# JOURNAL of FOOD SCIENCE AND TECHNOLOGY



ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS, INDIA

VOL. 20. NO. 2.

MARCH / APRIL 1983

0

### ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS

### (INDIA)

The Association is a professional and educational organization of Food Scientists and Technologists

AFFILIATED TO THE INSTITUTE OF FOOD TECHNOLOGISTS, USA

### **Objects:**

- 1. To stimulate research on various aspects of Food Science and Technology.
- 2. To provide a forum for the exchange, discussion and dissemination of current developments in the field of Food Science and Technology.
- 3. To promote the profession of Food Science and Technology.

The ultimate object is to serve humanity through better food.

### Major Activities:

- 1. Publication of Journal of Food Science and Technology (bi-monthly) and Indian Food Industry (IFI) (quarterly).
- 2. Arranging lectures and seminars for the benefit of members.
- 3. Holding symposia on different aspects of Food Science and Technology.

### Membership:

Membership is open to graduates and diploma holders in Food Science and Technology, and to those engaged in the profession. Each member will receive a copy of the Journal of Food Science and Technology, published by the Association. The Chapters of the Association are situated at Bangalore, Bombay, Calcutta, Delhi, Hyderabad, Karnal, Ludhiana, Madras, Nagpur, Parbhani, Poona, Palayamkottai and Trivandrum.

Membership Fee			Admission Fee	Annual Subscription		
Life Member	Rs	300	Rs 2	for each Journal		
Life Member (Resident abroad)	\$	150	<b>\$</b> 1	Inland Rs 100		
Corporate Member	Rs	300	Rs 5	Foreign:		
Member	Rs	20	Rs 2	Surface Mail \$ 30		
Member (Resident abroad)	\$	10	\$ 1	Air Mail <b>\$</b> 50		
Affiliate Member	Rs	30	Rs 2	For IFI		
Student Member		10	Re 1	Association members		
				Indian Rs. 20		

For membership and other particulars kindly address

Foreign

The Honorary Executive Secretary

Association of Food Scientists and Technologists, India

Central Food Technological Research Institute, Mysore-570 013, India

Editorial Board	
K. L. Chadha	
A. N. Bose	
Sunit Mukherjee	
I. S. Bhatia	
K. A. Ranganath	
L. A. Ramanathan	
S. N. Nigam	
N. Balasubramanyan	ı
P. Narasimham	
L. V. Venkataraman	(Ex-officio)
S. C. Basappa	( —do— )
Y. S. Lewis	( —do— )

### Editor

K. R. Sreekantiah

The Journal of Food Science and Technology is a bimonthly publication of the Association of Food Scientists and Technologists, India (AFST) issued in February, April, June, August, October and December.

The Editor assumes no responsibility for the statements and opinions expressed by the contributors.

Manuscripts for publication and books for reviewing in the Journal should be addressed to the Editor, Journal of Food Science and Technology, AFST, Central Food Technological Research Institute, Mysore-570013. The Editor reserves the privilege of editing the manuscript to make it suitable for publication in the Journal.

No part of this journal can be reproduced by any body without written permission of the Editor.

Correspondence regarding subscriptions and advertisements should be addressed to the Executive Secretary, AFST, Central Food Technological Research Institute, Mysore-570013, India. Payment may be made by cheque, draft, postal or money order in favour of Exec. Secretary, AFST.

#### Executives of the AFST(I)

#### President

S. K. Majumder

### Vice-Presidents

T. R. Sharma A. S. Aiyar A. G. Naik Kurade M. S. Laul G. A. Sulebele

Exec. Secretary

L. V. Venkataraman

### Joint Secretary

S. C. Basappa

### Treasurer

C. T. Dwarakanath

### JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Volume 20

Number 2

March/April 1983

47

### CONTENTS

### **Research Papers**

Comparative Studies on Volatile Components of Scented and Non-scented Rice 43 N. Ramarathnam, C Bandyopadhyay and P. R. Kulkarni

Studies on Lysine Earliched Plasteins from Oilseed Proteins N. S. Susheelamma

 Physico-chemical and Respiratory Changes in Dwarf Cavendish Variety of Bananas

 During Growth and Maturation
 51

Paul Thomas, Pushpa Paul, N. Nagaraja and V. B. Dalal

- Instrumental Quality Measures: Development, Standardization and their Correlation to the Sensory Attributes in Apple 57
- S. M. Ananthakrishna, S. Dhanaraj, M. B. Ramakrishnarajan and V. S. Govindarajan

Lipid Composition of Salted Sun-dried Indian Mackerel (Rastrelliger kanagurta) 62 B. Y. Krishnoji Rao and C. Bandyopadhyay

