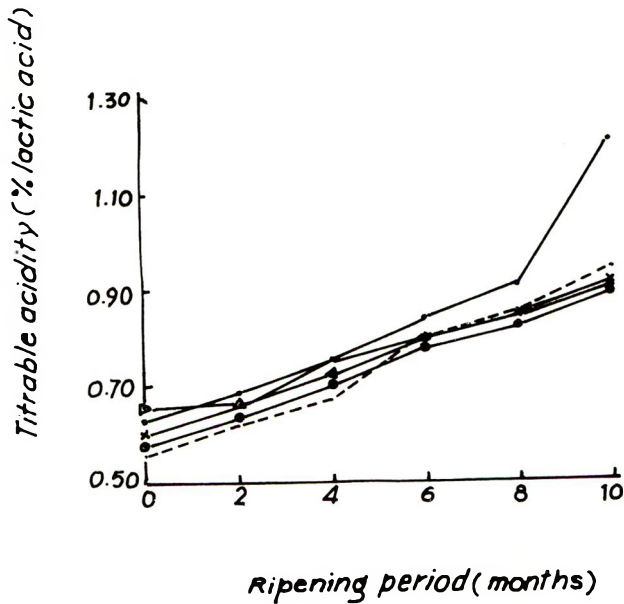


Fig. 2. Effect of additives and *L. casei* on titrable acidity in cheddar cheese during ripening at  $8 \pm 1^\circ\text{C}$ .

----- Control  
 X---X Sod. bicarbonate (0.015%)  
 o---o Sod. citrate (0.01%)  
 Δ---Δ Sod. citrate (0.015%)  
 ●---● *L. casei*

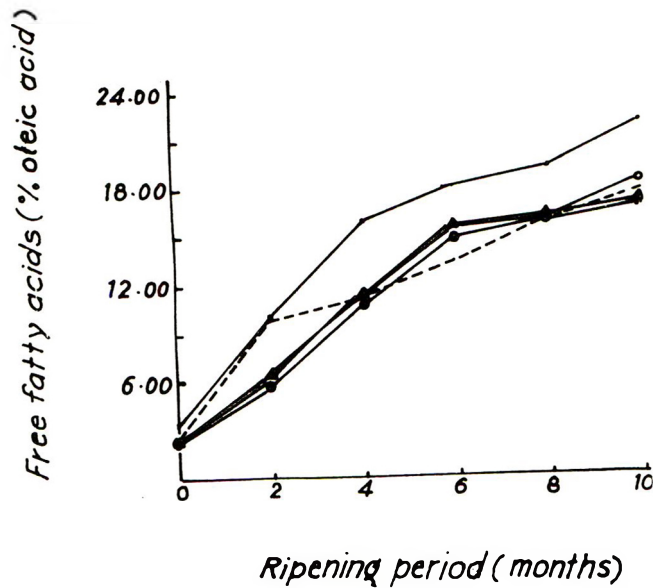


Fig. 4. Effect of additives and *L. casei* on free fatty acids in cheddar cheese during ripening at  $8/1^\circ\text{C}$ .

----- Control  
 X---X Sod. bicarbonate (0.005%)  
 o---o Sod. citrate (0.01%)  
 Δ---Δ Sod. citrate (0.015%)  
 ●---● *L. casei*

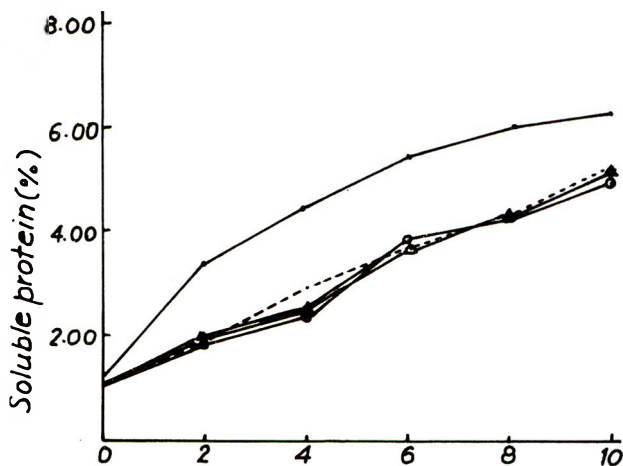


Fig. 3. Effect of additives and *L. casei* on soluble protein in cheddar cheese during ripening at  $8 \pm 1^\circ\text{C}$ .

----- Control  
 X---X Sod. bicarbonate (0.005%)  
 o---o Sod. citrate (0.01%)  
 Δ---Δ Sod. citrate (0.015%)  
 ●---● *L. casei*

Use of additives did not have significant effect on proteolysis. The initial value of soluble protein ranged from 1.09 to 1.22 per cent being highest in *L. casei* sample, which remained highest throughout the ripening period (Fig. 3). El-Safty *et al.*<sup>8</sup> used sodium citrate or sodium pyrophosphate (0.1M) to improve the cheese quality, the former being more effective. Schormuller *et al.*<sup>7</sup> noted increased proteolysis and reduced ripening period for skim milk cheese by addition of additives. *L. casei* cheese sample showed marked increase in soluble protein during ripening. The results of this study are in agreement with those of earlier workers<sup>18,19</sup>. The final soluble protein values in samples made from milk supplemented with *L. casei* was highest (6.29 per cent).

The effect of additives and *L. casei* on free fatty acids (FFA) initially ranged from 2.58 to 3.11  $\mu\text{M/g}$  fat, being highest in cheese supplemented with *L. casei*. (Fig. 4.) The increase in FFA of all experimental samples followed more or less similar pattern throughout the ripening, irrespective of additives. *L. casei* cheese samples markedly increased the rate of lipolysis and final concentration reached a level of 21.93  $\mu\text{M/g}$  fat. The present results are in agreement with those of earlier workers<sup>3,5,16</sup>.

is in agreement with well known fact that the lactobacilli establish and get activated during the latter part of ripening. Since they have high potential to produce lactic acid, the titrable acidity increased sharply<sup>3,4</sup>.

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## Changes in Carbohydrates and Free Amino Acids Caused by the Growth of Pure Cultures of Spoilage Bacteria on Meat Drip

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The influence of certain meat isolates when inoculated into sterile meat drips was studied. Analyses were carried out on the bacterial count, carbohydrate (glucose, glucose 6-phosphate, lactic acid) contents and amino acid levels. The isolates examined showed three different patterns in utilizing carbohydrates. *Pseudomonas* I and II preferentially utilized glucose, *Serratia liquefaciens* preferentially utilized glucose 6-p and *Moraxella*-like organism utilized glucose 6-p and glucose simultaneously. The levels of lactic acid were noted to fluctuate. *Pseudomonas* II was able to utilize the amino acid taurine. *Moraxella*-like organism attacked a different group of amino acids which were not really attacked by the other test organism. Little changes were observed in the levels of amino acids in *Serratia liquefaciens* inoculated samples.

The aerobic spoilage flora or fresh meat stored at chill temperature is usually dominated by species of *Pseudomonas* although other bacteria such as *Moraxella*, *Enterobacter* spp. may be present<sup>1,2</sup>. Substrate utilization of a fluorescent *Pseudomonas* sp. and *Lactobacillus* sp. growing on meat juice medium has been examined by Gill<sup>3</sup>. Gill and Newton<sup>4</sup> studied the development of aerobic spoilage flora on meat stored at chill temperature using meat juice as a substrate. There is no similar information on substrate utilization by aerobic meat spoilage flora using meat drip as a substrate. The importance of meat drip arises from its being released when meat is thawed or vacuum packed<sup>5</sup>. No information is available in the literature to show the spoilage pattern in meat drip when frozen meat is thawed. The present study determines the changes caused by pure cultures of some spoilage organisms in refrigerated drip fluid.

### Materials and Methods

The organisms were isolated from spoiling meat in a previous study by Babiker<sup>6</sup> and identified according to Cowan and Steel<sup>7</sup>. The selected isolates consisted of *Pseudomonas* I, II, *Moraxella*-like organism and *Serratia liquefaciens*.

Bovine striploin (*M. longissimus dorsi*) blocks stored at  $-20^{\circ}\text{C}$  for 3 days were allowed to thaw at  $4^{\circ}\text{C}$  overnight. The drip was collected and diluted five times with distilled water. It was filter sterilized using Seitz filters and the filtrate was distributed in 10 ml quantities in sterile test tubes. The tubes were held for 2 hr in a water bath adjusted to  $4^{\circ}\text{C}$  to attain the required temperature before inoculation with test organisms. Control samples were handled in the same way except they were uninoculated.

A pure culture of each organism was grown in 5 ml sterile nutrient broth overnight at  $25^{\circ}\text{C}$ . Using a dropper pipette,

one drop (0.02 ml) was sub-cultured into 10 ml of sterile nutrient broth and inoculated for 24 hr at  $25^{\circ}\text{C}$ . Cells were harvested by centrifugation at  $1.4 \times 10^3$  g for 15 min and then washed twice with 9 ml sterile distilled water and 0.02 ml from suitable dilutions was inoculated into the sterile meat drip. Following inoculation, all the tubes were incubated in a water bath adjusted to  $4^{\circ}\text{C}$ . Seven drip samples were analyzed for each organism and analyses of total count, pH, carbohydrate (glucose, glucose 6-p and lactic acid) were carried out at 0, 2, 5, 7, 9, 12 and 16 days, while analyses of amino acids were only at 0, 5 and 16 days.

**Microbiological analysis:** Growth of bacteria was followed by spread plate method. The plates were incubated at  $25^{\circ}\text{C}$  for three days before the number of bacteria was obtained.

The pH of the diluted drip was determined (after microbial sampling) using a Corning pH meter (Model 10).

**Carbohydrate analysis:** Samples for carbohydrate analysis were prepared by mixing 2 ml of spoiling diluted drip with 2 ml of 12 per cent (v/v) perchloric acid<sup>8</sup>. The cells and proteins were removed by centrifugation at  $2.2 \times 10^3$  g for 20 min. The supernatant was placed on ice and the pH was adjusted to 6.5 with 20 per cent (w/v) potassium hydroxide. The precipitated potassium perchlorate was removed by centrifugation and the supernatant was used for the determination of glucose, glucose 6-p and lactic acid. Boehringer kits (Boehringer Mannheim) were used for determination of carbohydrates<sup>8</sup>.

**Determination of individual free amino acids:** Five ml of the spoiling drip was treated with 25 ml of 1 per cent (w/v) picric acid to precipitate the proteins<sup>9</sup>. The samples were centrifuged at  $2.2 \times 10^3$  g for 20 min. The supernatant was decanted and stored at  $-20^{\circ}\text{C}$  prior to further preparation. The picric acid treated meat extracts were removed from the

freezer and allowed to thaw at room temperature. The picric acid was removed using Amberlite IRA 410 resin (Hopkin and William) in the chloride form.

**Amino acid analysis:** Amino acids were analyzed using LKB amino acid analyzer (Model 4400, LKB Instrument Ltd.). The determination of results as mg amino acid/100 ml sample was carried out as described by Babiker *et al.*<sup>10</sup>.

**Results and Discussion**

**Utilization of carbohydrates:** *Pseudomonas* I and II isolates showed preferential removal of glucose from meat drip. Preferential utilization of glucose by *Pseudomonas* has been reported by Jacoby<sup>11</sup> in liquid culture, by Gill<sup>3</sup> and Shelef<sup>12,3</sup> in meat and by Gill and Newton<sup>13</sup> in fat. *Pseudomonas* I and II showed a decrease in glucose 6-p in inoculated meat drip when the total count was almost 10<sup>7</sup> (Fig. 1 and 3). However, the same isolates utilized lactic acid in meat drip when the bacterial counts exceeded 10<sup>7</sup> organisms per ml (Fig. 2 and 4). Rhodes<sup>14</sup> and Stewart<sup>15</sup> reported that members of the genus *Pseudomonas* have the ability to utilize lactate and suggested that they might be responsible for the decrease in lactic acid in spoiled meat. Fig. 2, 4, and 6 show that the production of lactic acid was not followed by a decrease in the pH as was expected. This could be attributed to the production of alkaline substances as a result of utilization of amino acids.

In meat drip *Moraxella*-like organism metabolized glucose and glucose 6-p simultaneously (Fig. 5). *Serratia liquefaciens* preferentially utilized glucose 6-p, whereas the glucose concentration remained almost constant up to 8 days when the total count reached approximately 10<sup>7</sup> organism per ml (day 7) (Fig. 7). It was the only isolate examined that showed a continuous increase in lactic acid concentration (Fig. 8). The fact that *Moraxella*-like organism, *S. liquefaciens*,

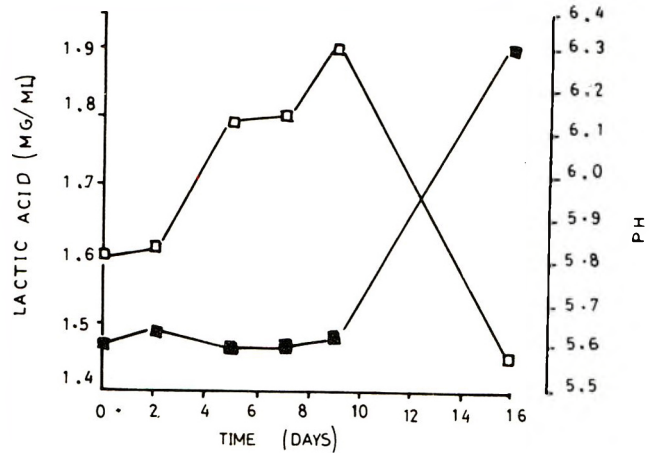


Fig. 2. Changes in lactic acid and pH by *Pseudomonas* I

■ — pH  
□ — lactic acid

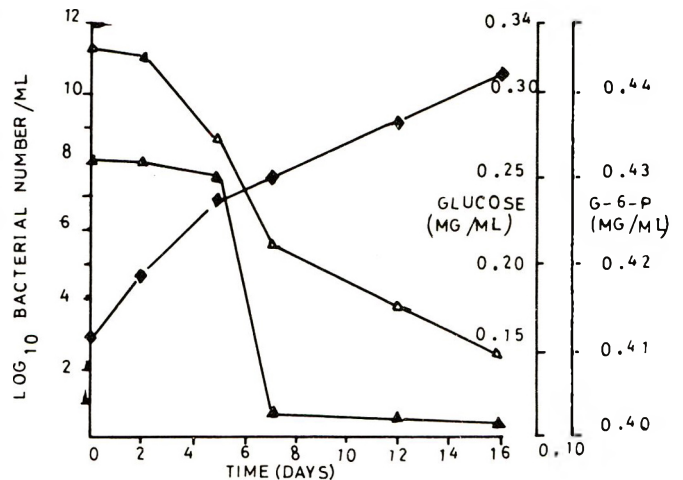


Fig. 3. Utilization of glucose by *Pseudomonas* II

◆ — bacterial count  
▲ — glucose -6p  
△ — glucose,

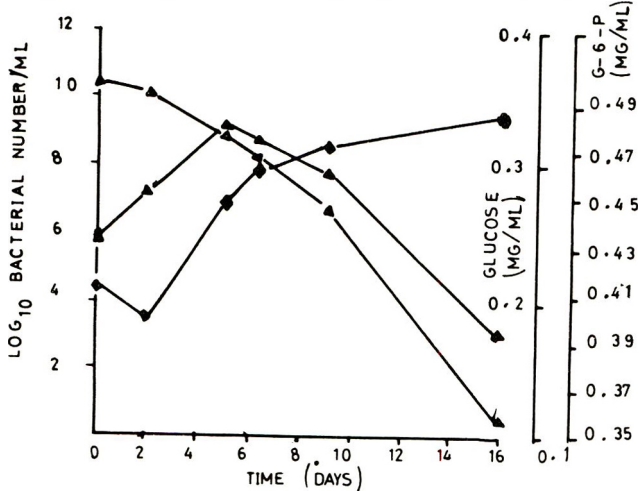


Fig. 1. Utilization of glucose by *Pseudomonas* I

◆ — bacterial count  
△ — glucose  
▲ — glucose 6-p

*Pseudomonas* I and II were found to utilize glucose 6-p does not agree to some extent with the findings of Gill and Newton<sup>4</sup> who reported that glucose 6-p is probably utilized only by members of the *Enterobacteriaceae*.

**Utilization of amino acids:** The increases noted in sterile control uninoculated sample throughout the storage period in glutamic acid, proline, valine, methionine, isoleucine, leucine, tyrosine, histidine and arginine concentrations (Table 1) suggest the presence of endogenous proteolytic enzymes. In studies on the proteolysis of beef, Locker<sup>16</sup> thought that amino exopeptidases of muscle which act only on N-terminal group and remove amino acid stepwise contributed to the increase in free amino acids in general and glutamic acid in particular. It is of importance to mention that drip released from meat contains lysosomal enzymes (cathepsins, nucleases, phosphatases) which are capable of breaking



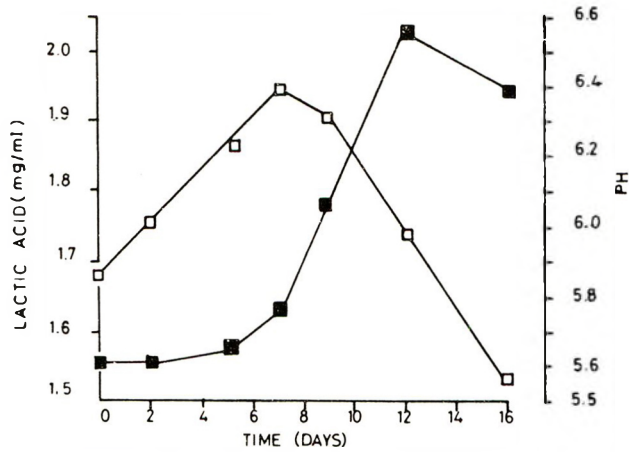


Fig. 4. Changes in lactic acid and pH by *Pseudomonas* II  
 ■ — pH □ — lactic acid

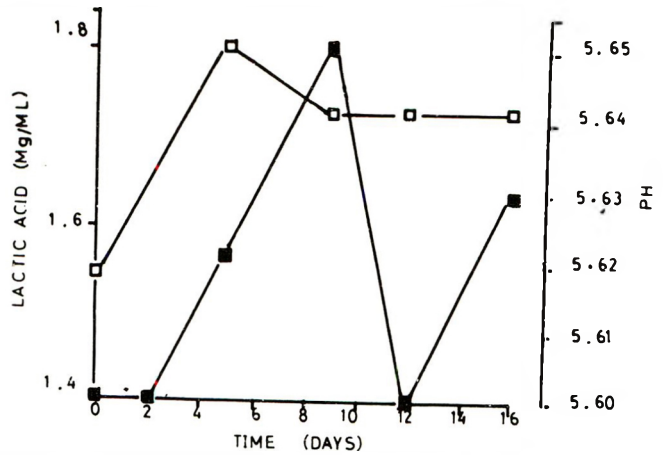


Fig. 6. Changes in lactic acid and pH by *Moraxella* - like organism  
 ■ — pH □ — lactic acid

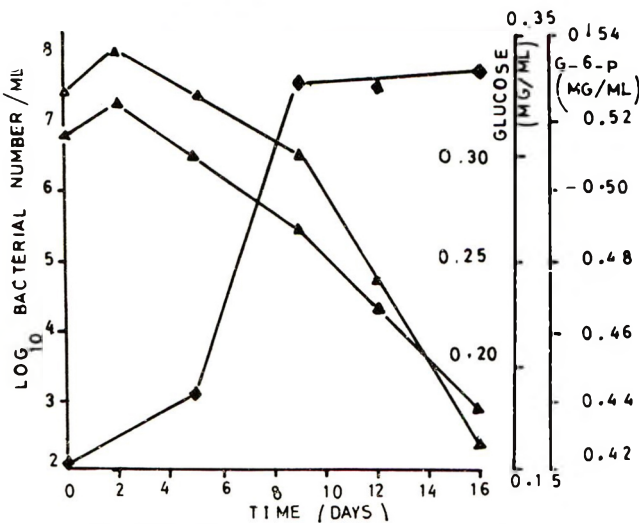


Fig. 5. Utilization of glucose by *Moraxella* - like organism  
 ◆ — bacterial count  
 ▲ — glucose -6p  
 △ — glucose

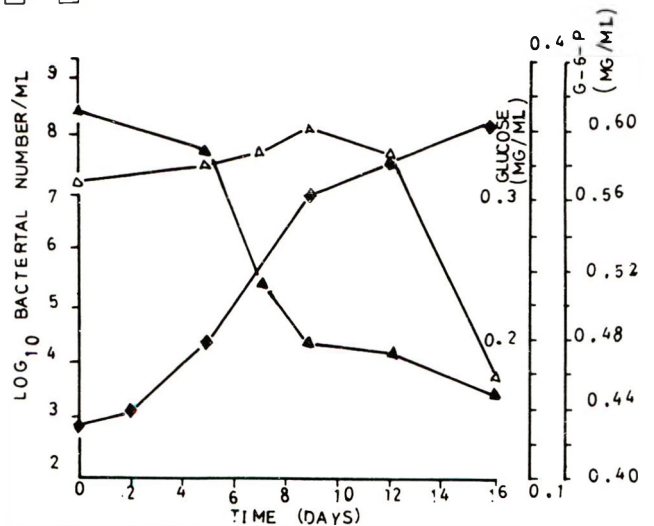


Fig. 7. Utilization of glucose by *S. liquefaciens*  
 ◆ — bacterial count  
 ▲ — glucose -6p  
 △ — glucose

down macromolecules to simple compounds. The latter are readily utilized by the spoilage flora and hence the bacterial multiplications<sup>17</sup>.

Results presented in Table 2 show that both *Pseudomonas* I and II exhibited marked reduction in asparagine, lysine, histidine and arginine levels during the storage period. However, glycine, valine, methionine, isoleucine, leucine and tyrosine were observed to increase in samples inoculated with *Pseudomonas* II. *Pseudomonas* II cannot completely be responsible for the increase noted in the concentration of these amino acids since similar increases were observed in the sterile control uninoculated samples. The reduction of arginine and increase of ornithine in samples inoculated with *Pseudomonas* I (Table 2) was not unexpected. Abdelal<sup>18</sup> reported that *Pseudomonas* sp. are commonly regarded as metabolizing arginine to ornithine via the so-called arginine dehydrolase system rather than via arginase enzyme.

Although taurine was reported to resist bacterial degradation<sup>19,20</sup>, *Pseudomonas* II was shown to be able to utilize this amino acid (Table 2). Taurine is a sulphur containing amino acid. Its importance arises from the fact that the sulphide-like odour can be detected at extremely low concentrations and it is the most important component of the spoilage aroma of meat. Sulphide-like odours have been reported in a wide variety of spoiled products including red meat<sup>21,2</sup>.

Little difference could be observed in the levels of the majority of amino acids in the sterile control uninoculated samples and *S. liquefaciens* inoculated samples, of specific importance was the pronounced increase in proline level in *S. liquefaciens* inoculated meat drip (Table 3). This suggests that *S. liquefaciens* might possess collagenase enzyme.

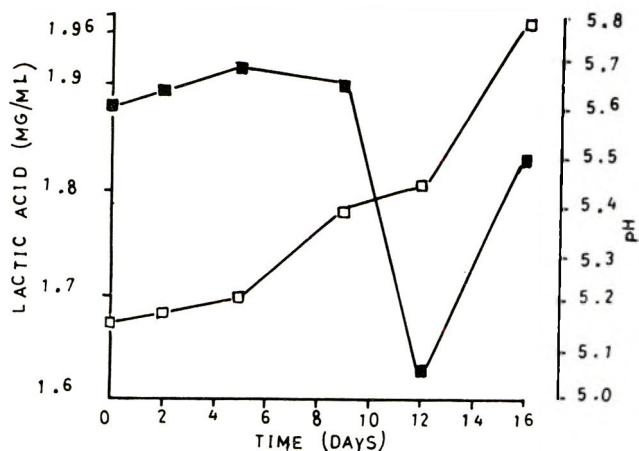


Fig. 8. Changes in lactic acid and pH by *S. liquefaciens*

■ — pH.  
□ — lactic acid

During the storage period *Moraxella*-like organism was able to utilize a different group of meat drip amino acids which were not really attacked by the other tested organisms. The amino acids and other related substances concerned were aspartic acid, glutamic acid, phenylalanine, ornithine and urea, (Table 3).