## Studies on the Extraction of Caffeine from Coffee Beans64K. Udaya Sankar, C. V. Raghavan, P.N. Srinivasa Rao, K. Lakshmi-<br/>Narayana Rao, S. Kuppuswamy and P. K. Ramanathan

### Investigations on Large Scale Preparation and Preservation of Milk Burfi 67

B. R. Ramanna, K. K. Bhat B. Mahadevaiah, C. T. Dwarakanath, A. Dhanaraj, V. H. Potty and D. P. Sen

### **Research Notes**

### Solvent Extraction of Whole Groundnuts

R. C. Belani and J. S. Pai

### **Bulk Densities of Oilseeds**

Y. Venkateswara Rao, G. Azeemoddin, D. Atchuta Ramayya and S. D. Thirumala Rao

### ห้องสมุดกรมวิทยาศาสตรบริการ

73

72

Post Harvest Control of Spoilage in Mango (Mangifera indica L.) with Hot Water and Fungicides 74 Shantha Krishna Murthy and K. P. Gopalakrishna Rao						
Steeping Preservation of Fruits G. S. Mudahar and B. S. Bhatia	77					
Use of Tomato Seed Powder as an Antioxidant in Butter and Ghee S. P. S. Guleria, P. Vasudevan, K. L. Madhok and S. V. Patwarahan	79					
Is Potassium Sorbate Necessary for Preserving Canaed Butter? R. Sankaran, M. S. Mohan and R. K. Leela	80					
<ul> <li>Aerobic Mesophilic Count of Fresh and Refrigerated Ground Mutton: Effect of Plating and Incubation Temperature</li> <li>T. R. K. Murthy</li> </ul>	83					
Microbial Degradation of Cellulosic Materials: Screening of Fungal Isolates K. Theja, T. R. Shamala, K. R. Sreekantiah and V. Sreenivasa Murthy	84					
A Note on Antibiotics Sensitivity of E. coli Isolated from Market Milk of Ludhiana City S. S. Kahlon and V. K. Joshi	86					
Book Reviews	89					
Association News	93					

### Comparative Studies on Volatile Components of Scented and Non-Scented Rice

N. RAMARARATHNAM

Food and Fermentation Technology Division, Department of Chemical Technology, University of Bombay, Matunga, Bombay-400 019, India

C. BANDYOPADHYAY

Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085, India

AND

### P. R. KULKARNI

Food and Fermentation Technology Division, Department of Chemical Technology, University of Bombay, Matunga, Bombay 400-019, India

Manuscript received 16 November 1981; revised 4 August 1982

These preliminary observations are concerned with the extraction, concentration and characterization of volatiles responsible for the unique flavor possessed by the highly priced 'Basmati' (scented) as well as 'Common' (non-scented) rice variety of Indian origin. Rice volatiles were isolated by distillation-extraction technique and analyzed by thin-Layer chromatography and gas-liquid chromatography techniques. Considerable similarities were noticed in the qualitative composition of volatiles of both the rice varieties. The aroma component(s) having characteristic 'Basmati' odour in scented rice appeared to be polar in nature and present in minute amounts.

Rice (Oryza sativa Linn) is a major cereal crop of India available in over 5,000 varieties which differ with respect to the size, texture, glutinous nature, aroma and cooking quality<sup>1</sup>. The different varieties are priced according to their qualities and are suited for various culinary purposes.

In India, there are two major scented rice varieties i.e. 'Basmati' cultivated in North India and Andhra Pradesh and "Ambemohor' cultivated in Maharashtra, which are highly priced for their characteristic flavour and cooking quality. Though the cooking qualities of common Indian rice varieties have been exhaustively studied<sup>2</sup>,<sup>3</sup>, no attempt has so far been made to characterise the volatiles of these scented rice varieties. Studies on the volatiles of cooked rice<sup>4</sup>,<sup>5</sup>, rice bran<sup>6-8</sup>, unprocessed rice<sup>9,10</sup>, polisehd rice<sup>11</sup> and stale flavour of cooked rice<sup>12</sup> of different origin have, however, been reported. In the present work, an attempt has been made to characterise the volatiles of 'Basmati' (scented) and a 'Common' (non-scented) rice of Indian origin.

#### Materials and Methods

Samples of polished 'Basmati' (scented) rice and

'Common' (non-scented) variety were procured from Delhi market.

*Extraction:* Distillation-extraction technique as described by Likens and Nickerson<sup>13</sup> was adopted for the isolation of volatile flavour components of rice. Isopentane (A.R.,) was used as the extracting solvent. Three Kilogram rice in twelve batches, each of 250 g was used, Prior to extraction, each batch was washed with water, transferred to a two-necked 2 L distillation flask connected to Nickerson-Likens's apparatus. Rice and distilled water in the ratio of 1:1.6 was added to the flask heated at 70°C for 10 min and steam was introduced to cook the mass. The flow of steam was continued for 40 min to drive off the volatiles. The extract thus obtained was concentrated under a slow stream of nitrogen gas and the yield was determined.

Thin-layer chromatography: Preparative silica gel G plates (20 cm  $\times$  20 cm) were prepared according to the method described elsewhere<sup>14</sup>. The aroma extracts, 30 to 35 mg in diethyl ether (5 per cent solution) were applied as a band, each 6 cm long, on the same plates which were allowed to develop at 23°C in a chamber containing petroleum ether (b.p. 40°-60°C : diethyl

ether :: 90:10 (v/v) as solvent. After the development and subsequent removal of solvent from the plates at room temperature, odour test of the separated components was done on the plate by a panel of four members, according to the method described by Bandyopadhyay *et al.*<sup>15</sup> The separated bands were then located successively by viewing under ultra violet lamp (254 nm), exposing to iodine vapours and finally by spraying with 0.2 per cent alcoholic solution of phosphomolybdic acid (PMA), followed by heating at 110°C for 10 min.

Gas liquid chromatography: The aroma concentrates of 'Basmati' and 'Common' rice were analysed by g.l.c. using BARC model gas chromatograph equipped with a flame ionisation detector and a glass column (0.625 cm o.d.  $\times 180$  cm) packed with 10 per cent carbowax 20 M supported on acid washed chromosorb W (60-80 mesh); the carrier gas was nitrogen with a flow rate of 40 ml/min., the temperature of the column and that of injection port was maintained at 150°C and 170°C respectively. Authentic samples of methanol, ethanol, acetaldehyde, propionaldehyde, butanol, pentanal, hexanal and hexanol were used for tentative identification. The aroma concentrates were also analysed by g.l.c. using Shimadzu GC 4A gas chromatograph equipped with a thermal conductivity detector and a dual column of stainless steel (3 mm o.d.  $\times$  3 m) packed with 20 per cent diethylene glycol succinate supported on acidwashed chromoscorb P (80-100 mesh); the flow rate of the carrier gas, helium, was maintained at 30 ml/min; the temperature of the column, the injection port and the detector oven was maintained at 160, 200 and 200°C, respectively. The filament current was 200 mA, at an attenuation of 1. The charateristic odour notes of the eluted fraction were subjectively evaluated by sniffing test at the column exit by a panel of four expert judges.

### **Results and Discussion**

The yield of total volatiles of the flavourful 'Basmati' and 'Common' rice varieties was found to be 28 to 30 ppm and 24 to 26 ppm respectively. It is interesting to

Table 1. description of odour notes and observation of changes in colour of tlc sep arated components of basmati (a) and<br/>common (b) rice volatiles on exposure to u.v. lamp, iodine vapour and on spraying with 0.2% pma solution

Band	Odour notes		Under U.V. lamp			Iodine vapour			Spray with 2% PMA solution				
No.	Value	Odour	Inte (A)	ensity (B)	Colour*	Inte (A)	ensity (B)	Colour	Inter (A)	nsity (B)	Colour	Inte (A)	ensity (B)
1	0.93	Sulphury	+++	+++	Strong blue	+++	+++	Dark brown	+++	+++	Blue	+++	+++
2	0.87	Sulphury	+++	+++	Strong green	+++	+++	—	_		Blue	+++	+ + +
3	0.67	Sulphury	++	++	Weak light green	+	+		—	_	-	_	_
4	0.57	Sulphury	+	+	-	-		Brown	+	+	Pink	+	+
5	0.50				Weak light blue	+	+	Faint grey	+	+	Light grey	+	+
6	0.47				Weak light green	+	+	—	_	_		_	_
7	0.43	Cooked rice	+ +	++	Strong blue	+ +	+ +	Brownish	+ <del>+</del> +	+ + +	Green	+ +	+ 🕈
8	0.40	Cooked rice	++	++	Strong blue	+ +	+ +	Brownish black	+++	+ + +	Faint red	+	+
9	0.37				Weak light green	+	+	Faint y <b>e</b> llow	+	+	Blue	+	+
10	0.27				_	—	—	_	—	_	Faint brown	+	+
11	0.13	Sulphury	+	+	Weak light green	+	+	Brown	+	+	Dark blue	++	+ +
12	0.067	Basmati	+ + +	_	Strong * green	+++	+ +		_		Light brown	+	+
13	0.033				Weak light blue	+	+	Brown	+ -	++	Dark blue	++	++

note that, although the difference between the yields for the two varieties was insignifican:, the levels in both the cases were six times higher than those reported for Japanese rice varieties<sup>5</sup>. This, could possibly be attributed to the varietal differences, as well as to the effect of other factors like soil, climate, etc. which are known to influence the composition of a crop.