**Acknowledgement**

We are grateful to Mr. D. Annette who carried out the

TABLE 1. CONCENTRATION OF AMINO ACIDS IN STERILE UNINOCULATED MEAT DRIP.

Amino acid	Amino acids (mg/100 g) at indicated periods		
	0 hr	5 days	16 days
Taurine	5.3	5.1	6.3
Aspartic acid	0.4	0.8	0.4
Threonine	4.4	5.8	6.0
Serine	5.9	7.9	8.7
Asparagine	1.9	2.3	1.4
Glutamic acid	13.2	15.9	19.9
Glutamine	29.6	46.6	28.8
Proline	3.7	5.3	5.9
Glycine	6.6	6.5	7.6
Alanine	27.0	38.7	36.7
Valine	6.4	7.4	9.0
Methionine	2.1	2.3	2.8
Isoleucine	3.6	3.9	4.2
Leucine	8.2	9.4	10.2
Tyrosine	3.7	4.6	5.1
Phenylalanine	4.5	5.1	5.4
Ornithine	1.1	1.3	1.2
Lysine	5.1	6.6	7.3
Histidine	3.1	3.9	4.1
Tryptophan	0.4	0.4	0.4
Anserine	43.6	48.2	45.6
Carnosine	257.6	481.6	425.8
Arginine	6.0	6.6	6.9
Urea	15.7	23.9	15.5
Ammonia	9.1	14.1	14.4

TABLE 2. CONCENTRATION OF AMINO ACIDS AND OTHER RELATED SUBSTANCES (MG/100 G) IN MEAT DRIP INOCULATED WITH *PSEUDOMONAS* I AND II.

Amino acid	Pseudomonas I			Pseudomonas II		
	0 hr	5 days	16 days	0 hr	5 days	16 days
Taurine	4.6	9.7	9.1	8.3	7.1	5.8
Aspartic acid	1.0	0.4	0.7	0.4	0.7	0.6
Threonine	4.7	6.8	6.6	4.6	5.5	6.5
Serine	5.7	8.3	8.1	6.3	8.0	7.8
Asparagine	1.0	0.0	0.0	3.8	2.0	0.2
Glutamic acid	19.4	32.5	36.5	17.2	16.2	34.9
Glutamine	13.5	25.9	0.0	24.8	38.6	0.0
Proline	4.6	5.9	4.4	5.2	6.0	5.3
Glycine	5.2	8.9	7.4	6.4	6.5	7.5
Alanine	29.8	43.8	40.6	39.2	52.9	40.8
Valine	4.6	8.2	8.1	6.2	7.8	9.7
Methionine	2.3	2.2	2.1	1.5	2.6	3.3
Isoleucine	2.9	4.5	4.4	3.7	4.6	6.8
Leucine	6.8	9.8	9.7	8.1	9.8	10.5
Tyrosine	3.0	9.3	5.1	4.2	4.5	6.0
Phenylalanine	5.4	11.5	5.6	5.6	5.4	6.7
Ornithine	1.3	1.5	6.1	1.3	1.2	6.6
Lysine	10.4	7.9	7.0	19.2	7.9	8.0
Histidine	15.2	8.5	3.0	8.7	3.8	3.2
Tryptophan	0.6	0.9	0.8	0.4	0.3	0.2
Anserine	34.3	49.2	45.0	54.0	44.0	44.0
Carnosine	403.5	566.4	534.0	495.2	554.4	401.8
Arginine	5.3	7.5	0.0	6.9	6.5	0.0
Urea	19.0	27.6	16.2	15.9	18.9	12.1
Ammonia	10.1	15.1	25.2	13.8	11.7	19.5

TABLE 3. CONCENTRATION OF AMINO ACIDS AND OTHER RELATED SUBSTANCES (Mg/100 g) IN MEAT DRIP INOCULATED WITH *S. LIQUEFACIENS* AND *MORAXELLA*-LIKE ORGANISM.

Amino acid	<i>S. liquefaciens</i>			Moraxella-like organism		
	0 hr	5 days	16 days	0 hr	5 days	16 days
Taurine	7.2	9.7	8.1	7.2	7.0	7.0
Aspartic acid	0.2	0.3	1.1	0.6	0.4	0.0
Threonine	4.6	6.5	2.0	5.1	5.3	5.4
Serine	6.8	8.2	5.2	7.0	7.5	7.1
Asparagine	1.8	2.1	0.0	1.3	1.2	2.1
Glutamic acid	13.6	15.5	28.5	18.5	16.0	13.2
Glutamine	35.1	52.8	26.0	33.6	34.7	30.8
Proline	4.5	3.6	9.0	4.6	4.8	6.5
Glycine	6.9	8.0	8.3	7.3	7.0	7.5
Alanine	38.0	45.6	50.4	37.8	36.3	40.5
Valine	6.2	7.4	9.2	6.4	6.6	8.4
Methionine	2.0	1.4	3.6	2.3	2.2	2.2
Isoleucine	3.4	3.9	4.1	3.8	4.0	4.3
Leucine	8.3	8.9	9.2	8.7	9.2	9.6
Tyrosine	4.4	4.9	5.9	3.8	4.5	6.0
Phenylalanine	5.9	5.3	8.7	4.8	4.6	5.7
Ornithine	0.9	1.3	0.0	1.3	1.3	0.8
Lysine	5.3	6.9	2.3	6.2	6.6	7.0
Histidine	3.2	4.0	9.8	4.2	2.7	4.2
Tryptophan	0.3	0.9	0.8	0.4	0.3	0.2
Anserine	48.7	54.7	50.3	50.2	44.2	37.5
Carnosine	420.6	622.8	569.9	463.8	471.3	492.2
Arginine	6.1	7.1	6.0	5.8	6.3	6.8
Urea	22.8	34.3	28.4	17.3	13.5	11.0
Ammonia	11.8	17.1	16.9	11.7	10.4	14.2

amino acid analysis and to members of the Technical Staff of the Agricultural and Food Bacteriology Division, the Queen's University of Belfast. This investigation was financed by a grant from the Sudan Government.

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# Preparation and Properties of Aroma Concentrates from Some Tropical Fruit Juices and Pulps

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A pilot plant model aroma recovery unit (Holstein and Kappert, W. Germany) was used for the preparation of aroma concentrates from some tropical fruit juices and pulps. The effect of some operating variables like vacuum, reflux ratio and condenser temperature on aroma recovery was studied. Operating the unit closer to atmospheric pressure and low condenser temperature was found to minimise loss of aroma volatiles. High reflux ratios increased the strength of aroma concentrates prepared.

Fruit juice concentrates are valuable semi-finished products for the production of respective fruit juices, fruit juice beverages, jams and jellies and fruit juice powders. It is well established that by concentrating fruit juices, considerable storage, transportation and packaging costs are saved in addition to providing microbiological stability in high solid concentrates. Development of multi-stage evaporators has rendered evaporation as the most economical method for the production of fruit juice concentrates. However, during fruit juice concentration volatile fruit aroma compounds are also evaporated together with water, which results in fruit juice concentrates having poor aroma. To overcome this, aroma recovery before or during fruit juice concentration is practised for several fruits<sup>1,6</sup>.

Tropical fruit juices and pulps constitute a major item of export from India. However, published literature on aroma recovery from tropical fruit juices is scarce. In this communication, results of a study on some tropical fruit juices and pulps are reported.

## Materials and Methods

**Raw materials:** Mangoes ('Alphonso' and 'Totapuri') purchased from the local market, were ripened at room temperature ( $25 \pm 5^\circ\text{C}$ ) under natural ripening conditions. Fully ripe guavas ('Allahabad' variety) and pineapples were purchased from the local market.

**Pulp and juice extraction:** One hundred kg of fully ripe fruits of mango and guava as well as 250 kg of pineapples were used for pulp/juice extraction. Mangoes were washed in tap water, stem portions and any black specks on the surface were removed using stainless steel knives, sliced and the pulp was extracted using APV pulper fitted with 30 mesh sieve. Guavas were washed in tap water and lye peeled (boiling 2.5 per cent NaOH solution for 2 min) and washed in water. Any

adhering peel was removed by scrubbing, followed by dipping in citric acid (0.5 per cent) solution for 1 min to neutralise any residual alkali on the surface and subsequently washed again in water to remove excess acid. Black specks were removed with stainless steel knives and fruits were crushed in a fruit mill. The pulp was extracted by passing the crushed mass through APV pulper fitted with 30 mesh sieve. Ripe pineapples after removing the crown and stem portions were washed in tap water and sliced using a mechanical slicer. The rind was peeled either with knives or punches, slices crushed in a fruit mill, and the juice recovered by pressing the crushed mass in a hydraulic press.

**Aroma recovery:** Mango and guava pulps were diluted with water (1:4) and used for recovery of volatiles in Holstein and Kappert aroma recovery unit by evaporation and fractional distillation. Dilution of pulps was necessary as the heat exchangers in the aroma recovery unit were plate type. However, pineapple juice was used without any dilution. The aroma recovery unit is designed to be operated near to atmosphere. It has a rectification column packed with raschig rings. Some experiments were carried out to determine the optimum operating pressure in the range of 50 to 75 KPa. Similarly, reflux ratios of 20, 30 and 40 and condenser temperature of 10 and  $30^\circ\text{C}$  were tried. A laboratory type steam distillation apparatus as described by Shah *et al*<sup>7</sup> was used for the isolation of volatile components from fresh fruit juice and pulps. The values obtained were used to calculate the percentage recovery of different groups of volatile components in the aroma concentrates.

**Analysis:** The steam distillates as well as the aroma concentrates were analysed for esters<sup>8</sup>, carbonyls<sup>9</sup>, alcohols<sup>10</sup>, total oxygenated terpenes<sup>11</sup> and chemical oxygen demand (C.O.D.)<sup>12</sup>.

## Results and Discussion

**Effect of operational variables:** Table 1 gives the chemical composition of aroma concentrates obtained from mango ('Alphonso' and 'Totapuri'), guava and pineapple by operating the aroma recovery unit at two pressures. At 50 KPa pressure, the recovery of esters, carbonyls and alcohols in the aroma concentrates was lower than at 75 KPa pressure. It is known that these groups of compounds are characterized by having high relative volatilities and hence are difficult to be condensed at lower pressure of operation leading to their loss through the vacuum pump<sup>13</sup>. Besides at lower pressure of operation, increase in the volume of non-condensable gases results in more loss of volatiles<sup>14</sup>. Oxygenated terpenes in general, have relatively lower relative volatilities and hence show better recovery even at lower pressures of operation.

Thus it is advisable to operate the aroma recovery unit close to atmospheric pressure. Table 2 gives the effect of condenser temperature on aroma quality. Retention of aroma volatiles in all the aroma concentrates prepared was better at condenser temperature of 10°C than at 30°C as indicated by C.O.D. values.

The results showed (Table 3) that as the reflux ratio increased the aroma strength also increased. Though the aroma quality was good at reflux ratios of 20 and 30, but at 40, the aroma quality was not satisfactory showing some altered notes. This could be due to loss of some aroma volatiles having typical fruit notes. At high reflux ratios, only very small quantities of vapour condensates were withdrawn and used as a wash liquid in the wash column. As the volume of the wash liquid decreased, efficiency of scrubbing

TABLE 1. EFFECT OF VACUUM ON THE AROMA RECOVERY FROM SOME TROPICAL FRUITS

Fruit juice/pulp	System pressure (KPa)	Aroma concentrate				
		Esters*	Carbonyls*	Alcohols**	Oxygenated terpenes*	COD**
Guava	75	770	1465	14	126	603
	50	705	1245	13	118	542
Alphonso mango	75	405	641	37	96	245
	50	380	595	35	94	230
Totapuri mango	75	590	300	35	150	677
	50	513	273	34	145	635
Pineapple	75	712	93	11	15	518
	50	570	81	10	14	487

\*µg per 100 ml; Average values are from duplicate measurements.

\*\* COD = Chemical Oxygen Demand = mg/100 ml.

TABLE 2. EFFECT OF CONDENSER TEMPERATURE ON AROMA QUALITY

Fruit juice/pulp	Chemical oxygen demand (mg/100 ml) at indicated condenser temp.	
	10°C	30°C
Guava	603	534
Alphonso mango	245	211
Totapuri mango	677	550
Pineapple	518	446

Aroma quality was typical to the fruits in all the cases.

decreased and there was likely loss of uncondensed vapours. Concentrations of esters, carbonyls, alcohols, oxygenated terpenes and C.O.D. in the fresh fruit juices/pulps (purees) are given in Table 4. Based on these values, the percentage of different groups of compounds recovered in the aroma concentrates were calculated. The results (Table 5) indicated that esters, carbonyls, alcohols and oxygenated terpenes could be removed to the extent of 78 to 83 per cent. The recovery in terms of chemical oxygen demand was in the range of 78 to 80 per cent. With the type of rectification column used,

TABLE 3. EFFECT OF REFLUX RATIO ON THE FOLD CONCENTRATION

Fruit juice/pulp	Feed rate (l/hr)	Evaporation* (%)	Aroma strength and quality at indicated Reflux ratio					
			20		30		40	
			C.O.D. fold	Aroma quality	C.O.D. fold	Aroma quality	C.O.D. fold	Aroma quality
Guava	140	38	10	Typical	14	Typical	20	Altered
Alphonso mango	150	40	11	„	17	„	23	More terpenic
Totapuri mango	160	40	13	„	18	„	25	„
Pineapple	120	50	40	„	61	„	82	Altered

\*Two passes C.O.D. : Chemical oxygen demand

TABLE 4. CHEMICAL ANALYSIS OF FLAVOUR COMPONENTS IN FRESH JUICES/PULPS (PUREES)

Fruit	Esters ( $\mu\text{g}/100\text{g}$ )	Carbonyls ( $\mu\text{g}/100\text{g}$ )	Alcohols ( $\text{mg}/100\text{g}$ )	Oxygenated terpenes ( $\mu\text{g}/100\text{g}$ )	C.O.D. ( $\text{mg}/100\text{g}$ )
Guava	69	134	1.20	11.4	54.5
Alphonso mango	30	48	2.80	7.0	18.5
Totapuri mango	42	21	2.50	10.3	47.0
Pineapple	15	20	0.18	0.303	10.7

TABLE 5. FLAVOUR COMPONENTS (AS PER CENT OF FRESH JUICE) IN AQUEOUS AROMA DISTILLATES FROM THE AROMA RECOVERY UNIT

Name	Fruit juice quantity (kg)	Aroma fold and quantity*	Esters	Carbonyls	Alcohols	Oxygenated terpenes	C.O.D.**
Guava	50	14 (3.6)	80	78	83	79	79
Alphonso mango	50	17 (2.9)	79	79	79	81	78
Totapuri mango	50	18 (2.8)	78	80	78	81	80
Pineapple	100	61 (1.6)	79	80	78	81	79

\*Figures in parenthesis refer to volume of aroma concentrates.

\*\*Chemical Oxygen Demand;

about 5 per cent of total aroma components were lost in the bottoms. However, when aroma concentrates were reconstituted with the juice concentrate and evaluated, they were found to be comparable with the fresh fruit juice.

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# Effect of Different Degumming Agents on the Physico-Chemical Characteristics of Rice Bran Oil

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Laboratory refined rice bran oil showed lower hydroxyl, acetyl and peroxide values and higher saponification and iodine values than industrial refined and unrefined oils. Citric acid was most effective in facilitating degumming and dewaxing. Decreases in hydroxyl and acetyl values were significant ( $P < 0.05$ ) with phosphoric, citric and oxalic acids by wet de-gumming in comparison to phosphoric acid by dry de-gumming. The peroxide value was also decreased with citric acid and phosphoric acid as degumming agents. Free fatty acid contents decreased significantly during neutralization and most of the phospholipids were removed at the stage of degumming. Refined oil contained lower amounts of phosphatidyl ethanolamine and phosphatidic acid.

A great deal of technological advancement has been achieved in the rice bran processing industry. Yet, the most useful method to minimize the formation of free fatty acids in the rice bran oil and to reduce refining losses are still a major constraint in the rice bran oil industry. Studies of Sah *et al.*<sup>1</sup>, Shah and Gupta<sup>2</sup>, Bhattacharya<sup>3</sup> and Bhattacharya *et al.*<sup>4</sup> have contributed in reducing the refining losses and enhancing the quality of oil. The normal method of degumming involves the use of phosphoric, sulphuric, oxalic or citric acid<sup>3,5</sup> while some have used hot water<sup>6</sup>. However, the work on physico-chemical characterisation of the oil during refining process has not been amply studied<sup>7</sup>. Recently, the use of polyelectrolytes in the refining of oil is receiving the attention of the oil technologists. Therefore, in the present study, apart from studying the physico-chemical characteristics of the raw, industrially refined and laboratory refined rice bran oil, the effect of different degumming agents on various chemical parameters of oil has been used to evaluate the process of refining for edible purpose.

## Materials and Methods

Raw and refined rice bran oils were obtained from M/S Oswal Vanaspati and General Industries, Ludhiana and were degummed by various chemicals based on the weight of the oil as per following details: (i) phosphoric acid (0.04 per cent) (dry degumming-DD), (ii) phosphoric acid (0.05 per cent) + water (7 per cent) (wet degumming-WD), (iii) citric acid (0.5 per cent) + water (5 per cent) and (iv) oxalic acid (0.5 per cent) + water (5 per cent).

These chemicals were applied by dissolving in water whenever necessary and then added to the oil. The degumming, dewaxing and neutralization were performed by following A.O.C.S. methods<sup>8</sup> and bleaching by the method

of Rede<sup>9</sup>. Determination of hydroxyl, acetyl, peroxide, iodine and saponification values were done by the A.O.C.S. methods<sup>8</sup>. The free fatty acid (FFA) contents were estimated by the method of Lowry and Tinsley<sup>10</sup> and total phospholipids by that of Ames<sup>11</sup>. Individual phospholipid fractions were separated by preparative TLC using the solvent system, chloroform: methanol:7N ammonia (65:25:4, v/v/v). The spots were visualized with iodine vapours and scrapped off individually for the estimation of phospholipids directly from silica gel. Individual spots were identified by chromatography of standard phospholipids and confirmed by colour reactions. Samples of oil were divided into three replications with duplicate observations. The statistical calculations were done by one way factorial classification.

## Results and Discussion

The hydroxyl, acetyl and peroxide values of the laboratory refined oil were lower than unrefined and refined (Table 1) and were similar to those of Jaiswal<sup>12</sup> and Ramaswamy and Gopalakrishna<sup>6</sup>. The saponification value and iodine number of laboratory refined oil were highest than the other two oils and meet the ISI specifications (I.S.:3448-1968). The content of phospholipids and FFA in laboratory refined oil was also lowest than the other two oils because the removal of gums and wax was maximum (data not given) using citric acid as the degumming agent. Such a comparison was considered necessary to evaluate the procedure of refining in the laboratory and to make further chemical estimations in the oil.

A marked decrease in hydroxyl and acetyl values was observed during neutralization. In addition, the removal of soap formed at this stage by centrifugation suggested that the partial glycerides were removed with the soap fraction<sup>4</sup>. The

TABLE 1. COMPARISON OF PHYSICO-CHEMICAL CHARACTERISTICS OF UNREFINED, INDUSTRIAL REFINED AND LABORATORY REFINED RICE BRAN OIL

Parameters	Raw oil	Industrial refined oil	Laboratory refined oil*
Hydroxyl value	13.4	9.6	6.8
Acetyl value	13.4	9.5	6.7
Peroxide value (meq/kg oil)	50.0	25.0	17.5
Sap. no. (mg FA released/g fat)	163.3	176.4	181.6
Iodine no.	88.1	96.5	98.1
Total phospholipids (%)	3.6	0.6	0.2
Free fatty acids	3.2	0.3	0.2

\* Using citric acid as degumming agent.

degumming agents used in the experiments eliminated the partial glycerides during degumming and neutralization (Table 2). The hydroxyl and acetyl values decreased significantly ( $P < 0.05$ ) with phosphoric acid (WD), citric acid and oxalic acid compared to phosphoric acid (DD), obviously due to the effect of hydration. The bleaching process did not influence appreciably these values. The citric acid and phosphoric acid (WD) brought about a significant decrease in peroxide value in meq/100 g oil (17.5 and 20.0) against raw (50.0) and industrial refined (25.0) oil and using phosphoric acid (DD) (25.0) and oxalic acid (27.5) as degumming agents. Thus, the use of citric acid and phosphoric acid (WD) may have contributed to the removal of peroxides during refining. Different degumming agents did not display any significant effect on iodine number of the refined oil. However, the use of these chemicals improved the iodine number of degummed

TABLE 2. EFFECT OF VARIOUS DEGUMMING AGENTS ON HYDROXYL, ACETYL, IODINE AND SAPONIFICATION VALUES OF RICE BRAN OIL AT EACH REFINING STAGE

Treatments	Degummed oil	Dewaxed oil	Neutralised oil	Bleached oil	Mean (B)
<b>Hydroxyl value</b>					
Phosphoric acid (DD)	13.5	13.3	9.7	9.5	11.5
Phosphoric acid (WD)	11.8	11.5	7.0	6.3	9.1
Citric acid	11.8	11.3	6.9	6.7	9.2
Oxalic acid	11.5	11.1	6.8	6.5	9.0
Mean (A)	12.1	11.8	7.6	7.3	—
<b>Acetyl value</b>					
Phosphoric acid (DD)	13.4	13.2	9.6	9.5	11.4
Phosphoric acid (WD)	11.0	11.4	6.9	6.2	8.9
Citric acid	11.7	11.2	6.8	6.6	9.1
Oxalic acid	11.4	11.0	6.7	6.5	8.9
Mean (A)	11.9	11.7	7.5	7.2	—
<b>Iodine number</b>					
Phosphoric acid (DD)	87.1	92.1	96.9	97.7	93.5
Phosphoric acid (WD)	87.6	93.2	97.4	98.3	94.1
Citric acid	88.5	92.6	96.1	98.1	93.8
Oxalic acid	84.9	90.8	97.4	98.7	92.9
Mean (A)	87.0	92.2	96.9	98.2	—
<b>Sap. no. (mg FA released/g fat)</b>					
Phosphoric acid (DD)	164.1	163.8	174.2	175.4	169.4
Phosphoric acid (WD)	173.8	176.0	183.3	181.0	178.5
Citric acid	171.5	176.7	183.9	181.0	178.4
Oxalic acid	178.0	182.8	181.5	181.4	180.9
Mean (A)	173.7	173.0	180.7	179.9	—
<b>Critical difference (<math>P &lt; 0.05</math>)</b>					
Parameters	Hydroxyl value	Acetyl value	Iodine number	Saponification number	
Treatments (T)	0.03	0.03	1.5	1.3	
Refining stage (S)	0.03	0.03	1.5	1.3	
T × S	0.10	0.10	4.2	3.7	

DD = Dry degumming; WD = Wet degumming

oil as some saturated fatty acids were removed with the gum due to hydration. The saponification number increased significantly in the neutralized oil and different degumming agents (aqueous) increased this value as compared to phosphoric acid (DD). The removal of higher quantity of wax (data not given) and partial glycerides as indicated by reduced

hydroxyl and acetyl values (Table 2) seems to have contributed to enhancing the saponification value. Similar results have been reported by Sah *et al*<sup>1</sup> and Bhattacharya<sup>3</sup>.