The thin layer chromatograms of the aroma concentrates are shown in Fig. 1. The  $R_f$  values of the t.l.c. separated constituents, their odcur notes and response to different detecting agents such as U.V. light, iodine vapours and PMA reagent are summarised in Table 1. It can be seen that both the aroma concentrates of 'Basmati' and 'Common' rice varieties show somewhat similar pattern with respect to the qualitative composition, with minor difference in the intensity of the fluorescence associated with band No. 12. Further, a new band No. 4 responded to iodine vapours by forming a brown coloured band which could not be located when viewed under U.V. lamp. The strong fluorescent bands-No. 2 and No. 12, in both cases, were not affected by exposing to iodine vapour. On spraying with PMA solution followed by heating for 10 min at 110°C, different colour-

### SOLVENT FRONT



Fig. 1. TLC separation of aroma concentrates of 'Basmati' (A) and 'Common' (B) rice on a silica gel plate using petroleum ether-diethyl ether (90:10) as developing solvent.



Fig. 2. Gas chromatogram of volatile components of 'Basmati' (-----) and 'Common' (.....) rice.



ed bands were observed (Table 1). It is interesting to note that the aroma components of the respective rice volatiles have similar odour notes except in the case of scented rice which had the characteristic 'Basmati' odour associated with the band having  $R_f$  value 0.067 (Table 1). The relatively low  $R_f$  value of this compound(s) indicates that the compound(s) responsible for 'Basmati' odour may be polar in nature.

The gas chromatographic separation of 'Basmati' and 'Common' rice volatiles is shown in Fig. 2. It appears that the 'Basmati' rice volatiles are richer in hexanol, propionaldehyde and acetaldehyde as compared to the 'Common' rice volatiles. The above compounds have also been reported in other varieties of rice<sup>10</sup>. Both the aroma concentrates resolved into 21 components which appeared to be similar in nature, but varied to some extent in their quantitative composition. Fig. 3 (A and B) represents the aromagram of the scented and non-scented rice volatiles. In both the aroma concentrates the separated components gave rise to similar odour notes, except in the case of scented rice, where the characteristic 'Basmati' odour was detected in the minor component(s) having retention time ca 2.5 min. The present g.l.c. studies using packed columns reveal that the "character impact" compound(s) of the scented rice having 'Basmati' odour are present in very minute concentrations, which, though sufficient enough for



Fig. 3. Gas chromatogram of aroma concentrate of 'Basmati' (A) and 'Common' (B) rice and details of the odour notes of the components eluted from the column of Shimadzu gas chromatograph.

subjective evaluation, are not distinctly identificable. Perhaps, it/they may belong to those class of volatile polar compounds having very low odour threshold values.

### Acknowledgement

The authors are indebted to Dr. G. B. Nadkarni, of B.A.R.C. for permitting to carry out the work in the Division. They also thank Shri B.Y.K. Rao, Shri A. S. Gholap and Shri G. M. Tewari of the Flavour Chemistry Section for their assistance. One of the authors (N.R.) is thankful to the C.S.I.R., New Delhi, for awarding a Junior Research Fellowship.

### References

- Vachhani, M. V., Butany, W. T. and Nair, C.P.K., A tentative commercial classification of rice. *Rice Newsletter*, 1962, 10, 15.
- Baldev, S., Juneja, P. and Kawatra, B. L., A study on the cooking quality of different varieties of Punjab rice. Fd Farm. Agric. 1977, 9, 80.
- Dutta, L. and Barua, J. N., Nutrient composition of glutinous and non-glutinous rice varieties grown in Assam. *Indian J. agric. Sci.*, 1978, 48, 610.
- Ayano, Y. and Furuhashi, T., Volatile carbonyl compounds of cooked rice. Chiba Daigaku Engeigakubu Gakujutsu Hokoku, 1970, 18, 53.