The content of FFA decreased significantly during neutralization (Table 3) since citric acid and phosphoric acid (WD) initially removed some FFA in degumming and,

TABLE 3. EFFECT OF VARIOUS DEGUMMING AGENTS ON FREE FATTY ACIDS AND PHOSPHOLIPIDS CONTENT OF RICE BRAN OIL AT EACH REFINING STAGE

Treatments	Degummed oil	Dewaxed oil	Neutralised oil	Bleached oil	Mean (B)
<b>Free fatty acids (%)</b>					
Phosphoric acid (DD)	2.3	2.1	0.3	0.3	1.2
Phosphoric acid (WD)	1.6	1.4	0.4	0.2	0.9
Citric acid	1.6	1.4	0.6	0.2	1.0
Oxalic acid	2.1	1.8	0.8	0.4	1.3
Mean (A)	1.9	1.7	0.5	0.3	—
<b>Phospholipids (%)</b>					
Phospholipids (DD)	0.6	0.6	0.4	0.4	0.5
Phospholipids (WD)	0.7	0.4	0.4	0.2	0.4
Citric acid	0.9	0.7	0.3	0.2	0.5
Oxalic acid	0.9	0.5	0.4	0.3	0.5
Mean (A)	0.8	0.5	0.4	0.3	—
<b>Critical difference</b>	(P<0.05)		Free fatty acids		Phospholipids
Treatment (T)			0.04		0.02
Refining stage (S)			0.04		0.02
T × S			0.11		0.05

therefore, FFA were reduced to minimum (0.2 per cent) during neutralization and this aspect is well known<sup>3,6,12</sup>. Most of the phospholipids were removed at the degumming stage and DD was also effective. However, little removal of phospholipids was observed at each stage of refining and all the aqueous systems were better in removing the phospholipids. In all, four major phospholipid fractions were present in rice bran oil. Phosphatidyl ethanolamine (PE) and phosphatidic acid (PA) were retained but at a lower level in the refined oil than the raw oil. Phosphatidylcholine (PC) and phosphatidyl inositol (PI) were found in traces in the refined oil (Table 4). Chakraborty *et al.*<sup>13</sup> showed that PC, PE and PI were the major phospholipids present in crude rice bran

oil. These phospholipids in small quantities are indeed useful as antioxidants as well as a dietary component<sup>14</sup> in the rice bran oil.

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TABLE 4. EFFECT OF VARIOUS DEGUMMING AGENTS ON THE CONTENT OF INDIVIDUAL PHOSPHOLIPIDS (mg/100 g oil) IN THE REFINED RICE BRAN OIL

Treatments	PA	PI	PC	PE
Raw oil	46.0	26.0	15.0	65.0
Phosphoric acid (DD)	7.5	Tr	Tr	12.1
Phosphoric acid (WD)	7.2	Tr	Tr	10.5
Citric acid	6.5	Tr	Tr	7.1
Oxalic acid	7.3	Tr	Tr	10.5
C.D. (P<0.05)	0.2	—	—	0.7

PA = Phosphatidic acid; PI = Phosphatidyl inositol; PC = Phosphatidyl choline; PE = Phosphatidyl ethanolamine



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## Comparative Behaviour of Cashew Kernel and Wheat to Phosphine Fumigation

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Cashew kernels desorb phosphine ( $\text{PH}_3$ ) in geometric progression till free  $\text{PH}_3$  is well within the  $\text{PH}_3$  holding capacity of cashew, and then the residue decreases regularly to zero over 9 to 20 days. This trend is reversed in the case of wheat. Desorption trends of  $\text{PH}_3$ , decrease in levels of computed residues of  $\text{PH}_3$  and negligible levels of phosphorus in cashew go to suggest that  $\text{PH}_3$  in cashew is retained by lipid portion. The latter desorbs retained  $\text{PH}_3$  completely on removal of cashew from the fumigation atmosphere. The extensive data provided here make us to conclude that  $\text{PH}_3$  can also become a suitable fumigant for cashew kernels.

The effects of dose, exposure period and behaviour of  $\text{PH}_3$  including its residues in some cereals, wheat types and their milled products have been extensively investigated<sup>1,15</sup>, while the available information about these parameters in case of oil seeds, and fatty foods is scanty. Tkachuk<sup>16</sup> at a dose of 6.6 p.p.m. (6.6 tablets/ton) employing  $\text{PH}_3$ , found the formation of higher amounts of non-volatile residues of  $\text{PH}_3$  in flax and rapeseed. Muthu *et al.*<sup>17</sup> in their experimental fumigation of fatty commodities at 10 g size dosed with 60 and 30  $\mu\text{g}$   $\text{PH}_3$  (corresponding to 6 and 3 tablets/ton), have found by determining the head space concentration, an uptake of 92, 97, 99 and 86.6% of dosed  $\text{PH}_3$  by peanut kernels, peanut cake, peanut oil and cashew kernels respectively. Sunflower seeds and walnut retained 100 per cent of dosed  $\text{PH}_3$ . In another study, Muthu *et al.*<sup>18</sup> have found a retention of 89 to 93 per cent of dosed  $\text{PH}_3$  by 6 days exposed cashew kernels fumigated with 0.15 to 0.6 mg  $\text{PH}_3/100$  g (1.5 to 6 tablets/ton). But all these studies have based their conclusions on the concentration of  $\text{PH}_3$  in the head space and not on the direct determination of  $\text{PH}_3$  residues in fumigated oil seeds and fatty foods.

A detailed study to compare dosage, exposure period, airing and behaviour of  $\text{PH}_3$  residue in cashew kernels and wheat is undertaken at the experimental size of 1 kg to assess the suitability of  $\text{PH}_3$  as fumigant for cashew kernels and the findings are reported here.

### Materials and Methods

Fumigation was done at two doses, by keeping 300 (100 mg  $\text{PH}_3$ ) and 36 mg (12 mg  $\text{PH}_3$ ) Celphos pellet in filter paper pack underneath the commodities in separate flasks for two weeks<sup>3</sup>. The commodities were then aired upto 10 days by spreading as thin layers.

*Pre-aired  $\text{PH}_3$  residue:* Pre-aired  $\text{PH}_3$  residue in wheat samples was determined by the method reported earlier<sup>19</sup>. Ten grams of cashew kernels were quickly weighed into a 150 ml tube whose mouth is fixed with B40 socket joint, containing 30 ml  $\text{AgNO}_3$  solution, which was then immediately closed with a B40 glass stopper, to determine the residue by a modified method<sup>20</sup>. The method would analyse 0.01 p.p.m.  $\text{PH}_3$  in cashew kernels. In all these studies, the corresponding crop control of the same sample size was employed as blank.

*Desorption experiments:* Desorption experiments with pre-aired samples of cashew kernels, wheat and those aired for 15 hrs and 1 to 10 days were done as reported earlier<sup>10</sup> by the detector strip method. Desorption pattern of  $\text{PH}_3$  from pre-aired, 15 and 24 hr aired samples of cashew kernels<sup>3</sup> and wheat was studied over 5 hr. With aired samples, desorption studies were continued till the red band ceased to form. From these values, levels of computed residues were worked out<sup>10</sup>.

*Phosphorus determination:* Inorganic phosphorus in cashew kernels and wheat samples and on the walls of glass test tubes used in desorption studies was determined by the method of Bruce as report earlier<sup>10</sup>, with minor modifications in case of cashew kernels. Ten grams of cashew kernels were blended well with 100 ml boiling water containing 1 ml 6N  $\text{H}_2\text{SO}_4$ . The blend was allowed to stand for 4-5 hr with intermittent stirring. It was then filtered through Whatman No. 1 filter circle and 5 ml clear (at times very slightly turbid) filtrate was used for colour development as per Bruce *et al.*<sup>4</sup> The reaction mixture after making up to 25 ml with water in a 25 ml graduated cylinder was allowed to stand for 40-60 min when a blue coloured precipitate was formed at the top of the bluish solution. The precipitate was

thoroughly dispersed in the solution by shaking the stoppered cylinder well and was then kept in boiling water bath for 15 min, when an uniform blue solution was formed with a few dark insoluble particles floating on the top of the solution. The solution was then cooled to room temperature and centrifuged for 10 min at 2500 rpm to suspend the floating particles. The clear blue solution was read at 820 nm against a reagent blank similarly prepared. In all these determinations, crop control of equal sample size was similarly treated and the absorbance of the crop control was subtracted from that of the  $\text{PH}_3$  fumigated sample to assess phosphorus formed due to oxidation of  $\text{PH}_3$  and expressed as mg/100 g.

### Results and Discussion

Residues of  $\text{PH}_3$  and phosphorus in fumigated (300 mg AIP/kg) cashew kernels during storage are shown in Table 1, while Table 2 shows a comparison of decrease of  $\text{PH}_3$  residues in cashew kernels and wheat during storage. Decrease in computed  $\text{PH}_3$  residues during storage of fumigated (300 mg AIP/kg) cashew kernels aired for different

periods is shown in Table 3. Comparison of residues of  $\text{PH}_3$  and phosphorus in 5 and 6 days aired samples of cashew kernels and wheat fumigated with 36 mg/kg during storage is shown in Table 4. Comparison of change in  $\text{PH}_3$  residue during storage of 5 to 6 days aired cashew kernels and wheat (36 mg AIP/kg) is shown in Table 5. Pattern of  $\text{PH}_3$  residue desorption from pre-aired and aired samples of cashew kernels and wheat is shown in Fig.1.

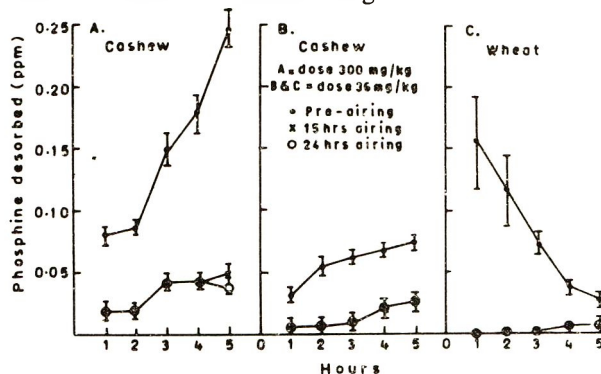


Fig.1.  $\text{PH}_3$  desorption pattern from pre-aired and aired cashew kernels and wheat.

TABLE 1.  $\text{PH}_3$  (P.P.M.) AND Pi (Mg/100g) RESIDUES IN FUMIGATED AND AIRED CASHEW KERNELS\*

Aeration period	$\text{PH}_3$ residue		Pi in kernels ( $\mu\text{g}$ )	Pi in tubes ( $\mu\text{g}$ )
	Determined	Computed		
Pre-aired	2.103 $\pm$ 0.050	—	0.051 $\pm$ 0.006	—
15 hr	1.764 $\pm$ 0.290	—	—	—
1 day	1.380 $\pm$ 0.110	—	0.066 $\pm$ 0.006	—
<b>2 days</b>				
Immediately	0.657 $\pm$ 0.059	0.991 $\pm$ 0.042	0.066 $\pm$ 0.006	—
After 20 days storage	BDL	0.001 (one)	0.075 $\pm$ 0.012	3.45 $\pm$ 0.22 (3.91)
<b>5 days</b>				
Immediately	0.199 $\pm$ 0.012	0.467 $\pm$ 0.051	0.139 $\pm$ 0.017	—
After 15 days storage	BDL	0.003 $\pm$ 0.001	0.137 $\pm$ 0.017	4.86 $\pm$ 0.32 (4.39)
<b>6 days</b>				
Immediately	0.235 $\pm$ 0.006	0.512 $\pm$ 0.051	0.151 $\pm$ 0.012	—
After 16 days storage	BDL	0.003 $\pm$ 0.001	0.164 $\pm$ 0.012	13.00 $\pm$ 4.78 (13.42)
<b>7 days</b>				
Immediately	0.049 $\pm$ 0.012	0.122 $\pm$ 0.029	0.129 $\pm$ 0.012	—
After 6 days storage	BDL	0.003 $\pm$ 0.012	0.115 $\pm$ 0.002	4.93 $\pm$ 0.6 (5.01)
<b>10 days</b>				
Immediately	0.018 $\pm$ 0.018	0.036 $\pm$ 0.006	0.185 $\pm$ 0.047	—
After 15 days storage	BDL	0.004 $\pm$ 0.002	0.168 $\pm$ 0.040	12.16 $\pm$ 4.68 (12.55)

Mean  $\pm$  SD of 6 replicates; BDL = Below detectable limit of the method.

Pi = inorganic phosphorus.

\*Dose: 300 mg/AIP/kg, 2 weeks exposure



TABLE 2. COMPARISON OF FALL IN PH<sub>3</sub> RESIDUES (p.p.m.) DURING STORAGE (dose 300 mg AIP/kg\*)

Days	Cashew kernels	Wheat <sup>10</sup>
0	0.99 ± 0.042	1.556 ± 0.004
2	0.841 ± 0.003	1.434 ± 0.001
4	0.414 ± 0.029	1.314 ± 0.007
6	0.227 ± 0.009	1.202 ± 0.003
8	0.146 ± 0.012	1.065 ± 0.001
10	0.078 ± 0.0006	0.925 ± 0.002
12	0.034 ± 0.0006	0.827 ± 0.010
14	0.010 ± 0.0003	0.774 ± 0.003
16	0.004 ± 0.0001	0.697 ± 0.002
19	0.001 (one)	0.6275 ± 0.001
21	0.0	0.512 ± 0.008

Mean ± SD 4 replicates

\* 2 Weeks exposure period; 2 days aired

At any given dose of fumigant (300 or 36 mg AIP/kg), pattern of PH<sub>3</sub> desorption from pre-aired and aired cashew kernels is quite different from wheat. (Fig.1). Figs 1A and 1B show the initial PH<sub>3</sub> desorption over 5 hrs, from cashew kernels dosed at 300 and 36 mg/kg respectively while Fig 1C shows that from wheat dosed at 36 mg/kg. Amount of desorbed PH<sub>3</sub> from pre-aired, 15 and 24 hr aired cashew samples is continuously increasing over 5 hr, this increase being more at a higher dose of 300 mg/kg (Fig.1A) than to that at 36 mg AIP/kg. From pre-aired samples at the end of 5 hrs, 0.25 p.p.m. PH<sub>3</sub> is desorbed from the former while it is only 0.055 p.p.m. from the latter. Desorption from 15 and 24 hr aired samples also shows a similar increase over 5 hrs. There is no difference in the amount of PH<sub>3</sub> desorbed from 15 and 24 hr aired cashew kernels (300 mg AIP/kg) over first 4 hr. The corresponding samples of cashew kernels

(36 mg/kg) also show the same pattern over 5 hr. It is surprising to note that the desorption from similar samples of wheat dosed at 36 mg/kg shows a continuous decrease over 5 hr. The pre-aired samples of wheat show a large desorption of 0.16 p.p.m. at 1st hr which decreases to a low value of 0.025 p.p.m. at the end of 5 hr. Wheat samples aired for 15 and 24 hrs show only a small desorption of 0.005 p.p.m. during 4th and 5th hr. These observations suggest that the behaviour of free PH<sub>3</sub> residue in cashew is entirely different from that in wheat. This suggestion is further strengthened by the residue values shown in Table 5. It is likely that in cashew, the free PH<sub>3</sub> residue is solely absorbed by the fatty portion, which when removed from the fumigation atmosphere can desorb large quantities of dissolved PH<sub>3</sub> in geometric progression till free PH<sub>3</sub> content is well within the PH<sub>3</sub> holding capacity of cashew, then the residue decreases regularly over a period<sup>20</sup>.

It was found in PH<sub>3</sub> fumigated vegetable oil (rich in unsaturated fatty acids as in cashew fat), large amount of PH<sub>3</sub> taken up by the oil was almost completely desorbed on airing for 1 day. This indirectly shows that PH<sub>3</sub> is largely retained by the fatty portion in cashew.

Free PH<sub>3</sub> residue determined (Table 1) shows a regular decrease during 0 to 10 days airing (2.1 to 0.018 p.p.m.) The residue in samples aired for 6 days is slightly higher (0.235 p.p.m.) than that in 5 days aired samples (0.199 p.p.m.) for reasons explained earlier<sup>12</sup>. In spite of the higher PH<sub>3</sub> holding capacity of cashew kernels<sup>20</sup> (69 per cent), compared to wheat<sup>19</sup> (62 per cent), the pre-aired cashew samples show slightly lower level of free PH<sub>3</sub> residue (2.1 p.p.m.) than wheat (2.69 p.p.m.)<sup>10</sup>. This decrease by about 0.6 p.p.m. is due to a longer duration of 4 min that has elapsed between opening of the flask and weighing the samples to

TABLE 3. FALL IN COMPUTED PH<sub>3</sub> RESIDUE (p.p.m.) DURING STORAGE OF CASHEW KERNELS AIRED FOR DIFFERENT PERIODS\*

Days	5 days aired	6 days aired	7 days aired	10 days aired
0	0.467 ± 0.050	0.512 ± 0.051	0.122 ± 0.029	0.036 ± 0.006
1	0.416 ± 0.048	0.485 ± 0.055	0.082 ± 0.023	0.025 ± 0.006
2	0.267 ± 0.029	0.434 ± 0.051	0.047 ± 0.016	0.016 ± 0.005
3	0.200 ± 0.030	0.283 ± 0.033	0.026 ± 0.011	0.009 ± 0.002
4	0.145 ± 0.017	0.0213 ± 0.023	0.015 ± 0.007	0.007 ± 0.002
5	0.109 ± 0.014	0.157 ± 0.020	0.006 ± 0.002	0.004 ± 0.002
6	0.086 ± 0.012	0.116 ± 0.016	0.003 ± 0.001	0.0
7	0.07 ± 0.011	0.091 ± 0.014	0	0
8	0.056 ± 0.010	0.074 ± 0.012	0	0
9	0.040 ± 0.009	0.057 ± 0.011		
10	0.026 ± 0.008	0.039 ± 0.008		
11	0.019 ± 0.006	0.024 ± 0.007		
12	0.013 ± 0.005	0.017 ± 0.005		
13	0.006 ± 0.003	0.010 ± 0.004		
14	0.003 ± 0.001	0.005 ± 0.003		
15	0	0.003 ± 0.001		
16	0	0		

Mean ± SD of 4 replicates

\*Fumigated 300 mg/ AIP/kg

TABLE 4. COMPARISON OF PH<sub>3</sub> (p.p.m.) AND Pi (mg/100 g) RESIDUES IN FUMIGATED AND AIRED CASHEW KERNELS AND WHEAT (dose 36 mg AIP/kg, 2 weeks exposure)

Aeration period	PH <sub>3</sub> residue		Pi in kernels/ grain	Pi in tubes µg
	Determined	Computed		
<b>Cashew Kernels</b>				
Pre-aired	1.097 ± 0.283	—	0.028 ± 0.007	—
15 hr	0.314 ± 0.014	—	—	—
1 day	0.294 ± 0.038	—	0.033 ± 0.005	—
<b>5 days</b>				
Immediately	0.036 ± 0.0007	0.087 ± 0.009	0.037 ± 0.006	—
After 9 days storage	BDL	0.002 ± 0.001	0.032 ± 0.008	5.31 ± 0.77 (5.82)
<b>6 days</b>				
Immediately	0.028 ± 0.0119	0.067 ± 0.005	0.038 ± 0.004	—
After 8 days storage	BDL	0.002 ± 0.002	0.038 ± 0.010	4.13 ± 0.45 (4.53)
<b>Wheat</b>				
Pre-aired	0.237 ± 0.133	—	0.032 ± 0.010	—
15 hr	0.093 ± 0.053	—	—	—
1 day	0.062 ± 0.019	—	0.030 ± 0.010	—
<b>5 days</b>				
Immediately	0.037 ± 0.006	0.0046 ± 0.001	0.029 ± 0.010	—
After 3 days storage	BDL	0.0026	0.029 ± 0.009	3.3 ± 1.7
<b>6 days</b>				
Immediately	0.033 ± 0.029	0.0050 ± 0.0	0.030 ± 0.009	—
After 3 days storage	BDL	0.0031	0.029 ± 0.010	1.5 ± 0.5

Mean ± SD of 6 replicates. BDL = Below detectable limit of the method

TABLE 5. COMPARISON OF FALL IN COMPUTED PH<sub>3</sub> RESIDUES (p.p.m.) DURING THE STORAGE OF COMMODITIES AIRED FOR 5 AND 6 DAYS (dose 36 mg AIP pellet/kg, 2 weeks exposure)

Days	Cashew kernels		Wheat	
	5 days aired	6 days aired	5 days aired	6 days aired
0	0.087 ± 0.009	0.067 ± 0.005	0.0046 ± 0.001	0.0050 ± 0.0
1	0.071 ± 0.008	0.052 ± 0.004	0.0046 ± 0.001	0.0031 ± 0.0002
2	0.053 ± 0.006	0.039 ± 0.004	0.0036 ± 0.0	0.0031 ± 0.0002
3	0.041 ± 0.005	0.029 ± 0.003	0.0026 ± 0.0	0.0031 ± 0.0002
4	0.030 ± 0.005	0.019 ± 0.002	0.0	0.0
5	0.018 ± 0.004	0.011 ± 0.002	0	0
6	0.010 ± 0.003	0.007 ± 0.001	—	—
7	0.006 ± 0.003	0.002 ± 0.003	—	—
8	0.002 ± 0.002	0.002 ± 0.0003	—	—
9	0.002 ± 0.002	0.0	—	—
10	0.0	0.0	0.0	0.0

Mean ± SD of 6 replicates.

the sixth replicate. The corresponding time for wheat is 1 min 20 sec. as wheat is poured from fumigation flask to the tube upto a mark precalibrated to 10 g<sup>15</sup>. So, more of PH<sub>3</sub> has lost from kernels during weighing due to combined effects of duration of sampling time and higher rate of desorption

from cashew kernels. When the time of weighing is reduced to 2 min, the pre-aired cashew samples show higher free PH<sub>3</sub> residue than in wheat (Table 4) which is in accordance with its higher PH<sub>3</sub> holding capacity. As observed in case of wheat<sup>13</sup> computed residue levels in cashew kernels are

also more than the determined residue levels (Table 1). This excess in  $\text{PH}_3$  residues, is not due to decomposition of interaction products of  $\text{PH}_3$  with constituents of cashew but perhaps is due to release of  $\text{PH}_3$  dissolved in fat. Computed residue (0.99 p.p.m.) in 2 days aired sample decreases to zero over 20 days in storage (Table 2), while those in 5 days (0.46 p.p.m.), 6 days (0.51 p.p.m.), 7 days (0.12 p.p.m.) and 10 days (0.036 p.p.m.) aired samples decrease to zero over 15, 16, 6 and 5 days of storage respectively (Table 3). These observations indicate that cashew kernels fumigated with such a high dose of 300 mg/kg are free of both free and dissolved residues of  $\text{PH}_3$  within about 22 days.

Several batches of cashew kernels examined showed phosphorus content of 40 mg/100 g, which is slightly less than the reported value of 45 mg/100 g<sup>21</sup>. The phosphorus values in Table 1 show that upto 20 days not much of  $\text{PH}_3$  is oxidised in 2 days aired samples. This again indicates that fat as solvent to  $\text{PH}_3$  is acting as barrier for oxidation of  $\text{PH}_3$ . On prolonged exposure to air over 5 to 10 days, only 0.06 - 0.18 mg/100 g of phosphorus is noticed, which is very much less than that found (0.28 - 1.2 mg/100g) in wheat<sup>13</sup>. Very low values (4-13  $\mu\text{g}$ ) of phosphorus in side tubes indicate the extent of oxidation of  $\text{PH}_3$  is negligible.

Comparison between cashew kernels and wheat in decrease of their levels of  $\text{PH}_3$  residues during storage (Table 2) indicates that on any day of observation, the former is holding a lower residue than the latter. As for example on 10th and 16th days the residues in cashew kernels are 0.078 and 0.004 p.p.m., while the corresponding residues in wheat are 0.925 and 0.697 p.p.m. respectively. The residue in cashew kernels decreases to zero in about 21 days, while the same in wheat on that day, is as high as 0.512 p.p.m.. These observations suggest that the rate of loss of  $\text{PH}_3$  residues from cashew kernels is faster than that from wheat indicating the differences in modes of holding  $\text{PH}_3$  by these two commodities.

The computed  $\text{PH}_3$  residues (Table 3) from 5 to 10 days aired cashew samples during storage also show fast decline in their levels. Barring slightly higher levels of  $\text{PH}_3$  residues in 6 days aired samples due to reasons already explained, the residue levels in all the samples decrease (0.46 to 0.036 p.p.m.) as the period of airing increases from 5 to 10 days. This is in contrast to those observed in wheat<sup>10</sup> which showed significant residue even after one year. Our experience has shown that such a fast decline does not occur in computed residue arising out of interaction of  $\text{PH}_3$  with constituents of wheat types<sup>10,11,13</sup>.

In case of cashew kernels (36 mg/kg; Table 4), the weighing time of pre-aired kernels is reduced to 2 min to minimise the loss of  $\text{PH}_3$  during sampling. Cashew kernels, in addition to holding a high pre-aired residue (1.09 p.p.m.), also show a regular decrease in residue levels over 0 to 6 days of airing. The samples show a zero residue at the end of 14 days. The computed residue levels in 5 and 6 days aired

samples are higher than those determined, indicating that  $\text{PH}_3$  held by fatty portion is not available for determination. Very low levels of phosphorus content of the kernels indicate that the oxidation of  $\text{PH}_3$  is negligible.

Under the same conditions of fumigation, wheat shows a low pre-aired residue level of 0.23 p.p.m. (Table 4) which is about 5 times lesser than that in cashew kernels. In spite of such higher pre-aired free  $\text{PH}_3$  residue in cashew kernels, the residue (0.036 p.p.m.) equals to that in wheat (0.037 p.p.m.) over 5 days of airing due to faster desorption of  $\text{PH}_3$  from cashew kernels. Interestingly, the computed residue levels in 5 and 6 days aired wheat are nearly 1/7th of the determinable residues indicating less interaction of  $\text{PH}_3$  in wheat at lower dosages of fumigation. Very low phosphorus levels are noticed in wheat fumigated with lower dosage. As seen in Table 5, the decrease in computed residues in cashew kernels compared to that in wheat is delayed only by a week in spite of significantly higher residues in the former confirming the rate of desorption of  $\text{PH}_3$  from cashew kernels is faster than from wheat.

Tkachuk<sup>16</sup> found that treatment of flax and rapeseed at 12 p.p.m. (12 tablets/ton)  $\text{PH}_3$  levels resulted in approximately 45 per cent of the  $\text{PH}_3$  reacting to form residues. With the same dose of fumigation (36 mg/kg = 12 tablets/ton)  $\text{PH}_3$  residue in cashew kernels not only decreases to negligible level over 2 weeks but also does not show any reaction as indicated by low levels of computed residues (Table 5) and negligible levels of phosphorus (Table 4). Our observations also contradict those of Muthu *et al*<sup>18</sup> who found very slow desorption of  $\text{PH}_3$  from fatty kernels.

$\text{PH}_3$  at 36 mg pellet/kg (12 tablets/ton) dose does not leave behind any free or bound residues in cashew kernels, in addition showing negligible levels of oxidation products. Fat portion (42-47 per cent) in these kernels not only appears to retain most of the sorbed  $\text{PH}_3$  but also desorb almost completely. In addition, fat appears to act as barrier not only for interaction of  $\text{PH}_3$  with reactive sites in cashew but also for its oxidation to phosphorus compounds. Extremely low levels (0.002 p.p.m.) of stratified  $\text{PH}_3$  in kernels packed in gas-tight containers will act as toxicant for any new emerged insects, during storage or transshipment. Even this residue escapes when the containers are opened. As a result of these observations, based on the extensive data provided, it may be concluded that similar to wheat,  $\text{PH}_3$  appears not only as an alternate fumigant but also a suitable fumigant for cashew.

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## Neutral Detergent Fibre from Various Foods and Its Hypocholesterolemic Action in Rats

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Dietary fibre from blackgram, barley, ragi and rice bran was isolated as neutral detergent fibre (NDF) and their effect on cholesterol metabolism studied in rats fed high fat-cholesterol diet. The chemical composition of each fibre was also determined and the cholesterol lowering action correlated with the chemical composition. It was seen that the hemicellulose content of the different fibre correlated with the effect on cholesterol metabolism. Barley NDF with its maximum hemicellulose content produced lowest concentration of serum and tissue cholesterol, highest concentration of hepatic and fecal bile acids and fecal sterols. *In vitro* binding studies also showed that barley NDF bound maximum amount of bile acids.

Investigations carried out by various workers indicate that not all dietary fibre has cholesterol lowering action<sup>1,2</sup> and that this effect varies with the source of the fibre. While wheat bran has been reported to have no cholesterol lowering effect, positive results have been reported for oat bran and soyabean hulls<sup>3-5</sup>. But no systematic investigation has been carried out so far to find out whether the chemical composition of the dietary fibre which varies from source to source has any effect on its cholesterol lowering action. In this paper, dietary fibres from different sources (*viz.*, blackgram, barley, ragi and rice bran) have been isolated as NDF and their cholesterol lowering action studied in rats fed high fat cholesterol diet. The chemical composition of each fibre has also been determined and attempts made to see whether the cholesterol lowering action is related in any way to its chemical composition.

### Materials and Methods

Male rats (Sprague - Dawley strain, weight 100-120 g) were divided into 5 groups of 12 rats each and fed as follows.

- Group 1 — Isocaloric fibre-free diet
- 2 — Blackgram NDF diet
- 3 — Ragi NDF diet
- 4 — Barley NDF diet
- 5 — Rice bran NDF diet

The diet had the following composition (g/100 g):

A	Fibre-free	NDF
Dextrin	61.5	31.5
Casein (vit. and fat-free)	16.0	16.0
Fibre	—	30.0
Coconut oil	15	15.0

B	Fibre-free	NDF
Salt mixture	4	4
Vitamin mixture	1	1
Cholesterol	2	2
Sodium cholate	0.5	0.5

The composition of the salt mixture and vitamin mixture was as given before<sup>6</sup>.

NDF diet (16.4 g of A) supplied the same calories as 12.0 g of A of fibre-free diet. The caloric intake of the rats of fibre-free and NDF diet groups was kept the same by adjusting the intake of A. The intake of salt mixture and vitamin mixture was kept the same in the various groups by giving the same amount of B (0.97 g/rat) mixed with the required weighed quantity of A. NDF was taken to contribute very little towards calorific value. Water was provided *ad libitum*. The rats were maintained on the respective diet for a period of 75 days. At the end of this period, they were deprived of food over night, stunned by a blow at the back of neck and killed by decapitation. The tissues were removed to ice-cold containers for various estimations. Before killing, 24 hr stool samples were collected from the rats of each group in metabolic cages.

NDF was isolated from blackgram, ragi, barley and rice bran according to the procedure of Goering and Van Soest<sup>7</sup>. Blackgram, ragi and barley were used with the seed coat removed. Rice bran free from husk was used. In each case, the NDF was subjected to digestion with  $\alpha$ -amylase to remove the residual starch.

Determination of the components of NDF was carried out using the successive extraction procedure of Goering and Van Soest<sup>7</sup>. Cholesterol and triglycerides were estimated in the serum, aorta, liver and heart as described earlier<sup>8</sup>. Fecal sterols and bile acids were extracted as described by Grundy

*et al.*<sup>9,11</sup>. Bile acids were extracted from the liver as described by Okishio *et al.*<sup>11</sup> and determined as described before<sup>8</sup>. The procedure of Warwick and Albers<sup>12</sup> was used to precipitate LDL and VLDL in the serum and the difference in cholesterol contents of the whole serum and the supernatant gave the LDL+VLDL cholesterol.

The NDF prepared from the different sources was passed through 20-30 mesh and powder of this particle size was used for *in vitro* studies. Five hundred mg of NDF was shaken with 0.3 M NaCl in a small stoppered conical flask for an hour and then with the solution of the material under study in 0.3 M NaCl for 2 hr at 37°C. It was then centrifuged and the material in the supernatant was estimated. In the control, NDF in 0.3 M NaCl was mixed with the test solution and centrifuged immediately and the material in the supernatant was estimated. The supernatant was acidified to pH 1.0 and the bile acids extracted with ethyl acetate and estimated as described earlier<sup>9</sup>.

## Results and Discussion

The caloric intake was similar in the rats of the various groups. The gain in weight was comparable in the rats of the various groups ( $90 \pm 5.8$  g). The liver weight was also comparable ( $6.5 \pm 0.62$  g).

There was significant variation in the percentage of the various components in the NDF from the different sources

(Table 1). Hemicellulose was maximum in barley NDF and minimum in rice bran. In order of decreasing hemicellulose content, the various NDFs are barley, blackgram, ragi and rice bran. Cellulose was maximum in blackgram and minimum in barley. Lignin was maximum in ragi and minimum in barley. Cutin + silica was maximum in rice bran and minimum in barley.

All the NDF (Table 2) fed rats showed lower cholesterol levels in the serum when compared to fibre-free group. Serum cholesterol was minimum in rats fed barley NDF and maximum in rats fed rice bran NDF. Cholesterol levels in the aorta, liver and heart were also lower in all the NDF fed rats when compared to those fed fibre-free diet, except in those fed rice bran. In all these tissues, lowest cholesterol levels were observed in rats fed barley NDF and highest in those fed rice bran NDF. Cholesterol in the LDL+VLDL fractions of serum lipoproteins also showed a similar pattern of change, lowest in the barley NDF group and highest in the rice bran NDF group.

Serum triglyceride levels were lower in rats fed NDF from barley, ragi and blackgram when compared to those fed fibre-free diet, while it was not significantly different in the rats fed rice bran NDF (Table 3). Lowest level of triglycerides was observed in barley NDF groups. Triglycerides in the aorta, liver and heart were also significantly lower in the rats fed barley NDF and blackgram NDF. The lowest level of

TABLE 1. COMPOSITION OF NDF (g/100 g NDF)

	Hemicellulose	Lignin	Cellulose	Cutin+Silica
Blackgram NDF	43.35 $\pm$ 1.26	15.07 $\pm$ 0.47	35.76 $\pm$ 1.09	5.82 $\pm$ 0.23
Ragi NDF	34.41 $\pm$ 0.99	29.98 $\pm$ 0.99	27.58 $\pm$ 0.85	9.02 $\pm$ 0.28
Barley NDF	70.82 $\pm$ 2.05	8.81 $\pm$ 0.31	20.01 $\pm$ 0.64	0.35 $\pm$ 0.01
Rice bran NDF	29.63 $\pm$ 0.82	28.55 $\pm$ 0.89	25.59 $\pm$ 0.79	16.23 $\pm$ 0.50

Average of values from 6 determinations  $\pm$  SEM

TABLE 2. CONCENTRATION OF CHOLESTEROL IN SERUM AND TISSUES (mg/100 g WET TISSUE)

Group	Serum (mg/100 ml)	Aorta ....	Liver	Heart ....	VLDL/LDL (mg/100 ml serum)
1	151.02 $\pm$ 3.78	394.12 $\pm$ 9.85	1357.12 $\pm$ 42.07	202.12 $\pm$ 6.27	39.32 $\pm$ 1.34
2	117.13 $\pm$ 2.93 <sup>a</sup>	329.56 $\pm$ 8.24 <sup>a</sup>	881.42 $\pm$ 25.56 <sup>a</sup>	127.31 $\pm$ 3.95 <sup>a</sup>	23.07 $\pm$ 0.89 <sup>a</sup>
3	121.73 $\pm$ 3.16 <sup>a</sup>	338.16 $\pm$ 9.13 <sup>a</sup>	1220.88 $\pm$ 28.12 <sup>b</sup>	100.15 $\pm$ 4.62 <sup>b</sup>	30.43 $\pm$ 0.87 <sup>a</sup>
4	111.59 $\pm$ 2.79 <sup>a</sup>	308.27 $\pm$ 7.71 <sup>a</sup>	827.03 $\pm$ 23.98 <sup>a</sup>	120.39 $\pm$ 3.73 <sup>a</sup>	22.83 $\pm$ 0.87 <sup>a</sup>
5	136.96 $\pm$ 3.47 <sup>b</sup>	368.91 $\pm$ 9.96	1288.41 $\pm$ 32.22	188.20 $\pm$ 5.83	35.81 $\pm$ 1.22

Group 1 has been compared with groups 2, 3, 4 and 5; a = p < 0.01, b = p between 0.01 and 0.05

Group 1 — Isocaloric fibre free-diet, Group 2 — Blackgram NDF diet

Group 3 — Ragi NDF diet Group 4 — Barley NDF diet

Group 5 — Rice bran NDF diet

Results are expressed as average of values from 6 rats  $\pm$  SEM



TABLE 3. CONCENTRATION OF TRIGLYCERIDES IN SERUM (mg/100 ml) AND TISSUES (g/100 g WET TISSUE)

Group	Serum	Aorta	Liver	Heart
1	9.21 ± 0.29	821.25 ± 25.46	814.28 ± 25.24	54.00 ± 2.11
2	7.23 ± 0.22 <sup>a</sup>	655.34 ± 19.01 <sup>a</sup>	730.31 ± 22.93 <sup>b</sup>	47.82 ± 1.54 <sup>b</sup>
3	7.42 ± 0.21 <sup>a</sup>	763.11 ± 23.66	750.58 ± 24.02	51.73 ± 2.02
4	5.36 ± 0.17 <sup>a</sup>	694.39 ± 19.14 <sup>a</sup>	720.17 ± 20.33 <sup>b</sup>	47.18 ± 1.84
5	8.65 ± 0.27	799.19 ± 22.38	794.00 ± 23.03	52.43 ± 1.84

The expression and statistical significance of the results are same as in Table 2; Superscripts as in Table 2.

triglycerides in these tissues, except in aorta, was observed in the barley NDF.

The rats of the various NDF groups showed significantly higher concentrations of hepatic bile acids (Table 4), when compared to those fed fibre-free diet. Hepatic bile acids were maximum in rats fed barley NDF and minimum in those fed rice bran NDF. Similar results were observed in the case of fecal bile acids and sterols.

In the case of chenodeoxycholic acid, again maximum amount was bound by NDF from barley and least by NDF from ragi (Table 5). The various NDFs in decreasing order of binding of chenodeoxycholic acid are barley, blackgram/ricebran and ragi. In the case of cholic acid, maximum amount was bound by NDF from rice bran and least by NDF from ragi. In decreasing order of cholic acid bound, the different NDFs are rice bran, barley, blackgram and ragi.

It may be mentioned that NDF represents only the insoluble components of dietary fibre. The soluble components of

dietary fibre viz., pectins, gum and mucilages are removed during extraction by the procedure of Goering and Van Soest<sup>7</sup>.

When the results on the effect of feeding the NDF from different sources on the concentration of cholesterol and triglycerides in the tissues and on the concentration of hepatic bile acids, fecal sterols and bile acids, are analysed in terms of their chemical composition, significant correlation is observed in the case of hemicellulose content of the different NDF and the effect on the above biochemical parameters. The various NDF in decreasing order of hemicellulose content are barley, blackgram, ragi and rice bran. The cholesterol and triglyceride levels in the serum and tissues are negatively correlated with the hemicellulose content, the more the hemicellulose content, the lower the tissue lipid levels. The concentration of hepatic bile acids and fecal excretion of neutral sterols and bile acids seem to be positively correlated, the higher the hemicellulose content, the higher the concentration of bile acids and the greater the excretion of

TABLE 4. CONCENTRATION OF HEPATIC BILE ACIDS AND FECAL EXCRETION OF NEUTRAL STEROLS AND BILE ACIDS

Group	Fecal sterols (mg/rat/day)	Fecal bile acids (mg/rat/day)	Hepatic bile acids (mg/100g wet tissue)
1	67.25 ± 2.42	16.28 ± 0.59	176.21 ± 6.17
2	106.92 ± 3.74 <sup>a</sup>	33.31 ± 1.29 <sup>a</sup>	281.34 ± 9.00 <sup>a</sup>
3	93.24 ± 3.17 <sup>a</sup>	25.57 ± 0.99 <sup>a</sup>	206.75 ± 6.56 <sup>a</sup>
4	133.89 ± 5.22 <sup>a</sup>	42.57 ± 1.62 <sup>a</sup>	284.75 ± 9.68
5	84.83 ± 2.45 <sup>a</sup>	19.72 ± 0.67 <sup>a</sup>	198.33 ± 6.06 <sup>b</sup>

The expression and statistical significance of the results are same as in Table 2

TABLE 5. *IN VITRO* BINDING OF NDF WITH BILE ACIDS

Source of NDF	Cholic acid	(% bile acids bound) Chenodeoxycholic acid
1 Blackgram	75.03 ± 2.33	85.68 ± 2.48
2 Ragi	73.73 ± 2.14	80.34 ± 2.01
3 Barley	77.52 ± 2.64	94.66 ± 2.65
4 Rice bran	80.00 ± 2.32	85.68 ± 2.23

Average of values from 6 determinations ± SEM

fecal sterols and bile acids. Some correlation is also seen in the case of cutin+silica content, but opposite to that in the case of hemicellulose. The various NDFs in increasing order of cutin+silica content are barley, blackgram, ragi and rice bran. The serum and tissue cholesterol and triglycerides correlate positively, the higher the content of cutin+silica, the higher the lipid levels. Hepatic bile acids and fecal excretion of sterols and bile acids' on the other hand' correlate negatively. In the case of lignin also, a similar correlation as in the case of cutin+silica is seen except in the case of ragi. In the case of cellulose, the different NDFs in increasing order of its content are barley, rice bran, ragi and blackgram. Correlation is observed only in the case of barley. Cholesterol and triglycerides correlate positively and the hepatic bile acids, fecal sterols and bile acids negatively. Thus, barley NDF which shows lowest lipid levels and highest hepatic concentration of bile acids and fecal excretion of neutral sterols and bile acids has the highest content of hemicellulose and the lowest content of lignin, cellulose and cutin+silica. In the case of other NDF, strict correlation is seen only in the case of hemicellulose.

As mentioned earlier, the mechanism of cholesterol lowering action of dietary fibre is believed to be the result of binding of the bile acids in the intestine<sup>13</sup>. It is generally recognised that the rate of hepatic conversion of cholesterol to bile acids is homeostatically controlled by the composition and concentration of bile acids in the enterohepatic circulation. Hence, bile acid sequestrants like dietary fibre would reduce the concentration of bile acids in the enterohepatic circulation thereby increasing the conversion of cholesterol to bile acids.

The results of *in vitro* binding studies indicate that correlation between the amount of bile acids bound and cholesterol lowering action exists in the case of NDF from barley. In this case, there is maximum binding of chenodeoxycholic acid and the cholesterol lowering action was also maximum. But in the case of cholic acid binding, it was next only to rice bran. Rice bran which showed the least cholesterol lowering action, however, bound significant amounts of cholic acid and chenodeoxycholic acid. One probable reason for the lack of correlation may be due to the amount of NDF digested *in vitro*. It is known that significant amount of NDF is digested in the intestinal tract by microbial action. Thus, the conditions *in vitro* and *in vivo* are different.

Not much information is available as to products of digestion of the NDF *in vivo*. It has been reported that various oligosaccharides may result and these may also adsorb the bile acids. The extent of digestion of NDF from different sources in the intestinal tract has not been studied.

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ON THE CAROTENOIDS OF RIPENED PEPPER BERRIES (*PIPER NIGRUM* L.)

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Three carotenoid pigments namely  $\beta$ -carotene, lycopene and leutin were tentatively identified in the pericarp of ripened berries of pepper (*Piper nigrum* L.) for the first time using TLC and spectrophotometric methods.

White pepper (*Piper nigrum* L.) is prepared from fully ripe bright red berries after the removal of pericarp<sup>1,2</sup> where the red coloured pigment is essentially located. The purpose of this work is to provide some informative data on the nature of pigments in ripened pepper berries which are unknown till date.

Spectral absorbance measurements were made with a varian DMS-100 uv/vis spectrophotometer.

Ripened pepper berries were procured from Kerala (India) by air and processed immediately on arrival. Red pericarp (21 g) obtained from 90 g fully ripened berries were homogenised with 25 ml of n-hexane in an omnimix grinder for 3 min. The slurry was filtered under suction and the residue was reextracted twice with n-hexane as above. The residue was then repeatedly extracted with 90 per cent aqueous methanol (6 × 25 ml) as described above till the filtrate was pale yellow. The filtrates were combined and extracted with n-hexane (6 × 100 ml). All n-hexane extracts were pooled together and concentrated on a rotary evaporator to obtain 69 mg of solid residue, which was made to 2 per cent in chloroform. This solution when analysed by spectrophotometry<sup>3</sup> showed characteristic absorption at 483, 456, 374, 608 and 668 nm suggesting the presence of carotenoid and chlorophyll pigments. The 90 per cent aqueous methanol fraction remaining after hexane extraction was found

to be devoid of carotenoids, as revealed by spectrophotometric analysis and hence was rejected. Total carotenoid content was estimated at 460 nm using  $\beta$ -carotene as standard and found to be 4.53 mg/100 g ripe pepper berries.

The chloroform solution containing carotenoid pigments was subjected to thin layer chromatographic (TLC) analysis on a preparative silica gel G plate (20/20 cm glass plate, 5 mm layer thickness) using petroleum ether : benzene (49:1 v/v) as developing solvent. Four coloured bands were detected at Rf 0.42 (yellow, I), 0.2 (yellow, II), 0.09 (red III) and 0.00 (greenish brown, IV). Each of these bands was scrapped from the plate, separately charged onto a short column (15×2 cm) containing 0.5 cm bed of celite and then eluted with chloroform. The solvent was removed from each fraction by blowing a stream of nitrogen and was analysed by spectrometry as stated above. Bands I, II and III when individually spotted on analytical silica gel G TLC (20×20 cm glass plate, 0.25 mm film thickness) in solvent systems of varying polarity gave a single spot indicating that these compounds are chromatographically pure. The absorption spectra and Rf values of each of these TLC separated compounds are given in Table 1. The band IV at Rf 0.00 showed absorption maxima at 419, 455, 481, 611 and 688 nm suggesting it to be a mixture of carotenoid and chlorophyll pigments. This band on further preparative TLC separation using more polar solvent system consisting of petroleum ether : benzene : methanol (49:49:2 v/v/v) resolved into a carotenoid band (yellow, IVA) at Rf 0.097 along with two chlorophyll bands at Rf 0.574 and 0.854 respectively. The yellow band (IVA) was scrapped, recovered and then analysed as described above (Table 1).

The compounds I, III and IVA were identified as  $\beta$ -carotene, lycopene and leutin respectively by comparison of Rf values on TLC and characteristic absorption maxima with available standard compounds and with literature values<sup>3,4</sup>. The compound II was not obtained in sufficient amount for further analysis and hence could not be identified. Thus, the carotenoids in ripened pepper berries consist

TABLE I. Rf VALUES AND SPECTRAL CHARACTERISTIC OF RIPENED PEPPER CAROTENOID PIGMENTS

Pigments bands	Rf value*			Absorption maxima (nm)*	
	Solvent A	Solvent B	Solvent C	Hexane	Chloroform
I	0.42	0.94	0.95	414 (inflection)	459.4, 491 (sh)
II	0.20	0.94	0.95	447, 473 (sh)	387, 409, 435
III	0.09	0.94	0.95	443, 468, 499	457 (sh), 482, 515
IVA	0.00	0.44	0.36	421 (sh), 443, 472	432 (sh), 455, 484

\* Each value is the average of three determinations; Solvent A : Petroleum ether (60-80°) : Benzene (49:1); Solvent B : Dichloromethane : Ethylacetate (4:1); Solvent C : Benzene : Methanol (25:1).



essentially of  $\beta$ -carotene, lycopene and leutin, where the latter two appear to be predominant.

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## THE STABILITY OF CELLULASE DURING SACCHARIFICATION OF BAGASSE

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The stability of a cellulase enzyme preparation of *Trichoderma viride*, *Aspergillus ustus* and their mixtures having cellulase and D-xylanase activities was compared with a Japanese enzyme (Onozuka) during saccharification of alkali treated bagasse. A varied trend with respect to stability of these enzyme preparations was observed. Substrate presence increases the thermal stability of the enzyme during saccharification.