- Izumi, Y., Tetsuya, Y., Mikio, W., Hidemasa, S. and Tsutomu, M., Volatile flavour components of cooked rice. *Agric. biol. Chem.*, 1978, 42, 1229.
- Mitsuda, H., Yasumoto, K. and Iwami, K., Analysis of volatile components in rice bran. Agric. biol. Chem., 1968, 32, 453.
- Fujimaki, M., Tsugita, T. and Kurata, T., Studies on the volatile components of rice bran. I. Fractionation and identification of volatile acids and phenols in the steam distillate of rice bran. Agric. biol. Chem., 1977, 41, 1721.
- Tsugita, T., Kurata, T. and Fujimaki, M., Studies on the volatile components of rice and rice bran-II. Volatile components in the steam distillate of rice bran: Identification of neutral and basic compounds. Agric. biol. Chem., 1978, 42, 643.
- Bullard, R. W. and Holguin, G., Volatile components of unprocessed rice (Oryza sativa L.). J. agric. Fd Chem., 1977, 25, 99.
- Legenore, M. G., Dupuy, H. P., Ory R. L. and McIIrath, W. O., Instrumental analysis of volatiles from rice and corn products. J. agric. Fd Chem., 1978, 26, 1035.
- Obata, Y. and Tanaka, H., The phytolysis of L-cysteine and L-cystine. Formation of the flavour of cooked rice from L-cysteine and L-cystine. Agric. biol. Chem., 1965, 29, 191.
- Yasumatsu, K., Moritaka, S. and Wada, S., Volatile carbonyl compounds of cooked rice. Agric. biol. Chem., 1966, 30, 478.
- 13. Likens, S. T. and Nickerson, G. B., Detection of certain Hop oil constituents in brewing products. Am. Soc. Brewing Chemists Proc. 1964, 5.

 Chakrabarty, M. M. Bandyopadhyaya, C., Bhattacharya, D. and Gayen, A.K., Detection of adulteration of fats by thinlayer chromatography of trisaturated glycerides-I. Detection of hydrogenated groundnut oil, tallow and mahua (mowrah) oil in butter fat (ghee). J. Chromatog., 1968, 36, 84.  Bandyopadhyay, C., Srirangarajan, A. N. and Sreenivasan, A., Studies on flavour components of onion (*Allium cepa*). Thin-layer chromatographic investigation of onion oil. J. Chromatog., 1970, 47, 400.

### Studies on Lysine Enriched Plasteins from Oilseed Proteins

### N. S. SUSHEELAMMA

Discipline of Biochemistry and Applied Nutrition, Central Food Technological Research Institute, Mysore-570013, India

#### Manuscript received 5 February 1982; revised 2 July 1982

Lysine enriched platteins have been prepared from groundnut and sesame protein using N- $\in$ -cbz-lysine methyl ester and also enzymatic hydrolyzates of casein or soybean protein as a source of lysine peptide. Plasteins obtained with N- $\in$ -cbz-lysine methyl ester had yields of around 40% for sesame and 70% for groundnut. Lysine content was 11-13% for groundnut and 16-19% for sesame plasteins. Those obtained with lysine peptides as a source of this amino acid had high yields (75-85%) and moderate lysine content (6-6.5% for groundnut and 7-9% for sesame plasteins.)

Groundnut (Arachis hypogaea) and sesame (Sesamum indicum) are valuable oilseeds containing about 50 per cent fat and nutritional studies on defatted meals have shown them to be good sources of protein, but for some limiting essential amino acids. Sesame protein is low in lysine<sup>1-5</sup> while groundnut protein is low in lysine and also methionine<sup>6-11</sup>. The problems encountered<sup>12</sup> during direct addition of limiting amino acids to achieve nutritional adequacy are overcome by covalent attachment and the application of plastein reaction for this purpose is well known<sup>13</sup>. Soy plasteins have been enriched with methionine<sup>14-16</sup> and microbial plasteins with lysine, methionine and tryptophan<sup>17</sup>. Preparation of lysine enriched plasteins from groundnut and sesame using lysine ester and also lysine peptides have been studied and the results are reported in this paper.

### Materials and Methods

Lysine monohydrochloride,  $N \in -cbz$ -lysine methyl ester, trinitro benzene sulfonic acid and the enzymes used in these studies were obtained from Sigma Chemical Co. Other chemicals and reagents used were of analytical grade. The oil seeds were purchased from the local market.

**Preparation of defatted flours:** Groundnut seeds were dried at 35-40°C for 2 hr, decuticled, flaked and solvent extracted in a soxhlet apparatus with petroleum ether (B.P. 40-60°C) to remove the fat. The residue was air dried and powdered in an Apex grinder to get groundnut flour. Sesame seeds were soaked in water for 10-12 hr at room temperature (22-25°C), drained and dehusked by rubbing over a gunny bag. Seeds were air dried and winnowed/aspirated to remove the husk, flaked, defatted and powdered to get the flour. Soybean seeds were dehusked, split and flaked after adjusting the moisture to 10-12 per cent, then defatted and powdered to get the flour.

**Preparation of proteins:** Groundnut, seasme or soybean flour was extracted with 0.01 N NaOH (pH of the dispersion was around 9.5) with a meal to solvent ratio of 1:10 for 4 hr at 5-7°C and centrifuged at 10,000  $\times$ G for 15 min. The extraction was repeated again with a contact time of 2 hr. The combined supernatant was adjusted to pH 4.6 and the precipitated protein centrifuged at 3000  $\times$ G for 15 min. It was then dispersed in water and adjusted to the desired pH with dilute acid or alkali. Amul casein was powdered (to about 80 mesh), washed with water and dispersed.

**Preparation of hydrolyzate:** Aqueous dispersions of proteins (1-1.5 per cent) were adjusted to pH 1 to 1.5 for peptic hydrolysis and to pH around 8.0 for tryptic and chymotryptic hydrolysis. Hydrolysis was carried out at 37°C, for 24 hr with an enzyme to protein ratio of 1:100, then they were neutralized and concentrated by flash evaporation (40°C).

Preparation of plasteins: They were prepared from different protein hydrolyzates, hydrolyzates of groundnut and sesame along with  $N \in -cbz$ -lysine methyl ester at three levels (100, 150 and 250 mg/400 mg of hydrolyzate) and also from combinations (1:1 w/w mixtures) of groundnut or sesame protein hydrolyzates along with those of casein or soy protein. Hydrolyzates concentrated to 25-30 per cent solids content were reincubated at pH 5.0 (Enzyme to protein ratio is 1:100) for 24 hr at 37°C. (a drop of chloroform or toluene was added as a preservative). Then they were diluted to three times their original volume with water and precipitated with ethanol at 70 per cent concentration, centrifuged at  $3000 \times G$  for 10 min washed with ethanol and dried.

The total and TCA (trichloracetic acid) precipitable nitrogen of proteins and plasteins was determined according to the microkjeldahl procedure<sup>18</sup>, and lysine according to Hall *et al.*<sup>19</sup> using trinitrobenzene sulfonic acid (TNBS). In the samples containing  $N-\in$ -cbz-lysine, the carbobenzoxy group was removed according to Merrifield and Wooley<sup>20</sup> and lysine estimated using TNBS.

### **Results and Discussion**

The plastein reaction has usually been described as a reversal of normal proteolysis and insolubility of the product in TCA or 70 per cent ethanol<sup>21,22</sup>, has been considered to be an index of the formation of plastein. As peptic hydrolyzates are commonly used for this reaction, initial experiments were carried out with peptic hydrolyzates of groundnut protein. Reincubation with pepsin, chymotrypsin and papain were carried out under similar conditions. The yield of 70 per cent ethanol precipitable material was more with pepsin-pepsin plastein (40 per cent) than with other plasteins which varied between 25 and 30 per cent.

Since trypsin has been reported to be poor in producing plastein at any pH value<sup>23</sup>, whether there would be any difference between tryptic and peptic hydrolyzate when used in the preparation of plastein was determined. Trypsin-pepsin (trypsin for hydrolysis and pepsin for plastein formation) and pepsin-pepsin plasteins were prepared separately from groundnut protein and casein under similar conditions. Results shown in Table 1 indicate that the yield of trypsin-pepsin plastein was higher in groundnut protein while that of pepsin-pepsin plastein was better in casein. The total nitrogen remained the same after 24 and 48 hr of reincubation, but the TCA precipitable nitrogen decreased after 48 hr of reincubation as compared to that at 24 hr. Reincubation for 24 hr was found satisfactory for both the protein hydrolyzates.

Whether hydrolysis with specific enzymes or at different pH values would have any effect on the yield of plastein was also tested. Chymotryptic, tryptic or peptic hydrolyzates was reincubated with pepsin under similar conditions. The yield of plasteins and their lysine contents are shown in Fig. 1. The yield of chymotrypsin-pepsin and trypsin-pepsin plastein was lowest (25-26 per cent) in the case of casein, moderate in the case of soybean protein (50 per cent) and high with groundnut protein (80 per cent). Trypsin-pepsin plastein from sesame was about 26 per cent. The lysine content of plastein was greater than that of protein in casein and soybean protein. The increase in lysine was marginal with groundnut and sesame plasteins. The yield of plasteins and the lysine contents were comparable with pepsin-pepsin plastein from all these proteins.

Experiments were also carried out to test the possibility of using enzymatic hydrolyzates of casein or soybean as a source of lysine peptide (as they are rich sources of lysine), along with groundnut or sesame protein hydrolyzate, to obtain lysine enriched plasteins. The results shown in Fig. 2.1 indicate that plasteins containing the tryptic hydrolyzate of casein gave lower yields but their lysine content was high. Peptic hydrolyzates of groundnut protein showed a similar trend. Both the yield and

	TABLE 1. EFFECT OF REINCUBATION TIME ON PLASTEINS									
	Dai shadar	T	rypsin-pepsin	plastein	Pepsin-pepsin plastein					
	Reincubation – time	Yield	Total N	TCA Precipitable N	Yield	Total N	TCA Precipitable N			
	(hr)	(%)	( <sup>0</sup> /)	(%)	(%)	(%)	(%)			
Groundnut protein	24	45	16	13	40	14	11			
hydrolyzate	48	50	14	18	45	15	8			
Casein	24	28	15	5	33	16	14			
hydrolyzate	48	35	14	7	45	16	12			

Proteins (0.5 g) hydrolyzed with trypsin or pepsin for 24 hr were reutralized, concentrated and reincubated with pepsin around pH 5 for 24 and 48 hr at  $37^{\circ}$ C. 70% ethanol precipitable plasteins from these were analysed. Values represent averages of three independent experiments.