With the rapid development of biotechnology, requirement for industrial enzymes has increased greatly. This is because of the fact that the enzymes are remarkably good catalysts which function selectively and under mild conditions. The industrial enzymes of microbial origin can be produced in unlimited quantities and also they can be potentially inexpensive. But in order to be suitable for technological applications, enzymes should be stable under operational conditions for weeks or months. But most of the enzymes do not satisfy these requirements.

During the studies on enzymic saccharification (using the crude enzyme preparations of *A. ustus* and *T. viride*) of alkali treated bagasse<sup>1</sup>, it was observed that saccharification of alkali treated bagasse was fast in the early stages and decreased gradually. The same trend was noticed with purified enzyme preparations (a Japanese enzyme preparation — Onozuka). Studies were carried out to find out the stability of the crude enzyme preparations of *A. ustus* and *T. viride* and the Japanese enzyme preparation in the presence or absence of substrate under conditions optimum for saccharification<sup>1</sup>.

Enzyme preparations of *A. ustus* and *T. viride* were produced in the laboratory according to the procedure of Shamala and Sreekantiah<sup>2</sup>. Precipitated crude enzymes were used in the investigations under conditions similar to those of optimum saccharification<sup>1</sup>. For comparison, a Japanese enzyme preparation (Onozuka) was included in the trials. Saccharification was carried out according to Manonmani and Sreekantiah<sup>1</sup>.

Reducing sugar, filter paper activity (FP'ase), carboxymethyl cellulase (CMC'ase),  $\beta$ -glucosidase and D-xylanase activities were determined<sup>1</sup>. Protein was determined by Lowry's procedure.<sup>3</sup>

Stability of enzymes was assessed by determining the enzyme activities during saccharification<sup>1</sup>.

Optimum pH and temperature for maximum saccharification of sugarcane bagasse were found to be 4.8 and 50°C respectively<sup>1</sup>. Same conditions were maintained in all the experiments reported here. When the enzyme alone was incubated (without substrate) both *A. ustus* and *T. viride* enzyme preparations were less stable (Fig.1). Initially *T. viride* enzyme was more stable retaining 100 per cent of its activity. As the period of incubation increased, the enzyme activity decreased with time (15 per cent in 72 hr). A mixture of the two enzymes of *A. ustus* and *T. viride* was not stable in the earlier stages of saccharification. The Japanese enzyme also showed initial drop in activity. The residual activity after 72 hr of incubation was 42 per cent.

In the presence of substrate, enzymes of *A. ustus*, *T. viride* and their mixtures were more stable after 72 hr (Fig.1). The Japanese enzyme showed only 35 per cent residual activity after 72 hr even in the presence of the substrate. The individual enzymes were observed to be more stable than their mixtures.

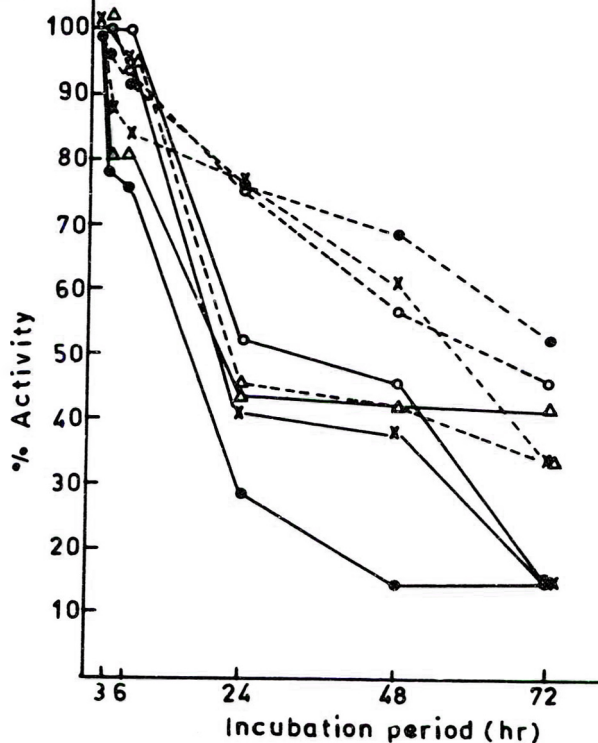


Fig.1. Stability of FPase of cellulase at different periods of saccharification.

○ *A. ustus* enzyme  
□ *T. viride* enzyme  
× Mixture of *A. ustus* and *T. viride* enzyme  
△ Japanese enzyme  
--- With substrate  
— Without substrate

(Enzymes in 0.1 M acetate buffer, pH 4.8, and incubation temperature 50°C).

Residual CMC'ase activities in all the enzyme preparations either alone or with the substrates are presented in Fig.2. In the initial stages CMC'ase activity was more in the enzyme-substrate mixture. However, the activity dropped rapidly after 6 hr of incubation.  $\beta$ -glucosidase in the Japanese preparation was more stable compared to the other two enzyme preparations (Fig.3). In the presence of the substrate, *A. ustus* enzyme was more stable than *T. viride* enzyme which had 4 per cent activity. Mixed enzyme preparations also showed similar trend in activity. The Japanese enzyme was less stable in the presence of substrate. D-xylanase was least stable compared to other enzyme activities. D-xylanase of *T. viride* was least stable and the enzyme became inactivated within 72 hr of incubation (without substrate). Even the Japanese enzyme showed only 41 per cent of residual activity after 1 hr of incubation (without substrate). (Fig. 4). In the presence of the substrate, enzyme activity was stable in the initial stages, but the activity dropped rapidly with the progress in saccharification.

It is clear that the Japanese enzyme was more stable compared to the enzymes of *A. ustus* and *T. viride*. One of the reasons might be due to the presence of proteases in the enzymes of *A. ustus* (102 U/min/mg enzyme) and *T. viride* (180 U/min/mg enzyme). Japanese enzyme showed very little protease activity (0.4 U/min/mg enzyme)<sup>4</sup>. Proteases have been reported to cause the loss of activity of the enzymes in the preparation. Correlation between half-life of the enzyme and susceptibility to proteolysis has been reported<sup>5</sup>.

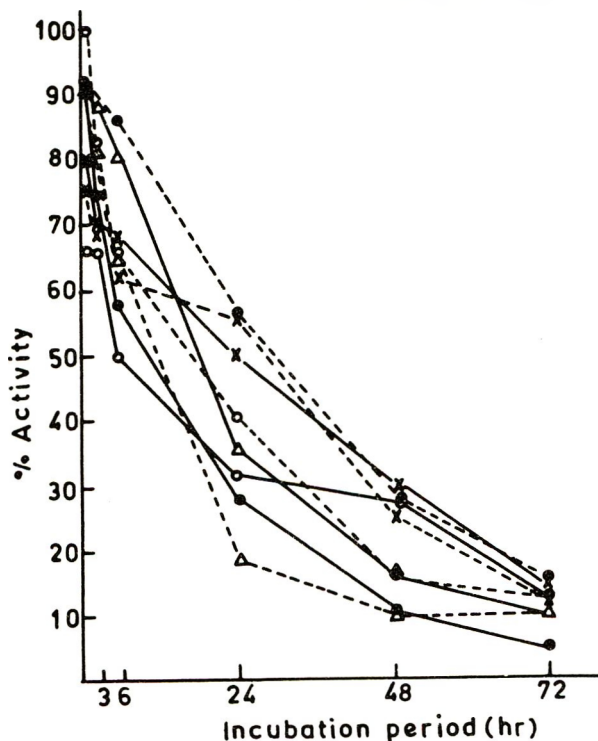


Fig.2. Stability of CMC'ase of cellulase preparations at different periods of saccharification.

Legend: As in Fig. 1.

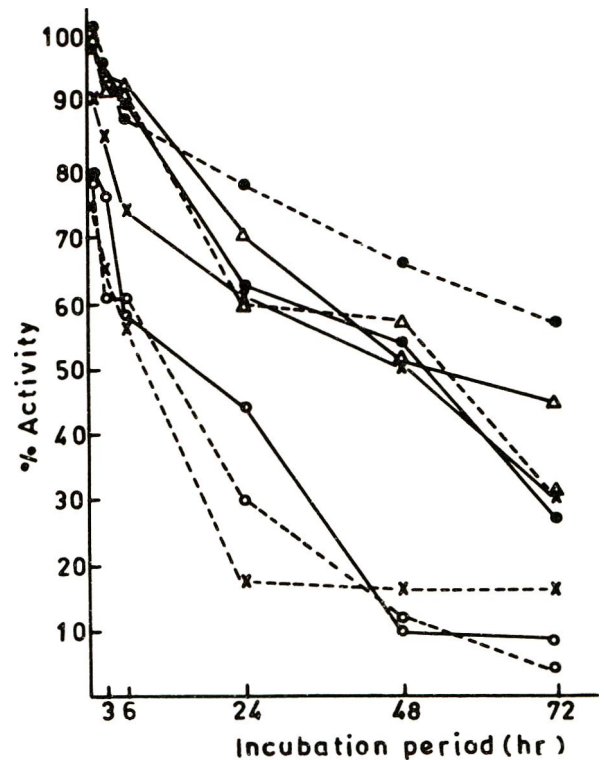


Fig.3. Stability of  $\beta$ -glucosidase of cellulase preparations at different periods of saccharification.

Legend: As given in Fig. 1.

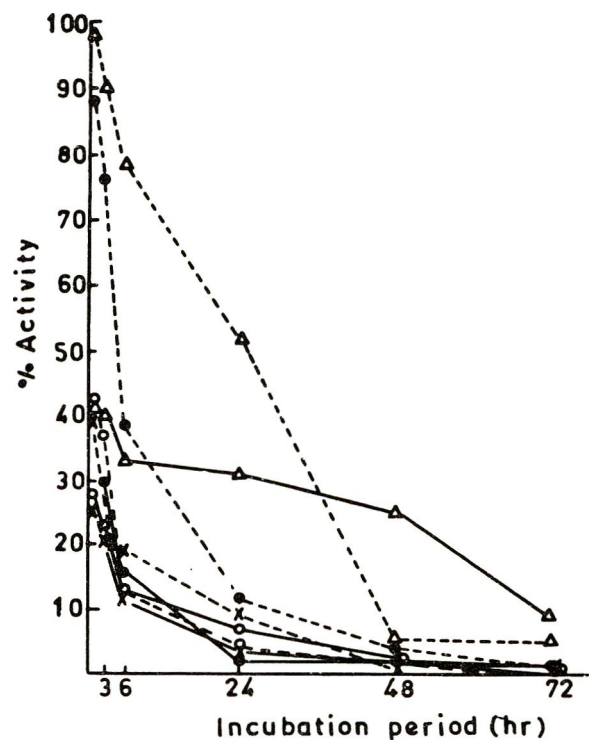


Fig.4. Stability of xylanase of cellulase preparations at different periods of saccharification.

Legend: As in Fig. 1.



Different enzymes of the cellulase complex and D-xylanase did not show the same trend with respect to their stabilities. FPase of *T. viride* appeared to be more stable than the enzymes of *A. ustus* (Fig.1.) and the mixture of the two, as complete activity could be detected upto 6 hr of incubation. D-xylanase of *T. viride* was least stable and the enzyme was completely inactivated within 72 hr of incubation. Aryl- $\beta$ -glucosidase of *T. viride* was less stable than that of *A. ustus*. D-xylanase of Japanese enzyme was least stable. Thus, the level of stability differed from one preparation to another. One of the reasons could be the variation in the susceptibility of different enzymes to proteases in the crude enzyme preparations.

The enzyme activities showed slightly better stability in the presence of substrate. Such a protection has been attributed to stabilization of tertiary protein structure when the enzyme combines with the substrate to yield a complex form<sup>6</sup>. The degree of protection of the enzyme also depends on the concentration of the substrate. So, the deactivation rate of the free enzyme is different from the enzyme-substrate complex. Ulbrich *et al*<sup>7</sup> have suggested that the substrate acts as a stabiliser thus reducing the inactivation of the enzyme.

Another probability could be due to product inhibition. Cellobiase has been reported to be inhibited by cellobiose accumulation<sup>8</sup>. Cellobiose was detected in the hydrolysate obtained from the Japanese enzyme while no cellobiose was detected in the other two enzyme preparations.<sup>4</sup> But glucose, the end product of saccharification has also been observed to be one of the strong inhibitors of cellulase<sup>8</sup>. Glucose or cellobiose might be responsible for the loss in enzyme activity.

Since the stability is more essential in technological applications, it has been suggested<sup>9</sup> that the thermal instability can be overcome in saccharification by continuously adding  $\beta$ -glucosidase<sup>10</sup> or by stabilising the

enzyme by immobilisation<sup>10</sup> or by the removal of end product, glucose, continuously<sup>11</sup>.

Thus, Japanese enzyme was more stable compared to the crude enzyme preparations of *A. ustus* and *T. viride*. The level of stability also varied from one preparation to another and also different enzymes of the cellulase complex showed variations in their stabilities. And in presence of the substrate, the enzymes showed slightly improved stability.

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## SHELF LIFE OF MAIZE (*ZEA MAYS* L.) FLOUR

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Biochemical changes in the flour of two untreated maize varieties viz. 'Vijay-normal' and 'Shakti-opaque-2' stored for 180 days, were studied under ambient conditions. Oil content of 'Vijay-normal' and 'Shakti-opaque-2' flours were 4.6 and 5.1 per cent respectively. Fatty acid composition in both the varieties almost remained constant during storage. Increase in peroxide and acid values during storage, ranged from 6.1 to 7.3 and 1.0 to 55.9 per cent in 'Vijay-normal' and 7.2 to 8.2 and 0.5 to 51.5 per cent in 'Shakti-opaque-2' respectively. Bacterial count in 'Shakti-opaque-2' flour decreased whereas that of fungal infestation increased after 180 days of storage. Acid value in flour prepared after heat treatment of kernels (80°C, 3 hr) was almost constant during 30 days of storage.

Maize occupies an important place next to wheat and rice in the world food supply map. In India, 90 per cent of this is directly consumed as human food. Maida, sooji, grits, flakes, pre-cooked flour and mill *atta* are different forms of its consumption<sup>1</sup>. Besides, it is also consumed as popped and boiled kernels<sup>2</sup>. The initial sources of degradation of maize are mostly from fungi, insect pest and enzymes. A suitable heat treatment to sterilize maize thus becomes inevitable to prolong the shelf life of maize flour and keep them free from foreign organism. Therefore, in the present investigation, studies have been made on fresh untreated maize flour to find out biochemical as well as microbiological changes and the flour prepared after heat treatment to find out only biochemical changes responsible for its poor keeping quality.

Two maize varieties viz. 'Vijay' (normal composite) and 'Shakti' (opaque-2 composite) used in the present study, were grown on the farms of Indian Agricultural Research Institute, New Delhi under identical agronomical conditions. Untreated dried samples were ground (60 mesh) and stored in air tight containers at ambient conditions (temperature, 27-29°C; relative humidity, 60-70 per cent) for a period of 180 days. Samples were drawn at 0, 2, 15, 30, 60, 120 and 180 days intervals. In another experiment, maize kernels of both varieties were heated at 80°C for 3 hr to sterilize before grinding (60 mesh). These samples of both varieties were stored for 30 days at ambient conditions and samples drawn

at 0, 2, 4, 6, 8, 10, 15, 20, 25 and 30 days intervals for determining acid values. Control (unheated) experiment was also run simultaneously.

Fat/oil from the flour was extracted and estimated by cold precolation method of Kartha and Sethi<sup>3</sup>. Moisture content, acid value (AV) and peroxide value (PV) of fat/oil were determined according to AOAC<sup>4,5</sup>. Methyl esters of fatty acids were prepared by transesterification using boron trifluoride (14 per cent in methanol) and sodium methoxide (0.4 N in methanol) as described by Metcalf and Schmitz<sup>6</sup>. These were analysed on gas liquid chromatography (GLC) using NUCON-AIMIL Model 5700 instrument fitted with glass column (1.5 m × 6 mm) packed with 20 per cent DEGS on 60-80 chromosorb W and fitted with FID detector, column temperature, 190°C ± 1, nitrogen flow, 30 ml/min. Peaks were identified using standard fatty acid methyl esters procured from Sigma chemicals. Relative proportions of fatty acids were determined according to Maddan and Bhatia<sup>7</sup>. Bacterial count was determined as recommended in Manual of microbiological methods<sup>8</sup>. Mould count was estimated by standard method using potato dextrose - agar medium.

Moisture content of opaque-2 flour at 0, 2, 15, 30, 60, 120 and 180 days intervals was 8.5, 9.0, 9.5, 10.2, 10.5, 10.9 and 11.5 per cent respectively. In the treated (80°C, 3 hr) opaque-2 samples, it was 3.5, 4.5, 4.8 and 5.3 per cent as against 8.3, 9.3, 10.1 and 10.3 per cent in control (untreated) at 0, 10, 20 and 30 days intervals respectively. In normal maize flour, moisture content matched with opaque-2 within ± 0.5 per cent limit. Oil contents of normal and opaque-2 maize flour were 4.6 and 5.1 per cent respectively. Among fatty acids of fat extracted from the samples at 0 and 180 days of storage of both the varieties, linoleic acid, which is essential for animal, was the major fatty acid, followed by oleic and palmitic acid whereas linolenic acid was present in traces (Table 1). The concentration of linoleic and stearic acid was found to be higher in normal maize and oleic acid was found to be higher in opaque-2 when compared to each other at both 0 and 180 days of storage. Similar trend of fatty acid composition in normal and opaque-2 maize was also reported by Gupta *et al*<sup>9</sup>.

PV of untreated opaque-2 samples was slightly more than that of normal maize samples at all the stages of storage. However, in both the varieties, it almost remained constant during storage. AV in untreated samples of both the varieties recorded an increase of 62 and 64 folds respectively in normal and opaque - 2 maize samples from 0 to 180 days of storage. This increase in AV was very rapid during first 10 days which declined thereafter (Table 2). There was no significant

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TABLE 1. FATTY ACID COMPOSITION OF OIL EXTRACTED FROM NORMAL AND OPAQUE-2 UNHEATED MAIZE FLOUR STORED FOR 0 AND 180 DAYS

Fatty acids	Normal		Opaque-2	
	0 day	180 days	0 day	180 days
Palmitic (16:0)	14.8	14.5	15.3	15.5
Stearic (18:0)	3.0	3.2	1.8	2.1
Oleic (18:1)	28.3	26.7	33.3	34.5
Linoleic (18:2)	54.9	55.6	49.5	47.8
Linolenic (18:3)	t	t	0.1	t
Linoleic/Oleic ratio	1.9	2.1	1.5	1.4
Saturated	17.8	17.7	17.1	17.7
Unsaturated	83.2	82.3	82.9	82.3
Saturated/Unsaturated ratio	4.67	4.65	4.85	4.65

Mean of duplicate analysis

difference in the rate and degree of increase between the two varieties. The appearance of acidity and its sharp increase could be attributed to the release of fatty acids from 1, 3 position of triacyl glycerol on fat hydrolysis due to its sudden contact with lipase<sup>10</sup>.

Colonies of *Aspergillus* and *Penicillium* increased nearly 4.2 and 5.0 fold, respectively whereas bacterial count decreased by 2.5 fold after 180 days of storage in untreated opaque-2 samples. Increase in fungal infection and decrease in bacterial count may be due to increase in moisture and acidity respectively in flour which in turn are due to increase in mould count and action of inherent lipase. Barton<sup>11</sup> also reported decrease in bacterial count as the period of storage increased. After heat treatment, AV marginally increased by 2.7 and 3.6 fold in normal and opaque-2 maize-samples from 0 to 30 days respectively in storage. This was only 8.1 and 7.7 per cent respectively of those in untreated normal and opaque-2 maize samples.

Bitterness leading to the unpalatability of maize flour within a short period could be attributed only to hydrolytic (rather than oxidative) rancidity which led to the accumulation of acids. Hydrolytic rancidity in the flour of cereal due to the lipase has been reported by many workers<sup>12,13</sup>. Therefore, the shelf life of the flour could be considerably improved by heat treatment which apart from inactivating enzymes, lowers the moisture content. Recently Gupta *et al*<sup>14</sup> reported rapid increase in AV in the flour of whole kernels than in its products like semolina and processed flour from three maize varieties.

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TABLE 2. ACID AND PEROXIDE VALUES OF OIL EXTRACTED FROM TREATED AND UNTREATED NORMAL AND OPAQUE-2 MAIZE FLOUR

Storage period (days)	Acid value				Peroxide value	
	Untreated normal	Treated normal	Untreated opaque-2	Treated opaque-2	Untreated normal	Untreated * opaque-2
0	0.9**	1.0	0.8**	0.7	5.9	6.5
2	12.7**	1.1	13.8**	1.1	6.1	7.8
4	11.8	1.3	12.0	1.3	—	—
6	15.1	2.0	15.3	1.8	—	—
8	18.3	2.0	18.9	2.0	—	—
10	23.2	2.4	22.7	2.4	—	—
15	23.7**	2.2	24.0**	2.1	6.2	7.2
20	27.7	2.4	27.2	2.5	—	—
25	29.8	2.5	29.4	2.4	—	—
30	33.2**	2.7	32.4**	2.5	7.2	8.2
60	36.0	—	35.0	—	7.2	7.2
120	50.9	—	49.0	—	7.2	8.2
180	55.9	—	51.5	—	7.3	8.1

Mean of duplicate analysis; \*\*Mean of 4 independent analysis



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## MICROSTRUCTURE OF COCONUT HAUSTORIUM

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Microstructure of coconut haustorium was studied by optical microscopy using histochemical staining and by scanning electron microscopy. Outermost layer of the haustorium was composed of small, compactly arranged parenchyma cells and contained colourless oil droplets which stained red with Sudan IV solution. Just below the outermost layer of the haustorium, two to three layers of the spongy tissue contained both oil droplets and starch grains. Subsequent five to six layers of the spongy tissue were rich in starch grains, their size ranging from 1.5-2.0  $\mu$ . Innermost region of the haustorium contained very little starch.

The ripe fruits of coconut (*Cocos nucifera* Linn.) when come into contact with water or moisture for sufficiently long time, they commence to germinate. The plumule of the embryo grows towards the soft eye and the other end of the embryo develops an absorbent spongy growth known as the haustorium<sup>1</sup>. The haustorium swells and continues to grow till it completely fills the cavity and is in close contact with the endosperm or the kernel. Its very finely corrugated surface exposes a very large absorptive surface area. In colour it is pale yellow outside and white inside, and in structure it consists of loosely connected thin-walled cells with large interspaces between them.

Changes in weight of the haustorium with the progress of germination also have been reported<sup>2</sup>. The weight of haustorium on sixteenth week after the ripe nut was put in the nursery, was found to be 25g, which increased to 44.3g, 72.8g, 89.0g and 125.2g in 18, 20, 22 and 24 weeks. The weight of shoot increased from 7.9 to 33.2g during this period. Biochemical changes during the germination of the coconut was reported by Balasubramaniam *et al.*<sup>3</sup> Studies carried out on microstructure of coconut haustorium are presented here.

Germinating coconut of about 18 to 20 weeks after sowing was used in these studies. It was cut transversely into two halves and the haustorium was scooped out as a single mass. The weight of the haustorium was about 72g and the volume occupied was around 3/4th of the cavity in the nut. The shoot had no well-formed green leaves. Hand sections of the haustorium, both transverse and longitudinal were made and examined under the microscope (PZO Warzava biological microscope MB10). Due to extreme spongy nature of the haustorium, freeze-microtome sections could not be made.

Sudan IV in ethyl alcohol was used to stain oil droplets<sup>4</sup>. Iodine-potassium-iodide<sup>5</sup> was used for staining starch. Scanning electron micrographs were taken with Jeol 80 Model Scanning Electron Microscope after the sections were sputtered with gold ions.

Diagrammatic representation of the coconut haustorium is shown in Fig.1. Cross section of the haustorium showed an outermost compact layer followed by a spongy and aerenchymatous tissue inside. Outermost layer of the haustorium was composed of small, compactly arranged parenchyma cells. Many conspicuous wrinkles were characteristics of this outermost layer. This layer contained colourless oil droplets. When stained with Sudan IV, which is essentially a lipid stain, oil droplets were stained red. Inner to this layer, the spongy and aerenchymatous tissue consisted of elongated cells, irregular in shape and loosely arranged enclosing air spaces between them.

Just below the outermost layer of the haustorium, two to three layers of the spongy tissue contained both oil droplets and starch grains. Subsequent five to six layers of the spongy tissue were rich in starch grains. Starch grains were small and globose, their size ranging from 1.5 to 2  $\mu$ . Innermost region of the haustorium contained very little starch. All the elongated cells of the spongy tissue had a thin coating of oil inner to the wall of each cell.

Scanning electron micrograph of coconut haustorium is shown in Fig.2. Few starch grains are seen.

Balasubramaniam *et al.*<sup>3</sup> reported that during germination of coconut, the reducing and non-reducing sugars of kernel

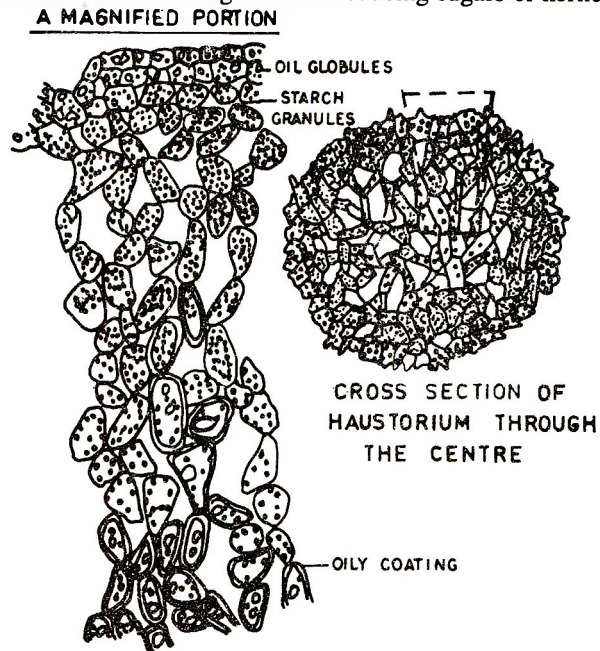


Fig.1. Cross section of coconut haustorium showing the distribution of starch grains and oil globules.

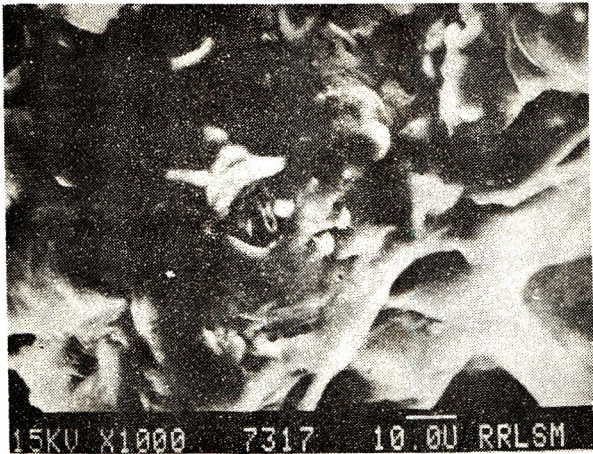


Fig.2. Scanning electron micrograph of coconut haustorium.  
Few starch grains are seen.

decreased. In the haustorium, starch increased linearly, whereas the reducing and non-reducing sugars initially increased very rapidly and then remained constant. The results suggest that during germination, embryos utilized the stored carbohydrates of the kernel and that the excess

carbohydrates mobilised from the kernel are stored in the haustorium as starch. The starch content at 24 weeks of germination was around 1.6 per cent. They further report that during the early stages of germination upto 18 weeks, the embryo utilises the stored carbohydrates only and not fat. Nathanael<sup>6</sup> has shown that the weight of oil per nut does not decrease during the first 18 weeks of germination.

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## PROCESSING OF PLUMS

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**Method of extraction of juice from plum (*Prunus salicina* L) was standardised. Pulp and juice yield by hot break method was higher in all three varieties of plum as compared to cold break process. Addition of water increased the juice yield but reduced the total solid content. Pectinolytic enzyme treatment increased the juice yield but reduced total soluble solids, total solids, pectin and relative viscosity of the juice.**

Plum (*Prunus salicina* L) is a major stone fruit of Jammu and Kashmir, Himachal Pradesh and Uttar Pradesh. It is a good source of carbohydrates and minerals<sup>1-3</sup>. Fruits are mainly utilized for table purpose during its limited period of availability but its storability at room temperature is poor because of its soft texture and high moisture content. Processing of plums into jam, juice and wine has been reported<sup>4-8</sup>. The present investigation was carried out to standardize the technique of extraction of juice from plums.

Three varieties of plum namely 'Alubukhara', 'Satsuma' and 'Santa-Rosa' were selected for the present study. The fruits were temporarily stored at low temperature (0-4°C) till processing. Cold stored fruits were washed in tap water and pulp was extracted by following straight extraction procedures. Half of the lot was extracted by hot break process. Extraction was carried out by superfine stainless steel pulper (Raylon India) fitted with 1/32" sieve. Extraction of pulp by hot break extraction procedure involved mashing of fruit with 4 per cent of water to facilitate heating, crushing and extraction. Extracted pulp was heated in a steam jacketed kettle held at 80°C for 2 min, hot filled in 650 ml bottles, crown corked and processed in boiling water for 40 min. Crushed pulp was heated to 75°C and cooled to 37°C and Triz-yne (obtained from Triton Chemicals, Mysore) was added at 0.5 per cent and kept for 12-16 hr at 37°C. Then, it was subjected to extraction with hydraulic press at a pressure of 100 psi.

Physico-chemical analysis of fresh pulp/juice was conducted. Total soluble solids (TSS) were determined by using hand refractometer and were expressed as degree Brix at 20°C using Reference Tables. Total solids (TS) were determined gravimetrically taking 10 g of sample for drying at 70°C in hot air oven to a constant weight. Acidity and pH

were determined by standard methods of AOAC<sup>9</sup>. Sugars were estimated by Lane and Eynon method and pectin was determined by modified Carre and Haynes method as described by Ranganna<sup>10</sup>. Viscometer was used to determine viscosity of samples by taking distilled water as standard reference. Determinations were carried out at 20°C and results expressed in sec for 100 revolutions of the rotor. Extent of browning was determined by taking a known quantity of sample (2g) which was extracted with 20 ml of 60 per cent ethanol and filtered. Absorbance of filtrate was measured at 440 nm using Bausch and Lomb Spectronic 20 spectrophotometer.

Recovery of pulp and juice from different varieties is given in Table 1. Pulp and juice recovery varied with variety, treatment and methods used for extraction. Pulp and juice yield by hot break process was higher in all three plum varieties as compared with cold break extraction process. In 'Santa-Rosa' variety, enzyme treatment resulted in recovery of juice as high as 90 per cent on pulp basis in hot break extraction process (Table 1).

An increase of 5.8 per cent of pulp was achieved by application of heat. Clarified juice yield was higher by 3.4 per cent by hot break process as compared to cold break process (Table 1). Juice from enzyme clarified pulp was clear and bright with thin consistency.

Approximate composition of pulp obtained with and without enzyme is presented in Tables 2 and 3. Pulp of 'Alubukhara' recorded maximum TSS and total solids i.e. 14 and 14.7 per cent respectively as compared to 'Satsuma' (Table 2). TSS in all three varieties ranged between 9-14 per cent. Pulp seems to contain an appreciable proportion of soluble solids which depends upon variety, mode of extraction and treatment given. Average total solids ranged from 10.6-14.7 per cent for pulp and 9.2-12.2 per cent for juices as shown in Tables 2 and 3 which indicated that a slight decrease in TS was observed in enzyme clarified pulp, where suspended matter settled down after pectinase treatment and was subsequently eliminated during further processing.

Maximum acidity of 1.67 per cent was found in 'Santa-Rosa' pulp among the varieties tested followed by 'Alubukhara' (1.64 per cent) and 'Satsuma' (1.30 per cent) respectively (Table 2). pH of juice ranged between 3.3 and 3.15 (Tables 2 and 3). The increase in acidity in plum juice was attributed to the de-esterification and degradation of pectin by enzyme. Maximum total sugars were observed in 'Santa-Rosa' (7 per cent) and minimum in 'Alubukhara' (5 per cent). Pectin content was maximum in 'Alubukhara' (1.37 per cent) followed by 0.95 per cent and 0.87 per cent in 'Satsuma' and 'Santa-

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TABLE 1. EFFECT OF VARIETY AND PROCESSING ON THE YIELD OF PULP AND JUICE\*\*

Variety	Pulp yield (%) in control			Juice yield (%) in enzyme treatment		
	Cold pressed	Hot pressed	Increase (%)	Cold pressed	Hot pressed	Increase (%)
Alubukhara	67.0	71.0	4.0	82.0	84.0	2.0
Santa-Rosa	73.0	77.1	4.1	87.8	90.0	2.2
Satsuma	62.0	71.3	9.3	79.0	85.0	6.0
Mean	67.3	73.3	5.8	82.9	86.0	3.4

Trizyme enzyme was treated at 0.5%; \*On whole fruit basis; \*\*On pulp basis (w/v)

TABLE 2. EFFECT OF VARIETY AND PROCESSING ON THE COMPOSITION OF PLUM PULP

	Alubukhara		Santa-Rosa		Satsuma	
	Cold pressed	Hot pressed	Cold pressed	Hot pressed	Cold pressed	Hot pressed
TSS (%)°Brix	14.0	11.0	13.0	11.0	10.0	10.0
Total solids (%)	14.7	11.8	13.7	11.6	10.7	10.6
Total sugars as dextrose (%)	5.6	5.0	7.0	5.8	5.5	5.5
Reducing sugar as dextrose (%)	4.7	4.8	5.7	4.3	4.9	4.3
Titrate acidity as malic acid (%)	1.46	1.54	1.67	1.64	1.25	1.30
pH	3.07	3.08	3.06	3.06	3.08	3.07
Pectin as Ca Pectate (%)	1.37	1.37	0.87	0.87	0.95	1.01
Browning absorbance at 440 nm.	0.18	0.17	0.20	0.19	0.20	0.20
Stormer viscosity (SEC)	15	12	18	16	16	11

Rosa' respectively (Table 2). Although the pulp of 'Santa-Rosa' appeared to be more viscous than other varieties it contained minimum amount of pectin among three varieties tested. Pectinolytic enzyme was most beneficial for producing clear juice, though it imparted some brown tints to the juice extract. There was no residual pectin substance in enzyme treated pulp (Table 3).

A tremendous change in viscosity of the juice was observed by enzyme application as compared to their counterparts

(Tables 2 and 3). A significant decrease in relative viscosity in all the three plum varieties was observed (Table 2). Change in stormer viscosity may be related to the elimination of the suspended matter during processing by enzyme action. Viscosity of the juice was more or less similar in all varieties of plum because of the effective pectin degradation in the juice of all varieties. Maximum browning was shown by 'Satsuma' pulp juice followed by 'Santa-Rosa' and 'Alubukhara'. Natural colour native to plum variety present seemed to affect the

TABLE 3. EFFECT OF VARIETY AND PROCESSING ON THE COMPOSITION OF PLUM JUICES

	Alubukhara		Santa-Rosa		Satsuma	
	Cold pressed	Hot pressed	Cold pressed	Hot pressed	Cold pressed	Hot pressed
TSS (%)°Brix	12.0	10.0	11.0	10.0	8.0	9.0
Total solids (%)	12.2	10.6	10.3	10.3	9.2	9.2
Total sugars as dextrose (%)	5.0	4.6	6.0	6.0	5.0	5.7
Reducing sugar as dextrose (%)	5.06	4.1	4.6	4.6	4.4	4.9
Titrate acidity as malic acid (%)	1.67	1.60	1.67	1.64	1.34	1.17
Browning absorbance at 440 nm.	0.20	0.20	0.15	0.14	0.16	0.16
Stormer viscosity (SEC)	5	5	5	5	5	5

Pectin was not detected in any of the samples.

absorbance of brown pigment at 440 nm. The high soluble solids, total solids, high pectin and acidity are characteristic of all the three varieties as discussed.

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## EFFECT OF SUGAR SYRUP CONCENTRATION AND TEMPERATURE ON THE RATE OF OSMOTIC DEHYDRATION OF APPLES

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The effect of different concentrations of sugar solution viz. 60 to 75%, different temperatures i.e. 40 to 80°C for different durations (0.5 to 4.5 hr) on the rate of osmosis of apple slices (d = 20 mm & ht = 15 mm) is reported here. Based on these results, an empirical equation was derived which can predict rate of osmosis (F), percentage of dehydration of any given fruit slices of specific size, time (T), given concentration of sugar (%B) and temperature (t) in the above referred parameters.

Drying is one of the oldest methods of preservation of fruit and vegetables and the achievements in this field led to the development of more improved methods of drying. Relatively a new trend in this area is the preliminary dewatering of fresh fruits and vegetables to 50 per cent moisture by osmotic means and subsequent drying by convection or vacuum<sup>1</sup>. The method is called osmo-convection drying. It provides some advantages over the conventional method of drying such as conservation of flavour, high nutritive value, decrease in polyphenoloxidase activity and less energy consumption<sup>1-5</sup>, but there is scanty information in the literature on the theoretical and empirical equations for the determination of the rate of osmotic dehydration under the given conditions. The objective of the present investigation was to carry out experimental studies on the osmotic drying of sliced apples under various conditions including temperature, sugar concentration and the contact time and to work out an equation of the process which can help to predict the rate of osmosis under varied conditions.

Apples ('Golden Delicious') of technological maturity were used in these studies. These were preliminarily peeled with stainless steel peeler and then shaped in cylindrical slices of 20 mm in diameter and 15 mm in thickness. The osmotic dehydration was achieved under laboratory conditions in a water bath fitted with a thermostat. Various concentrations of sugar syrup viz., 60, 65, 70 and 75 per cent and at temperatures of 40, 50, 60, 70 and 80°C were tried. The samples were placed on a special mould (Fig.1) to ensure fixing separate slices at an equal distance. The ratio of fruit to syrup was kept at 1:26 throughout the experiment.

Observations on the mass flow i.e. loss in weight of slices during the process were made. The experiments were repeated four times.

The percentage decrease in the mass in relation to the initial mass of fruit in the sugar syrups of various concentrations for different times and temperatures is shown in Fig.2. It is quite evident that there was an increase in the weight loss (measured in terms of mass flow) with the increase of sugar syrup concentration, temperature of osmotic bath as well as the duration of dipping. The Figure shows that as the duration of dipping time was increased, the total weight loss of the slices was also increased, but not with equal proportion throughout the experiment i.e. initially the per cent weight loss was higher and decreased slowly as the dipping time was increased. It is, therefore, concluded that osmotic drying rates (per cent weight loss) are controlled only by partial pressure differentials which are directly correlated with the concentration of sugar syrup and temperature. Sugar concentration from 60 to 75 per cent was effective in removing moisture from the treated fruits. However, from practical and handling points of view, 70 per cent sugar syrup is suggested for commercial scale application. The Figure also shows that with increase in temperature of the osmotic bath, the weight loss of the fruits was also increased. However, there is a limit of temperature used for osmotic dehydration of apple rings, as the higher temperature above 50°C causes internal browning of the pieces on one hand and loss of fruity flavour on the other.

The experimental data were computerised and the following equation was derived:

$$F = 31.8 - 0.307B - (0.56 - 0.016B) t + 2.10^{9.26/B} - 1 (T - 0.3)^{0.54} - 0.00425 t$$

where

F = decrease in mass, %

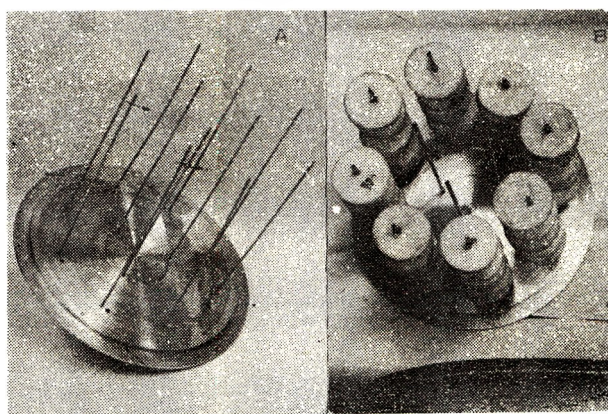


Fig.1. A) Specially designed and fabricated mould. B) Sliced apple rings placed in a mould.

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$$F = \frac{M - M_1 \times 100}{M}$$

M,  $M_1$  = initial and final mass, kg; B = sugar syrup concentration, %; t = temperature of osmotic process °C; T = duration of process, hr.

The equation is valid for the values within the following intervals

B = (60 — 75) per cent

t = (40 — 80) per °C

T = (0.5 — 4.5) per hr

In the case of the cylindrical shape of slices (dia. = 20 mm, ht = 15 mm), the mean error of equation was (5 ± 3%) at a confidence probability of 0.95.

It may be applied to calculate the percentage dehydration at a given duration of the process, temperature and

concentration of the solution, or to estimate the duration at a given percentage dehydration of apple fruits.

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## COMPOSITION OF SOME UNCONVENTIONAL HIMALAYAN WILD FRUITS

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Fifteen species of unconventional wild fruits are identified and collected for documentation and chemical examination during survey of food habits of tribals residing in North-Western Himalayan range. Next to water component, the fruits are rich in sugars (9.95 – 35.42%) and mineral matter (0.5 – 4.7%). Potassium, calcium, iron and phosphorus contents varied from 34 to 998, 51 to 671, 2 to 160 and 3 to 201 mg/100g fruit pulp, respectively. Fruits of *Rosa macrophylla* (769 mg/100g), *Rosa webbiana* (751 mg/100g) and *Hippophae rhamnoides* (509 mg/100g) are rich source of vitamin C content. *Artocarpus lakoocha* fruits are used in making chutney and as pickle while sun-dried *Diospyros lotus* fruits are used throughout the year by local hill communities.

The primitive societies viz. Gujjars, Bakarewals, Gaddis, Ladakhis and Kinnars are residing in the Himalayan range of Jammu and Kashmir, and Himachal Pradesh States, where temperature falls upto  $-40^{\circ}\text{C}$  during winter and the intensity of ultraviolet radiations are comparatively high. The tribal

communities in the remote areas still prefer to remain in wilderness. During adverse climate and scarcity of food, the tribals of this region consume leaves, roots and rhizomes of numerous wild plants<sup>1-3</sup> as emergency food. The aim of the present communication is to document some lesser known Himalayan wild fruits and study their chemical composition.

The information on the wild edible fruits were gathered through interviews with local tribal people of different areas. The fruits were collected from the forests/fields and botanically identified. Chemical studies were conducted on the edible parts of following fifteen fruit species. 1. *Aegle marmelos* Linn. 2. *Artocarpus lakoocha* Roxb. 3. *Berberis umbellata* Wall. 4. *Carissa spinarum* Linn. 5. *Cordia myxa* Roxb. 6. *Diospyros lotus* Linn. 7. *Ephedra gerardiana* Wall. 8. *Fragaria vesca* Sch. 9. *Hippophae rhamnoides* Linn. 10. *Momordica tuberosa* Roxb. 11. *Prunus domestica* Linn. 12. *Rosa macrophylla* Lindl. 13. *Rosa webbiana* Royle 14. *Rubus niveus* Thunb. 15. *Ziziphus nummularia* Wight.

The edible parts of fruits were analysed for their moisture, ash, crude protein, fat and fibre by AOAC methods<sup>4</sup>. Total digestible carbohydrate content was computed by difference. Calcium, potassium and sodium contents in fruit pulps were determined flame photometrically while phosphorus by the method of Fiske and Subba Row<sup>5</sup> and iron by Andrews and Felt<sup>6</sup> method. The vitamin C content in fresh fruits was estimated titrimetrically<sup>7</sup> by using 2,6-dichlorophenol-indophenol dye.

TABLE 1. PROXIMATE COMPOSITION OF HIMALAYAN WILD FRUITS

Name of fruits	Physical characteristics (size, shape, colour, taste)	Moisture (%)	Ash (%)	Crude protein (%)	Crude fibre (%)	Petroleum spirit extractives (%)	Total carbo- hydrates (%)
<i>Aegle marmelos</i>	70-100 mm, globose, yellowish, sweet	76.7	0.59	1.44	2.10	1.17	18.00
<i>Artocarpus lakoocha</i>	80-100 mm, spherical, dirty yellow, sweetish sour	75.8	1.76	2.50	3.95	1.65	13.71
<i>Berberis umbellata</i>	9-12 mm, spherical, red, mild sweet	84.0	0.53	1.49	1.48	1.28	11.00
<i>Carissa spinarum</i>	12-18 mm, ellipsoid, purple, sourish sweet	85.9	0.71	0.72	1.41	0.26	11.01
<i>Cordia myxa</i> (unripe)	15-18 mm, ovoid, yellowish brown, sour, ripe sweetish	80.4	2.14	2.13	5.78	0.13	9.95
<i>Diospyros lotus</i>	16-22 mm, ovoid, purple, sweet	67.1	1.82	1.48	1.57	0.21	27.80
<i>Ephedra gerardian</i>	5-7 mm, ovoid, red, sweet	41.0	4.74	4.94	10.70	2.52	35.42
<i>Fragaria vesca</i>	9-11 mm, spherical, red, light sweet	72.8	2.20	1.04	7.18	3.03	15.10
<i>Hippophae rhamnoides</i>	5-7 mm, ovoid, orange yellow, sweetish sour	52.4	1.76	2.23	4.75	6.94	32.37
<i>Momordica tuberosa</i>	75-100 mm, long, pendulous, ribbed with tubercles, bitter	65.6	1.43	3.08	8.17	4.88	16.57
<i>Prunus domestica</i>	18-21 mm, spherical, yellowish red, sweet	82.2	2.52	0.57	1.36	1.40	11.61
<i>Rosa macrophylla</i>	20-28 mm, ovoid, red, mild sweet	55.6	2.85	4.05	9.83	1.85	24.98
<i>Rosa webbiana</i>	12-19mm, globose, red, mild sweet	56.4	2.98	3.57	10.12	2.37	24.91
<i>Rubus niveus</i>	8-14 mm, spherical, black, mild sweet,	58.2	2.52	3.56	5.54	4.19	26.25
<i>Ziziphus nummularia</i>	20-25 mm, elliptical, greenish yellow, or red, sweetish sour	76.6	1.17	0.65	2.29	0.05	18.94

Each value is the mean of three independent replicates.



The individual sugars in the fruits were separated by paper partition chromatography. The solvent systems used were ethyl acetate-pyridine-water (12:5:4, v/v) and phenol-ammonia (80 per cent, w/v). Different sugars were identified by spraying with aniline hydrogen phthalate and simultaneous cochromatography of authentic sugar samples.

The physical characteristics of the fruits viz. size, shape, colour and taste are summarised in Table 1. The fruits of *B. umbellata*, *E. gerardiana*, *F. vesca* and *H. rhamnoides* are smaller in size and usually eaten by the children during their

movement in the forests. Mostly, the fruits have pleasant flavour. Water constitutes the major component of wild and fresh fruits i.e. 41.0-85.9 per cent whereas carbohydrate content varies from 9.95 to 35.42 per cent. Fat (petroleum spirit extractives) is found in lesser concentration except in *H. rhamnoides* (6.94 per cent), *M. tuberosa* (4.88 per cent) and *R. niveus* (4.19 per cent) as shown in Table 1. Among the mineral constituents, potassium is dominant over others (Table 2). The fruits of *F. vesca*, *R. macrophylla* and *R. webbiana* are good sources of phosphorus i.e. 201, 141 and

TABLE 2. MINERAL AND VITAMIN C (mg/100 g) COMPOSITION OF HIMALAYAN WILD FRUITS

Name of fruits	Na	K	Ca	Fe	P	Vitamin C
<i>Aegle marmelos</i>	12	168	107	3	24	63
<i>Artocarpus lakoocha</i>	7	250	85	52	3	9
<i>Berberis umbellata</i>	17	161	75	34	37	3
<i>Carissa spinarum</i>	4	34	51	2	11	32
<i>Cordia myxa</i> (unripe)	16	998	87	97	35	27
<i>Diospyros lotus</i>	10	242	148	9	23	31
<i>Ephedra gerardiane</i>	45	770	301	8	72	36
<i>Fragaria vesca</i>	44	520	233	102	201	46
<i>Hippophae rhamnoides</i>	69	622	671	16	74	509
<i>Momordica tuberosa</i>	17	339	69	98	8	161
<i>Prunus domestica</i>	20	670	110	36	26	2
<i>Rosa macrophylla</i>	49	740	62	80	141	769
<i>Rosa webbiana</i>	49	730	75	81	139	751
<i>Rubus niveus</i>	18	704	345	160	73	27
<i>Ziziphus nummularia</i>	18	275	155	2	31	71

Each value is the mean of three independent replicates

139 mg/100g fruit pulp respectively whereas *F. vesca* (102 mg), *R. niveus* (160 mg), *M. tuberosa* (98 mg) and *C. myxa* (97 mg) are rich in iron. Fruits of *R. macrophylla*, *R. webbiana* and *H. rhamnoides* contain high concentrations of vitamin C among the fruits studied i.e. 769, 751 and 509 mg/100 g fruit pulp respectively.