Fig. 1. Yield and lysine contents of plasteins from proteins

Proteins (0.5 g) were hydrolysed with different enzymes (Enzyme to protein ratio is 1-100) at  $37^{\circ}$ C for 24 hr, neutralized, concentrated and reincubated with pepsin at pH 5 for 24 hr. Proteins and 70% ethanol precipitable plasteins were analysed for lysine. Values represent averages for three independent experiments.

1.1 Groundnut protein, 1.2 Casein, 1.3 Soybean protein, 1.4 Sesame protein.

a) Chymotryptic hydrolyzate, b) Tryptic hydrolyzate,c) Peptic hydrolyzate.

lysine content were high with chymotryptic hydrolyzate of groundnut protein along with peptic hydrolyzate of casein followed by plastein formation with pepsin. When soybean hydrolyzates (Fig. 2.2) were used along with groundnut protein hydrolyzate, the yield of plastein was greater than 60 per cent in all the samples and relative increase in lysine varied between 1.5 and 2.1 (range being comparable to that obtained with groundnut protein and casein). Tryptic hydrolyzate of groundnut protein along with peptic hydrolyzate of soybean gave rise to plasteins with high yield and also lysine content.

Sesame protein hydrolyzates along with casein hydrolyzate (Fig. 2.3) gave rise to plasteins in about 40-50



Proteins (0.3 g) were hydrolysed with different enzymes (Enzyme to protein ratio is 1:100) at  $37^{\circ}$ C for 24 hr, neutralized and concentrated. Mixtures of different hydrolyzates were reincubated with pepsin at pH 5 for 24 hr. 70% ethanol precipitable plasteins were analysed for lysine. Values are averages of three experiments.

a, a', A, A' represent chymotryptic hydrolyzates of groundnut, sesame, casein and soybean protein respectively.

b, b', B, B' represent tryptic hydrolyzates of groundnut, sesame, casein, and soybean proteins respectively.

c, c', C, C' represent peptic hydrolyzates of groundnut, sesame, casein and soybean protein respectively.

per cent yield but the lysine content was greater (8-10 per cent) than that obtained with a mixture of groundnut protein and casein hydrolyzate. Chymotryptic hydrolyzate of casein and peptic hydrolyzate of sesame gave rise to plasteins with good yield and also lysine content. When soybean protein hydrolyzate was used in place of casein hydrolyzate (Fig. 2.4), the yield of plastein was slightly higher (50-65) per cent instead of 40-50 per cent), but the lysine content was slightly less. The relative increase in lysine varied between 2.1 and 2.3, Chymotriptic hydrolyzate of sesame with tryptic hydrolyzate of soybean gave rise to plasteins with higher yield and also lysine content.

The trypsin-pepsin plasteins were chosen from groundnut and sesame (due to their high lysine content) for further studies with lysine ester. At the three levels tested, the yield and lysine contents were higher with 150 mg of the ester (Table 2). The yield was more in case of groundnut plastein (70 per cent) but less with sesame plastein (40 per cent), while the lysine content was higher in sesame plasteins (17-19 per cent) than

Amount of ester (mg)	Yield (%)		Lysiı (%)	in	elative ncrease Lysine
	Groundnut	protein	tryptic	hydrolyzate	
100	7	5	10.6	5	3.53
150	7	0	13.6	5	4.53
250	e	55	12.2	2	4.06
	Sesame p	rotein	tryptic	hydrolyzate	
100	3	2	16.0	)	5.33
150	4	10	19.2	2	€.40
250	4	ю	17.2	2	5.73

TABLE 2. PREPARATION OF PLASTEINS WITH N- $\in$ -cbz lysine methyl ester

25-30% tryptic hydrolyzate of groundnut or sesame protein (400 mg) was mixed with 100, 150, and 250 mg of N- $\in$ -cbz-Lysine methyl ester and reincubated with pepsin at 37°C for 24 hr at pH 5.0. Lysine content of 70% ethanol precipitable plasteins was determined and relative increase in lysine expressed as the ratio of lysine in plastein to that of protein. Values represent averages of three independent experiments.

that of groundnut plasteins which was around 11-13 per cent.