During survey, it was observed that the ripe fruits of *A. lakoocha* are used in making *chutneys* by local hill communities. The fruits are sliced, dried and used in place of tamarind. *A. lakoocha* fruit is also used in pickled form. The sun-dried fruits of *D. lotus* are widely used throughout the year by the Himalayan inhabitants. Tender fruits of *M. tuberosa* are used as vegetable. Among the sugars identified, glucose, fructose and sucrose are predominant.

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## FUNCTIONAL AND CHAPATI MAKING PROPERTIES OF HULL-LESS BARLEY SUPPLEMENTED WHEAT FLOUR\*

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Chapati making characteristics of two recently evolved hull-less barley varieties were studied. Replacement of whole wheat flour upto 25% of barley flour did not affect the chapati quality adversely. The farinograph and amylograph characteristics of four of these varieties are also reported.

Three types of barley are, hulled six-rowed; hulled two-rowed and hull-less. The use of hull-less barley in malting is not feasible due to handling problems; but are useful as food, due to their higher digestibility (94 per cent) than hulled type (83 per cent). Besides, the yield of barley flour from hull-less barley is about 10 per cent more than that of hulled barley<sup>1</sup>. In North Africa, South Arabia and Far East, barley is consumed as pearled grain for soups, as flour for flat bread and ground grains are used for porridge'. At the Indian Agricultural Research Institute Regional Station, Karnal, a few promising varieties of hull-less barley have been developed. The lysine content of two barley varieties viz. 'Karan 16' and 'Karan 19' and 'C 306' variety of wheat have been reported to be 3.96, 3.24 and 2.39 per cent (on protein basis), respectively<sup>2</sup>. Studies were, therefore, undertaken to determine the suitability of hull-less barley flour either alone or in combination with bread wheats, for the preparation of *chapatis*.

Commercially grown wheat (cv 'PBW 154') was procured from Punjab Agricultural University, Ludhiana farm during 1987-88 crop year. The hull-less barley cultivars 'Karan 16'

and 'Karan 19' were obtained from IARI Regional Station, Karnal. The wheat and barley samples were cleaned and ground in commercial disc mill (*chakki*) to obtain whole grain meal. The moisture content of wheat, 'Karan 16' and 'Karan 19' was 8.4, 8.0 and 8.5 per cent, respectively. The samples were then sieved through 24 mesh sieve to remove 5-7 per cent of coarse bran.

Chemical characteristics of flour samples such as moisture, ash, protein, falling number, diastatic activity and damaged starch were determined according to AACC procedure<sup>3</sup>. Tannins were estimated by Folin-Denis method of AOAC<sup>4</sup>. Functional properties of the samples were determined by amylograph and farinograph according to AACC<sup>3</sup> procedure. All the above observations were taken in quadruplicate and average values are reported. Test baking of chapati was carried out using a standard procedure of Haridas Rao *et al.*<sup>5</sup> and the *chapatis* were evaluated for quality characteristics such as hand feel, flexibility, appearance, eating quality, colour and acceptability, using a score of 1 to 6. The sensory data were analysed statistically and critical differences are reported<sup>6</sup>.

It is interesting to note that both of these barley varieties required nearly half of energy as compared to wheat for grinding into meal similar granulation. During milling, the energy requirement (Ammeter reading of *chakki*) for barley samples was 11 amperes, compared to 20 amperes for wheat, indicating that barley grains of these varieties were softer than wheat. The flours were sieved and the extraction rate was 93.0, 94.3 and 94.7 per cent for wheat, 'Karan 16' and 'Karan 19', respectively. Thousand grain weight and hectolitre weight of wheat were more than those of barley (Table 1). The protein content of wheat was 1 and 2 per cent lower than that found in 'Karan 16' and 'Karan 19', respectively; similar trend was also observed in flour samples. The diastatic activity and damaged starch were higher in wheat flour than in barley flour.

The falling number values were similar for barley and wheat (Table 2). From the amylograph paste characteristics

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF WHEAT AND BARLEY CULTIVARS AND THEIR FLOURS

Wheat/barley (var)	Whole grain			Flour				
	Thousand grain wt (g)	Hectolitre wt (kg)	Protein (N×6.25) (%)	Ash (%)	Protein (N×6.25) (%)	Diastatic activity (mg maltose/10g flour)	Tannins as tannic acid (mg/100g)	Damaged starch (%)
PBW 154	49.3	86.9	10.4	1.2	10.0	379	84	8.0
Karan 16	34.3	81.2	11.5	1.5	10.9	249	110	4.3
Karan 19	35.2	81.6	12.5	1.4	11.7	291	116	5.3

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TABLE 2. PASTE CHARACTERISTICS OF WHEAT AND BARLEY FLOUR USING FALLING NUMBER AND AMYLOGRAPH

Variable	Falling no. (sec)	Gelatinization temp (°C)	Peak viscosity (BU)	Peak viscosity temp (°C)	Viscosity at 95°C (BU)
PBW 154	461	67.3	510	87.8	315
Karan 16	499	59.6	1055	90.5	805
Karan 19	486	58.3	1045	91.3	830
PBW 154 + Karan 16*	490	66.5	550	89.0	340
PBW 154 + Karan 19*	481	66.5	565	87.7	360

\*Wheat (75 %) + Barley (25 %)

it is inferred that the wheat flour had higher gelatinization temperature and lower peak viscosity than barley flour, while the peak viscosity temperature of barley flour was higher. Supplementing barley at 25 per cent level did not affect significantly the pasting behaviour of wheat flour.

Farinograph water absorption was notably higher in wheat flour (70 per cent) compared to 'Karan 16' (63.1 per cent) and 'Karan 19' (61.3 per cent) flour (Table 3). High water absorption by wheat flour may be due to the highly damaged starch in wheat compared to barley flour<sup>7</sup>. Supplementing barley flour at 25 per cent level to wheat flour improved the mixing characteristics of wheat flour. The dough development time of wheat flour increased by 101.4 and 116 per cent when

supplemented with 25 per cent 'Karan 16' and 'Karan 19', respectively. The mixing tolerance values decreased from 70 to 20 BU in case of 25 per cent barley supplemented wheat flour indicating better handling properties. Barley flour showed greater stability and less softening upon mixing compared to wheat flour. Therefore, the supplementation of whole wheat flour with 25 per cent barley flour resulted in improved stability of dough.

The dough of 'Karan 16' was crumbly and hard to roll compared to wheat flour which was smooth and pliable. The dough from 'Karan 19' had sheeting characteristics quite similar to wheat dough. The addition of 25 per cent barley flour to wheat flour further improved the dough sheeting

TABLE 3. RHEOLOGICAL PROPERTIES OF WHEAT AND BARLEY FLOURS USING FARINOGRAPH TECHNIQUE

Variable	Water absorption* (%)	Curve characteristics				
		Arrival time (min)	Dough development time (min)	Mixing tolerance index (BU)	Stability (min)	Softening (BU)
PBW 154	70.2	2.1	3.7	70	3.0	80
Karan 16	63.1	1.3	4.8	28	19.5	63
Karan 19	61.3	0.8	2.5	33	17.2	35
PBW 154 + Karan 16**	69.3	2.8	7.5	20	12.5	45
PBW 154 + Karan 19**	69.1	1.8	8.8	20	14.6	40

\*Water absorption to center 500 BU; \*\*Wheat (75%) + Barley (25%)

TABLE 4. SENSORY EVALUATION OF WHEAT, BARLEY AND BARLEY SUPPLEMENTED WHEAT CHAPATIES

Variable	Quality parameters						
	Hand feel	Appearance of spots	Flexibility	Eating quality	Colour	Acceptability	Total score
PBW 154	4.5	5.0	5.2	4.8	5.2	5.2	29.9
Karan 16	4.0	2.8	2.8	3.0	3.0	2.7	18.3
Karan 19	4.0	3.5	3.2	3.0	3.2	3.0	19.9
PBW 154 + Karan 16a	4.5	4.3	4.8	3.8	4.5	4.3	26.2
PBW 154 + Karan 19a	4.5	4.3	4.7	3.8	4.5	4.3	26.1
PBW 154 + Karan 16b	4.2	4.3	4.2	3.5	4.2	3.8	24.2
PBW 154 + Karan 19b	4.5	4.2	4.7	3.8	4.3	4.2	25.7
F ratio	1.00	7.08**	14.05**	5.44*	8.48**	15.86**	
CD at 1%	—	1.43	0.92	1.05	1.03	0.83	

\*\*Significant at 1% level

Key to score: Excellent 6, Very good 5, Good 4, Fair, 3, Poor 2, Very poor 1

a = Wheat (75%) + Barley (25%), b = Wheat (50%) + Barley (50%)



properties of wheat flours. With 50 per cent barley supplemented wheat flour, the dough was smooth and extensible but showed a reduced resistance to extension. Wheat, barley and combinations showed full puffing. Sensory evaluation showed that barley chapatis were slightly greyish in colour, had typical flavour probably due to tannins and were slightly hard to bite but crumbled easily in mouth. Such typical barley flavour was not perceptible in 25 per cent barley supplemented chapatis, and the colour was also light brown like wheat chapati. The differences among the various samples for quality parameters were found to be statistically significant ( $P < 0.01$ ) except for hand feel (Table 4). The total score was highest for wheat chapati (29.9) and was lowest for 'Karan 16' (18.3) and 'Karan 19' (19.9), respectively. But in case of 25 per cent barley supplemented chapati, the score nearly approached wheat chapatis. Thus, it can be concluded that barley flour from these varieties can be supplemented at 25 per cent level to wheat flour without affecting the quality of chapati adversely, thus extending our wheat supply by 25 per cent. The 'Karan 19' variety had superior dough handling

and chapati making characteristics as compared with 'Karan 16' variety.

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## EFFECT OF ADDITION OF BUFFALO MILK PROTEOSE AND PROTEOSE-PEPTONE COMPONENTS 3, 5 AND 8 ON THE WHIPPABILITY OF CREAM

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**Addition of buffalo milk proteose (BP) and proteose-peptone (BPP) components 3, 5 and 8 like fractions to both cow's and buffalo creams did not reveal any appreciable change in the whippability upto an added concentration of 2.0 mg/ml. Cow's milk cream attained lower whippability (64.3%) in comparison with buffalo milk cream (93.7%) under identical conditions of processing.**

Whipping consists of incorporating air in cream to produce a stable foam of fairly rigid structure. The rate of air incorporation and gain of over-run are reflected by the term whippability. A higher whippability makes the air incorporation possible in the form of small air cells. As the whipping continues, the bigger cells are sub-divided into smaller nuclei which, in turn, are again surrounded by an adsorbed layer of protein.

Aggarwal<sup>1</sup> found that a minimum of 2.3 per cent protein is essential to achieve good standability (stiff in over-run) in a whipping cream. A good whipping cream should come to maximum over-run within 1.5 min. If it fails to do so in 3 min, it should be considered as unsatisfactory. Addition of different additives as emulsifiers and stabilizers is in practice to produce a good whippable cream<sup>2,3</sup>. Jelen<sup>4</sup> and Morr<sup>5</sup> have proposed that proteose-peptone fraction is probably responsible for the excellent whipping properties of deproteinated whey. Proteose-peptone is thermostable and not precipitated along with caseins and denatured whey proteins of milk at pH 4.6. It is a heterogeneous protein fraction and consists of three major components named as proteose-peptone components 3, 5 and 8 on the basis of ascending electrophoretic mobilities in total whey proteins<sup>6</sup>. Proteose-peptone components 5 and 8 are reported to be typical soap like molecules and largely responsible for good foaming properties of proteose-peptone in whey<sup>7</sup>. However, the effect of addition of minor protein components of buffalo milk proteose-peptone fraction on the whippability of cream has not yet been studied. Hence the present study was undertaken.

Buffalo milk proteose (BP) was precipitated at 35 per cent ammonium sulphate concentration from acid whey (pH 4.6) while buffalo milk proteose-peptone (BPP) was obtained at

12 per cent trichloroacetic acid concentration from acid whey (pH 4.6) of buffalo milk. The proteose-peptone components were obtained by gel filtration on sephadex G-75 column with 0.2 M phosphate buffer pH 6.5 as the eluting buffer<sup>8</sup>.

Whippability of cream samples with and without added proteose-peptone components was determined according to the method adopted by Kumar and Srinivasan<sup>9</sup>. Skim milk was pasteurised at 72°C and cooled to 35°C. Cream of 40 per cent fat was prepared by mixing proportionate quantities of cream having higher fat content and skim milk using Pearson's square formula<sup>10</sup>. It was pasteurized at 82°C and homogenised at 600 psi twice in a laboratory hand operated (Gremac mark III Model) homogeniser at 66°C. It was cooled to 20°C by placing in chilled water. Proteose-peptone component fractions at concentrations of 50, 100, 150 and 200 mg were mixed in 2 ml of pasteurized skim milk at 35°C with the help of a glass rod in separate test tubes. These were then mixed with pasteurised cream at 20°C and volume made to 100 ml with cream in the nickel cup of Mohr's apparatus. The whipped cream without added proteose-peptone was taken as control. The volume of the whipped mix was determined in the whipping container with the help of a graduated dip. Buffalo and cow's milk cream samples with or without added buffalo milk proteose-peptone components were examined for their whippability and the results are shown in Table 1 and 2.

It is quite apparent from Table 1 and 2 that there is no appreciable change in the whippability of cream on additions of BP and BPP components fractions. Our results do not support the views expressed by Jelen<sup>4</sup> and Morr<sup>5</sup> that PP fraction might be responsible for excellent whipping properties of deproteinated whey. This can be accounted on the basis of the following probabilities.

- (i) The concentrations of BPP components existing in cream might be sufficient enough to impart their maximum effect on its whippability.
- (ii) The BPP components at the concentrations tried out may not have any effect in cream.
- (iii) The BPP components may not have any role to play on the whippability of cream in view of the cumulative effect of other constituents like casein,  $\beta$ -lactoglobulin,  $\beta$ -lactoalbumin, bovine serum albumin, phospholipids, calcium etc.<sup>1,3,5,11-14</sup>

Sulphydryl groups are one of the best predictors of over-run<sup>11</sup> and  $\beta$ -lactoglobulin is the main source of free sulphydryl groups in milk<sup>15</sup>. Commonly cited literature values for  $\beta$ -lactoglobulin are in the range of 2-4 g/l which are higher than those reported for proteose-peptone fraction in skim milk<sup>7,15</sup>.  $\beta$ -lactoglobulin has been found to correlate well in whipped toppings over-run (correlation coefficient

0.92) whereas  $\alpha$ -lactalbumin and bovine serum albumin were not significantly correlated with any of the functional properties tried out<sup>11</sup>. Thus the two possibilities (i) and (ii) are ruled out. It is quite likely that proteose-peptone components may have something to do with the foaming

characteristics of cream as has been reported by some workers<sup>5,14</sup>.

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TABLE 1. EFFECT OF ADDITION OF BP AND BPP FRACTIONS ON THE WHIPPABILITY OF BUFFALO MILK CREAM

Fraction added	Amount added (mg/ml)	Range	Mean $\pm$ SE	Change %
Control	—	90 — 99	93.7 $\pm$ 2.7	Nil
Buffalo milk proteose	0.5	90 — 100	94.0 $\pm$ 3.1	+ 0.3
	1.0	90 — 98	93.3 $\pm$ 2.4	— 0.4
	1.5	91 — 99	94.3 $\pm$ 2.4	+ 0.6
	2.0	92 — 98	94.7 $\pm$ 1.8	+ 1.0
BPP Component 3 like fraction	0.5	92 — 96	93.3 $\pm$ 1.3	— 0.4
	1.0	90 — 99	93.0 $\pm$ 3.0	— 0.7
	1.5	91 — 98	93.3 $\pm$ 2.3	— 0.4
	2.0	89 — 99	92.7 $\pm$ 3.2	— 1.0
BPP Component 5 like fraction	0.5	91 — 99	94.3 $\pm$ 2.4	+ 0.6
	1.0	90 — 101	94.3 $\pm$ 2.4	+ 0.6
	1.5	90 — 100	94.7 $\pm$ 2.9	+ 1.0
	2.0	90 — 102	94.7 $\pm$ 3.7	+ 1.0
BPP Component 8 like fraction	0.5	88 — 99	93.7 $\pm$ 3.2	Nil
	1.0	92 — 100	95.0 $\pm$ 2.5	+ 1.3
	1.5	90 — 98	94.0 $\pm$ 2.7	+ 0.3
	2.0	92 — 100	94.7 $\pm$ 2.3	+ 1.0

Mean  $\pm$  SE of 3 replicates; BPP — Buffalo milk proteose-peptone; + Increase; — Decrease

TABLE 2. EFFECT OF ADDITION OF BP AND BPP FRACTIONS ON THE WHIPPABILITY OF COW'S MILK CREAM

Fraction added	Amount added (mg/ml)	Range	Mean $\pm$ SE	Change %
Control	—	62 — 68	64.3 $\pm$ 1.9	Nil
Buffalo milk proteose	0.5	61 — 68	63.7 $\pm$ 2.2	— 0.6
	1.0	60 — 67	64.0 $\pm$ 2.1	— 0.3
	1.5	61 — 70	65.0 $\pm$ 2.7	+ 0.7
	2.0	62 — 69	64.3 $\pm$ 2.3	Nil
BPP Component 3 like fraction	0.5	60 — 68	63.3 $\pm$ 2.4	— 1.0
	1.0	62 — 66	63.7 $\pm$ 1.2	— 0.6
	1.5	61 — 67	63.0 $\pm$ 2.0	— 1.3
	2.0	62 — 66	63.7 $\pm$ 1.2	— 0.6
BPP Component 5 like fraction	0.5	62 — 68	64.3 $\pm$ 1.9	Nil
	1.0	62 — 71	65.7 $\pm$ 2.3	+ 1.4
	1.5	63 — 69	65.0 $\pm$ 2.0	+ 0.7
	2.0	62 — 70	65.3 $\pm$ 2.4	+ 1.0
BPP Component 8 like fraction	0.5	64 — 66	65.0 $\pm$ 0.6	+ 0.7
	1.0	61 — 70	64.3 $\pm$ 2.9	Nil
	1.5	63 — 68	65.0 $\pm$ 1.5	+ 0.7
	2.0	61 — 70	64.7 $\pm$ 2.7	+ 0.4

Mean  $\pm$  SE of 3 replicates; BPP — Buffalo milk proteose-peptone; + Increase; — Decrease



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**RESIDUES OF FENVALERATE IN PIGEON PEA  
(CAJANUS CAJAN (L.) MILLSP.)**

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The levels of fenvalerate residues from pigeon pea grains as well as podshell were determined during 1986-87 and 1987-88. Three emulsion sprays (0.005%, 0.01%, 0.02%) and a dust formulation (0.4%) were applied @ 25 kg/ha. The levels in pigeon pea grains at harvest did not exceed the maximum residue limit of 1 ppm. However, more than 1 p.p.m. of residues were found in the podshells from all the treatments except 0.005% spray.

Fenvalerate is a potential synthetic pyrethroid having high efficacy against lepidoptera, hemiptera and diptera larvae infesting several economic crops<sup>1</sup>. It has also been proved effective in checking the pest infestation of some pulses<sup>2-4</sup>. It becomes imperative to evaluate the safety of fenvalerate from the view point of its toxic residues. Several researchers have worked out its residue levels in greengram, blackgram, chickpea and cowpea<sup>2-5</sup>. Report on residues of fenvalerate in pigeon pea grains is limited in India<sup>6</sup>. Investigation was, therefore, attempted to determine fenvalerate residue levels in pigeon pea grains and podshells.

**Sampling:** Samples of 1 kg mature pods were collected randomly from the plants in field experiment layout in RBD in which EC formulation of fenvalerate at 0.005, 0.01 and 0.02 per cent concentrations and its 0.4 per cent dust formulation @ 25 kg/ha were applied with knapsack hand sprayer (liquid formulation) and hand rotary duster (dust formulation) twice at fortnightly interval initiating at 50 per cent pod formation stage during both the years (i.e. 1986-87 and 1987-88) of experimentation.

**Extraction:** The samples from each treatment were powdered/chopped, mixed and 50 g and 25 g sub-sample of seed and podshell were extracted with solvent mixture of hexane and acetone (1:1 v/v)<sup>7</sup> respectively keeping solvent to sample ratio 3:1. The extract was filtered through Buchner funnel and residual matter was re-extracted. The filtered extracts were pooled and diluted with 200 ml of 2 per cent sodium sulphate solution in a separating funnel. The hexane layer was separated and lower acetone layer was re-extracted with 100 ml hexane. The combined hexane extract was drained through anhydrous sodium sulphate and was concentrated to about 5 ml at 40°C under vacuum.

**Clean-up:** The extracts obtained were cleaned by column chromatography using 5 g each of anhydrous sodium sulphate,

florisil and neutral alumina (grade I). The residues were eluted with 200 ml solvent mixture (9:1), hexane : acetone, v/v). The eluate was concentrated to dryness under reduced pressure at 40°C and final volume was made up to 5 ml with iso-octane for GLC analysis.

**Gas-chromatographic analysis:** The fenvalerate residues were estimated by gas liquid chromatography (GLC) equipped with an electron capture detector Ni<sup>63</sup> using metal column (50 cm long × 3.2 mm i.d.) filled with 5 per cent OV-101 stationary phase on 100-120 mesh Chromosorb G(HP). The column was operated at 250°C, injection port 280°C and detector 350°C. The minimum detectable limit for fenvalerate was 0.001 p.p.m. Recoveries of the chemical from fortified samples at 0.1 and 0.5 p.p.m. were 80 and 95 per cent respectively. Data presented have not been corrected for recovery.

The results on fenvalerate residues in pigeon pea grains and podshells without grains obtained during 1986-87 and 1987-88 are presented in Table 1. The residues of fenvalerate detected in the grains sampled from the plots treated with fenvalerate (Fenval<sup>(R)</sup> 20 EC) 0.005, 0.01, 0.02, and fenvalerate 0.4 per cent dust @ 25 kg/ha, during 1986-87, were on an average 0.052, 0.050, 0.122 and 0.055 p.p.m. respectively; whereas, during 1987-88 the levels were 0.008, 0.010, 0.017 and 0.012 p.p.m. respectively.

In the podshells, the residues detected from the samples from different treatments viz., fenvalerate (Fenval<sup>(R)</sup> 20 EC) 0.005, 0.01, 0.02 and fenvalerate 0.4 per cent dust @ 25 kg/ha, during 1986-87 were on an average 0.91, 1.53, 4.09 and 5.50 p.p.m. respectively, whereas, during 1987-88 the levels were 0.967, 1.937, 4.164 and 6.017 p.p.m. respectively.

The levels of fenvalerate residues in the grains of all treatments with fenvalerate in its emulsifiable concentrate as well as dust formulations were thus quite below the tolerance limit of 1 p.p.m. as prescribed by FAO<sup>8</sup>. Both the formulations of fenvalerate at the recommended doses are found to be safe from consumers' view points. However, the residues in the podshells without grains were more than 1 p.p.m. in all the treatments except fenvalerate 0.005 per cent.

TABLE 1. FENVALERATE RESIDUES IN PIGEON PEA GRAINS AND PODSHELLS

Treatment	1986-87		1987-88	
	Grain (p.p.m.)	Podshell (p.p.m.)	Grain (p.p.m.)	Podshell (p.p.m.)
Fenvalerate (0.005%)	0.052	0.91	0.008	0.967
Fenvalerate (0.01%)	0.050	1.53	0.010	1.937
Fenvalerate (0.02%)	0.122	4.09	0.017	4.164
Fenvalerate (0.4% dust)	0.055	5.50	0.012	6.017

Values are average of four replicates

Values are moisture free basis.

Maximum residues in the podshells were found when fenvalerate was applied in its dust formulation, indicating its slow dissipation and longer persistence in the podshells. This warrants precautionary measures before podshell is being fed to the animals. Besides this, the tur fodder (leaves and twigs) also need to be evaluated for such residues to safeguard the animal health.

The authors are thankful to Searle (India) Ltd., Bombay for generous supply of fenvalerate in EC and dust formulations and also for providing financial support to the research project.

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## BOOK REVIEWS

*J. Fd. Sci. Technol.*, 1990, Vol. 27, No. 5, 319 — 320

*Rapid Assessment of Community Nutrition Problems:* by Purnima Kashyap and Richard H. Young, International Development Research Centre (IDRC), P.B. No. 8500; Ottawa, Canada; KIC 3 HG, 1989; pp. 60 Price: not mentioned.

In this booklet (59 pages with 48 pages text) the authors have collected some information in a few villages (exact no. not given) in Parbhani district (Maharashtra) on social pattern, on food crops and food preparation, on infant feeding practices, nutrition and health facilities.

“Rapid rural appraisal (RRA)”, a new slogan coined: This “appraisal” was done in villages in a radius of 25-30 km of an agricultural university (MAU) which exists since more than 20 years and another agricultural university in a semi-arid area of Maharashtra (Rahori), approximately 400 km south. It is rather improbable that these universities had not collected information on agricultural subjects such as cropping, grain storage, processing, marketing, etc. applicable to the area.

The team who endured the rigours of the climate, waited for buses in the hot sun and walked through slushy fields and muddy roads deserves all credit. But, since they were only 25-30, maximal 54 km away from the district town Parbhani with the university campus they could return after a day's work. The reader has to find out by inference the number of villages surveyed. A map of the respective area would have been useful for the reader who is not altogether unacquainted with the geography of India. Since reference is made to the traditionally big teams of surveying experts, the reader would like to know how small this team has been. We learn about the 2 authors and 2 M.Sc. students from the Home Science Dept. of Marathwada Agricultural University (MAU) and assume that the team consisted of four.

For the authors, the survey must have been a good learning experience and, hopefully, has also some outcome for the villages, as they claim. Otherwise, the booklet remains one of the innumerable, non-consequential studies. Unfortunately, the authors have only studied the recent literature (earliest reference 1974 and earliest Indian from 1981). Had the authors taken the trouble to study the Indian literature from the 1920's onwards they would have realised that much work has been done and much knowledge acquired through Home Science Colleges, Agricultural Universities, Medical Colleges and National Institute of Nutrition (NIN), Hyderabad, which had its origin in 1919. NIN has a Nutrition Monitoring Bureau which surveys different areas of the country at intervals (a Nutrition Atlas and a Diet Atlas of India were published since 1969 and 1971, respectively by NIN). Equipped with some of this knowledge, the authors would probably have been more cautious with their statements and perhaps also more modest in giving suggestions and advice.

This “appraisal” has definitely not led to any new insight or revelations. The continuous and multiple advice on what should be done is quite exasperating, the claims exaggerated (17 out of 48 pages are devoted to advice + recommendations, plus interspersed advice in the running text). Everybody concerned with the field of nutrition and nutrition intervention is aware of the problems and also the conditions in the villages, the regional staple foods, etc.

The survey or “appraisal” would have been useful in preparation of an intervention programme in the surveyed villages. It is common knowledge that most of the programmes specifically governmental, suffer from ill-execution. It is well known what has to be done, the question is how can it be done best and who can implement it. Had the authors taken up the implementation of one of their many suggestions much would have been achieved e.g. instead of advising education programmes (to be done by whom?) it would have been extremely laudable had they taken up one programme with a single message only. The need of the hour is action and proper implementation, not advice and consultation.

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

*Safety of Irradiated Foods:* by J.F. Diehl, Marcel Dekker Inc, 270, Madison Avenue, New York, N.Y. 10016, 1990; pp 368, Price: US \$125 (US & Canada), \$150 (All other countries).

A grain preserved is a grain produced! It has been the endeavour of scientists to develop techniques to preserve the food, produced after much toiling, to feed the teeming millions of world population. Irradiation, one of the technologies is hotly debated by the proponents and opponents for its potential, economics and safety. Anti nuclear lobbying, world over, holding sway over the minds of politicians and general public, should not keep this technology at bay without rational discussion.

In this context “Safety of Irradiated Foods” by J.F. Diehl is an excellent book, for the people who are non-specialists, yet genuinely interested in knowing about the FACTS of the subject. The book is nicely structured to introduce the reader to the subject of Food Irradiation and also enable him to know about the potential of this technology and partake in rational discussions on the subject. Exhaustive, up-to-date bibliography helps people to delve deep into the subject, if one aspires to do so. The book, thus seeks to dispel the misgivings, fears dogging the minds of general public by presenting the facts as they are and accord “Food Irradiation” its due share in the field of Food Preservation and quality improvement.

C.S.R. PRASAD  
INDIAN RARE EARTHS LTD., MYSORE.

*Improving Young Child Feeding in Eastern and Southern Africa-House hold level food technology: Proceedings of a workshop held in Nairobi, Kenya October 1987; pp.380; Price: not mentioned.*

The proceedings of a workshop Co-sponsored by International Development Research Centre, UNICEF and Swedish International Development authority, illustrating the papers presented with recommendations and conclusions constitute the content of this book. Scientists from 19 countries (South African countries, Nepal, India, Sweden, U.K. and USA) have contributed 35 papers.

The book begins with an introduction of executive summary of the workshop which illustrates the objectives of workshop followed by general and specific recommendations and conclusions aimed at improving the feeding of young children. The specific recommendations are grouped under five important topics for consideration with respect to infant and young child feeding practices namely breast feeding, anorexia, supplementary foods for young children, fermented foods for young children, fermented foods and germinated flour. These recommendations and conclusions have pragmatic approach to solve some of the feeding problems. The workshop had five sessions with specific topics. Each session had papers related to the topic followed by useful discussions. These topics of five sessions are the five chapters of this book.

The first session includes papers on Issues in improving child feeding. This session elaborates on examination of real solutions for young child malnutrition, complementary food problem, the importance of breast feeding as weaning food resource. Further, papers throw light on use of sorghum and millets as weaning foods in East Africa and provision of weaning foods in refugee situations.

The second session has the subject 'Weaning practices and promoting change' which includes nine papers. These papers

enlighten the reader on weaning practices available in Ethiopia, Zimbabwe, Swaziland and Mozambique and weaning foods of Kenya, Uganda, Rwanda and Botswana. It also highlights the importance of reintroducing the traditional weaning foods.

The third session focusses on 'Fermented foods in child feeding'. Here a revision on fermented foods for improving child feeding in Eastern and South Africa is presented. Details with regard to different fermented foods of South African countries namely 'Ugi', maize based mahewa, cereal and legume-based weaning food, cassava-based fermented foods are illustrated.

The fourth session entitled 'Food contamination and lactic fermentation' includes papers on Weaning food hygiene in Kenya, contamination of weaning food in Zimbabwe, formulation and microbiological safety of cereal-based weaning foods and bacteriological properties of traditional sour porridge in Lesotho.

The fifth session deals on 'Experiences in East Africa and Asia namely Nepal, India, Tanzania, Sweden and UK'. The papers throw light on dietary bulk and its effect on food and energy intake, high nutrient density weaning foods from germinated cereals, feeding pattern with reference to feeding frequency and dietary bulk, food consistency and nutrient intake of young children. Further, experiences on bulk reduction of traditional weaning gruels, malted weaning foods in India, Nepal, and improved iron bioavailability in weaning foods are recorded.

The book is a useful reference book for all those who are interested and deal with the practical aspects of feeding young children.

G. SARASWATHI

UNIVERSITY OF MYSORE, MYSORE.

### ANNOUNCEMENT

As a consequence of the increase in the cost of printing, publication and postal tariff, it has now become absolutely necessary to enhance the SUBSCRIPTION RATES of the Journal of Food Science and Technology. The new rates are as follows:

#### Annual Subscription of Journal

##### India

Rs. 300/-

##### Foreign

Air Mail US\$ 125/-

Surface Mail US\$ 100/-

This will come into effect from the beginning of Volume 28, 1991. I solicit your kind cooperation.

— Editor

## AFST(I) NEWS

### Bombay Chapter

The following office-bearers were elected at the Annual General body Meeting held on 22nd June 1990 for the year 1990-91.

President	: Dr. S. R. Padwal-Desai
Vice-Presidents	: Dr. H. R. Adhikari Dr. J. S. Pai
Hony. Secretary	: Dr. S. B. K. Warriar
Hony. Jt. Secretary	: Dr. M. Y. Kamat
Hony. Treasurer	: Dr. A. S. Gholap

### Calcutta Chapter

The Annual General Body Meeting was held on 20th April 1990. The following office-bearers were elected for the year 1990-91.

President	: Shri R. N. Ghosh
Vice-Presidents	: Shri O. P. Dhamija Shri S.K. Das Gupta
Hony. Secretary	: Dr. Pratap Chakraborty
Hony. Jt. Secretaries	: Mrs. Maya Mitra Shri Rajesh Mehta
Hony. Treasurer	: Dr. S. Sil

### Delhi Chapter

The Annual General Body Meeting was held on 17th June 1990, in which following office-bearers were declared elected for the year 1990-91.

President	: Shri D. S. Chadha
Vice-Presidents	: Dr. S. M. Ilyas Dr. D. S. Khurdiya Shri Y. K. Kapoor Shri V. B. Oberoi
Hony. Secretary	: Shri B. L. Kapoor
Hony. Jt. Secretary	: Shri S. K. Checker
Hony. Treasurer	: Shri. P.N. Narang

### Jammu Chapter

The Annual General Body Meeting was held on 28th June 1990 and the following office-bearers were elected unanimously for the year 1990-91.

President	: Shri S. S. Langer
Vice-President	: Dr. B. L. Raina
Hony. Secretary	: Shri Virander Kaul
Hony. Treasurer	: Shri R. P. Singh
Councillors	: Shri G. S. Gaur Shri A. K. Gupta

## SYMPOSIUM

Defence Food Research Laboratory, Mysore is organising a three day symposium in the third week of January 1991 on "**Convenience Foods - Emerging Technologies, Quality Assurance and Nutritional Evaluation Techniques**". There will be both oral and poster presentations and technical sessions spread on three days will deal with convenience foods from (a) fruits and vegetables; (b) cereals, pulses and oil seeds; (c) meat, fish, poultry and dairy products; (d) chemical and microbial quality assessment techniques; (e) nutritional and safety assessment of convenience foods and (f) packaging requirements and marketing aspects. An exhibition on Convenience Foods will be also be organised on the eve of symposium. Those desirous of attending the symposium may contact Dr. S. S. Arya, Organising Secretary, D.F.R.L. Mysore-570011 (Phone: 26828) for further particulars.



# ANNOUNCING FELLOWSHIPS ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS (INDIA)

Central Technological Research Institute Campus, Mysore - 570 013

## Subject:- FELLOWSHIPS OF AFST(I)

The Association has pleasure in announcing conferring of Fellowships of AFST(I) entitled "Fellow of Association of Food Scientists and Technologists (India)" (FAFST) to honour persons who have contributed significantly to the progress of Food Science and Technology.

The following are the highlights of the Fellowship:

### General:-

1. The awardee will be called as Fellow of Association of Food Scientists and Technologists(India) and in an abbreviated form will be termed as FAFST.
2. The total Fellowships of the Association will not exceed 5% of total membership including regular and life members of the Association in any given year or 100, whichever is lower.

Fellowships have been awarded to 22 AFST(I) members and 4 non-members who have contributed to the progress of Food Science and Technology.

### Eligibility:-

1. The Fellowship is to honour persons of outstanding merit who have contributed significantly in the field of Food Science and Technology including R&D, Product/Project Development, Industry, Transfer of Technology and Marketing. The merit of Contribution should be the main criterion.
2. Among the Fellows to be nominated every year 70% will be from ASFST(I) and remaining 30% may be from non-members who have contributed significantly for the development of Food Science and Technology.

### Nominations:-

1. The nominee for Fellowship should be proposed by five AFST(I) members of good standing for a minimum of 5 years or by 2 Fellows of the Association. This is applicable to AFST(I) members as well as non-members.
2. Any regular or life member of AFTS(I) who has been continuously a member of the Association can sponsor the nomination for only one Fellowship in a particular year.
3. The nomination shall be accompanied by acceptance of the person proposed.
4. The nomination shall be in the format given. A brief biodata of the nominee with highlights of Scientific or Technological achievements in the area of Food Science and Technology supported by list of publication not exceeding 10 important research papers or other supporting documents not exceeding 20 pages must accompany the nominations.

5. The nomination duly proposed and accepted by the nominee's consent shall be sent to the Hony. Executive Secretary AFST(I) by January of each year.

### Selection of Fellows:-

The nominations received will be placed before an expert committee appointed by CEC for suitable recommendations to CEC each year. CEC by majority decision will finalise Fellowships for each year. The decision of the CEC in this matter will be final.

### Privileges of a Fellow:-

The Fellow shall be entitled to the following rights:-

1. The awardee will be entitled to add FAFST after his name as short title.
2. To be present and vote at all general body meetings.
3. To propose and recommend the candidates for Fellowship of the Association.
4. To receive gratis copies of one of the publications of AFST(I)
5. To fill any office of the AFST(I) duly elected.
6. To be nominated to any committee of AFST(I)
7. To offer papers and communications to be presented before the meeting of the Association.

### Cessation of Fellowship:-

1. Any Fellow may withdraw from the Fellowship of the Association by signifying his wish to do so by a letter addressed to the Hony. Executive Secretary, AFST(I), which will be placed before CEC for acceptance.
2. Fellowship will be for life time of the member.
3. If the Association comes to know of any activity prejudicial to the interest and well being of the Association, the CEC will have the right to withdraw the Fellowship.

### Conferring of Fellows:-

The Fellowship will be conferred with a Citation at the time of AGBM or at any other suitable function of the Association.

The Association may invite some fellows nominated each year to deliver special lectures in the area of their specialisation either at the AGBM or any other function arranged by AFST(I).

Please write to Hony. Executive Secretary, Association of Food Scientists & Technologists (India), CFTRI Campus, Mysore - 570013, India for application forms for nominations.

Last date for receiving the nominations is 15th January 1991

**Dr. K. Vidyasagar**  
Hony. Exec. Secretary

**ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (INDIA)**  
**CFTRI CAMPUS, MYSORE — 570 013.**

**Nomination Form**

We, the following members of AFST(I) wish to propose

---

Full name and academic distinction

FULL NAME :

ACADEMIC QUALIFICATION :

---

Date of Birth

Areas of specialization

for election of the Fellowship of AFST(I). We append below the statement of his/her claims for election as Fellow and certify that in our opinion he/she is fully qualified for that distinction. We also certify that he/she has been informed of the obligations attaching the fellowships of the AFST(I) and agreeable, if elected to abide by them.

Statement of the proposer (not to exceed 100 words) setting out the discovery, invention or other contribution to newer or process/ products or the industrial development of the knowledge made by the candidate.

.....  
Seconder's name & Signature

Date:

Station:

Proposer's name & signature

Date:

Station:

(Signature of supporters from personal/general knowledge)

(1)

(2)

(3)

I agree for the above nomination

(name & signature)

.....

Note: (1) Five copies of the candidate's bio data and list of important scientific publications not exceeding 10 pages and one set of reprints or supporting documents not exceeding 20 pages shall be attached to this form.

(2) Additional information that would be of assistance in considering the nomination may be supplied in separate sheet.

(3) Last date for receipt of nomination at the office is 15th January 1991

## **ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS (INDIA)**

*CFTRI Campus, Mysore — 570 013, India*

### **NOMINATIONS FOR AFST (I) AWARDS FOR 1990**

Nominations for the following awards of the AFST (I) for the year 1990 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 15th January 1991

#### **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. Subrahmanyam Industrial Achievement Award — 1990

#### **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 specification.
- (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 1990.



## **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 1990

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 — 1990

## **YOUNG SCIENTIST 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 1990 working in the area of Food Science and Technoogy.
  - (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 1990.

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# INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (in triplicate) should be typewritten in double space on one side of bond paper. They should be complete and in final form. The paper should not have been published or communicated for publication anywhere else. Research Notes should clearly indicate the scope of the investigation and the salient features of the results. Only *invited* review papers will be published.
2. The typescript should be arranged in the following order: Title (to be typed in capital and small letters for Research Papers and all capitals for Research Notes), Authors' names (all capitals) and Affiliation (capitals and small letters). Also give a short running title not exceeding 10 words as a footnote.
3. **Abstract:** The abstract should indicate the principal findings of the paper and typed in single space. It should not be more than 200 words and in such a form that abstracting periodicals can readily use it.
4. Use names of chemical compounds and not their formulae in the text. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Footnotes especially for text should be avoided as far as possible.
5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables 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 twice the size of the printed letter. Photographs must be on glossy paper and must have good contrast; **three copies** should be sent.
7. **References:** Names of all the authors along with title of the paper should be cited. Abbreviations such as *et al.*, *ibid*, *idem* should be avoided. References should be serially numbered as superscripts in the order they are cited in the text and the same order should be maintained in the reference list. The titles of all scientific periodicals should be abbreviated in conformity with the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.

Citation should be as follows (note the underlines also):

- (a) *Research Paper:* Jadhav S S and Kulkarni P R, Presser amines in foods, J Fd Sci Technol, 1981, 18, 156.
  - (b) *Book:* Venkataraman K, The Chemistry of Synthetic Dyes, Academic Press, Inc, New York, 1952, Vol, II, 966.
  - (c) *References to article in a book:* Joshi S V, in The Chemistry of Synthetic Dyes, by Venkataraman K, Academic Press Inc, New York, 1952, Vol, II, 966.
  - (d) *Proceedings, Conferences and Symposia Papers:* Nambudiri E S and Lewis Y S, Cocoa in confectionery, Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India, Mysore, May 1979, 27.
  - (e) *Thesis:* Sathyanarayan Y, Phytosociological Studies on the Calicolous Plants of Bombay, 1953, Ph.D. Thesis Bombay University.
  - (f) *Unpublished Work:* Rao G, unpublished, Central Food Technological Research Institute, Mysore, India.
8. Consult the latest issue of the *Journal* for guidance. For "Additional Instructions for Reporting Results of Sensory Analysis" see **issue No. 1** of the *Journal*.



# JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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Contents of forthcoming issue

Nov./Dec. 1990

## Research Papers

MIGRATION STUDIES ON SOME SELECTED COMMERCIAL PLASTIC PACKAGING MATERIALS FOR FOOD CONTACT APPLICATIONS by *Baldev Raj, R. A. N. Murthy, N. S. Vijayalakshmi, A. R. Indiramma, N. Balasubrahmanyam and P. Veeraj*

INTERACTION OF PLASTIC FILMS WITH FOODS. II. EFFECT OF POLYETHYLENE AND POLYPROPYLENE FILMS ON THE STABILITY OF VEGETABLE OILS by *G. K. Sharma, C. V. Madhura and S. S. Arya*

STUDIES ON STABILIZATION OF WHEAT BRAN by *R. Vetrmani and P. Haridas Rao*

STUDIES ON DIFFERENT METHODS OF EXTRACTION OF BETALAINES FROM RED BEET (*BETA VULGARIS*) by *M. Abeyskere, S. R. Sampathu and M. L. Shankaranarayana*

A STUDY ON THE BEHAVIOUR OF AIR MICROFLORA IN FOOD INDUSTRIES by *Shabbir A. Sayeed and R. Sankaran*

STUDIES OF SOME ASSAM RICE VARIETIES FOR PROCESSING AND NUTRITIONAL QUALITY by *L. Saikia and G. S. Bains*

CHANGES IN THE FUNCTIONAL CHARACTERISTICS OF WHEAT DURING SOAKING AND SUBSEQUENT GERMINATION by *K. Leelavathi, R. Vetrmani and P. Haridas Rao*

DEVELOPMENT OF SHELF STABLE READY-TO-EAT INDIAN SWEET MEATS BASED ON SUGAR AND COCONUTS by *T. S. Satyanarayana Rao, N. M. Kaverappa and K. S. Jayaraman*

DEVELOPMENT OF READY-TO-EAT TRADITIONAL INDIAN SWEET DISHES BASED ON JAGGERY AND COCONUT by *T. S. Satyanarayana Rao, N. M. Kaverappa, T. Hemaprakash Reddy and K. S. Jayaraman*

EFFECT OF PROTEASE ON FLAVOUR DEVELOPMENT AND BIOCHEMICAL CHANGES IN BUFFALO MILK CHEDDAR CHEESE by *S. K. Kanawjia and S. Singh*

EFFECT OF COOKING ON LIPID COMPOSITION OF BUFFALO BONE MARROW by *N. Sharma, R. C. Keshri and G. Gandemer*

STORAGE STUDIES ON COORG MANDRIN JUICE CONCENTRATES PACKED IN VARIOUS CONTAINERS by *R. S. Ramteke and W. E. Eipeson*

CONFORMATIONAL CHANGES IN LYSOZYME DUE TO FOOD DYES by *J. M. Teijon, J. A. Onrubia, M. D. Blanco and Issa Katime*

## Research Notes

EFFECT OF PROCESSING CONDITIONS ON EXTRUSION COOKING OF SOY-RICE BLEND WITH A DRY EXTRUSION COOKER by *R. T. Patil, D. S. Singh and R. E. Tribelhorn*

COMPARATIVE STUDY ON THE ESTIMATION OF ETHANOL IN FERMENTED SAMPLES BY DIFFERENT METHODS by *O. Sreekumar and S. C. Basappa*

SOME STUDIES ON RAPESEED MILLING by *A. K. Tikko, U. S. Agrawal and D. K. Gupta*

EVIDENCE FOR THE PRESENCE OF A LIPOPROTEIN LIPASE IN ULTRA HEAT TREATED BOVINE MILK AND ITS IONIC BINDING TO HEPARIN by *Deepa Pande and M. T. Mathur*

NON-COAGULABILITY OF COW'S MILK WITH CHYMOSIN by *Leslie M. Nsofor*

USE OF PRESERVATIVES FOR IMPROVING SHELF LIFE OF CURD (DAHI) by *M. K. Sanyal, P. L. Yadav and P. C. Dubey*

STUDIES ON THE PHYSICO-CHEMICAL PROPERTIES OF ICE CREAMS FORMULATED WITH DIFFERENT SUBSTITUTION LEVELS OF ARROW-ROOT POWDER SOLIDS-NOT-FAT by *J. P. Venkateswarlu, P. M. Sastri, M. R. Rao and M. Rangadham*

QUALITY CHARACTERISTIC OF FREEZE-DRIED EDIBLE OYSTER *CRASSOSTREA MADRASENSIS* (Preston) by *G. Indra Jasmine, C. B. T. Rajagopalasamy, G. Sugumar and P. Jayachandran*

ORANGE CONCENTRATE BASED CARBONATED BEVERAGE by *D. S. Khurdiya*

DETOXIFICATION OF AFLATOXIN B<sub>1</sub> IN MAIZE IN DIFFERENT COOKING METHODS by *Fasiha Rehana and S. C. Basappa*

QUALITY CHARACTERISTICS OF CAKES PREPARED FROM DIFFERENT FATS AND OIL by *N. Shrestha, S. A. Vali and P. N. Choudhary*