The lysine content of sesame plasteins enriched with either lysine peptides or with N-∈-substituted lysine ester was higher than those obtained with groundnut plasteins under similar conditions, but the yield was lower. Groundnut plasteins enriched with lysine had comparable values for relative increase in lysine using hydrolyzates of either casein or soybean protein as a source of lysine peptide. Lysine enriched sesame plasteins had higher lysine with casein hydrolyzates than with soybean protein hydrloyzates. For both these proteins (groundnut and sesame), the lysine contents of plasteins with  $N-\varepsilon$ -cbz-lysine esters were nearly twice that of plasteins obtained with lysine peptides as a source of this amino acid.

These studies indicate that plasteins enriched with lysine could be prepared using either esters of lysine or lysine peptides (as a source of this amino acid) from proteins of vegetable or animal origin. The relative increase in lysine of the plasteins is comparable to or slightly greater than that obtained with proteins of photosynthetic microorganisms<sup>17</sup>. Fortification of proteins or peptides with essential amino acids through the application of plastein reaction has the dual advantage of removing unwanted colouring or flavouring materials associated with the protein and eliminating the necessity for safety evaluation or acceptability of the final product, when obtained through modification with enzymes whose properties have been well studied. This type of plastein production may be economically feasible only on a small scale, but still they may find use as therapeutic foods when used with other proteins or protein hydrolyzates. Factors which influence the yield and relative increase in lysine of plasteins are not fully known. Probably among others, the amino acid composition of the proteins may play an important role and thus conditions best suited may have to be determined with each protein individually.

### Acknowledgement

Thanks are due to Shri M.V.L. Rao for his valuable suggestions and to Dr. M. R. Raghavendra Rao for his encouragement.

#### References

- 1. Evans, R. J. and Bardmer, S. L., Nutritive value of some oil seed proteins. *Cereal Chem.*, 1967, 44, 417.
- Cuca, M. and Sunde, M. L., Amino acid supplementation of a sesame meal diet. *Poultry Sci.*, 1967, 46, 1512.
- Taskar, P. K., Joseph, K., Rao, D.R., Rao, N. M., Indiramma K., Swaminathan, M., Sreenivasan, A. and Subrahmanyan, V. Effect of feeding peanut protein fortified with limiting essential amino acids on growth and composition of liver, blood, carcass and certain liver enzymes in rats. *Annal Biochem. expt. Med.*, 1963, 23, 279.
- Joseph, K., Supplementary values of the proteins of Bengal gram and sesame to groundnut proteins. Food Sci., 1958, 7, 186.
- Kik, M. C., Effect of amino acid supplements, votamin B<sub>12</sub> and buffalofish on the nutritive value of proteins in sesame seed meal. J. agric. Fd Chem., 1960. 8, 327.
- Duckworth, J., Woodham, A. A. and McDonald, I., The assessment of nutritive value in protein concentrates by the gross protein value method. J. Sci. Fd Agric., 1961. 12, 407.
- Black, A. E. and Cuthbertson, W. F. J., The limiting amino acids of groundnut flour and meal as the sole protein source for the rat. Proc. Nutr. Soc., London, 1963, 22, XX1.
- Ellinger, G. M. and Boyne, E. B., Limiting amino acids in cereal; groundnut—cereal diets for chicks. *Proc. Nutr. Soc.*, *London.*, 1963, 22, XXIII.
- Milner, C. K. and Carpenter, K. J., The limiting amino acids of groundnut flour and meal as the sole protein source for the chick. *Proc. Nutr. Soc.*, *London.*, 1963, 22, XXII.
- Anderson, J. O. and Warnick, R. E., Amino acid deficiencies in peanut meal and corn peanut meal rations. *Poultry Sci.*, 1965, 44, 1066.
- 11. Carpenter, K. J., McDonald, I. and Miller, W. S., Protein quality of feeding stuffs. Brit. J. Nutr., 1972, 27, 7.
- Lis, M. T., Crampton, R. F. and Mathews, D. M., Effect of dietary changes on intestinal absorption of L-methionine and L-methionyl-L-methionine in the rat. Brit. J Nutr., 1972, 27, 159.

- Fujimaki, M., Arai, S. and Yamashita, M., in Food Proteins: Improvement through Chemical and Enzymatic modification. R. E. Feeney and J. R. Whitaker (Ed.). American Chemical, Society, Washington D. C. 1977. 156.
- Yamashita, M., Arai, S., Aso, K. and Fujimaki, M., Location and state of methionine residues in a papain-synthesised plastein from a mixture of soybean protein hydrolyzate and L-methionine ehtyl ester. *Agric. biol. Chem.*, *Tokyo*, 1972, 36, 1353.
- Arai, S., Yamashita, M., and Fujimaki, M., Plastein reaction and its applications. *Cereal Foods World*, 1975, 20, 107.
- Yamashita, M., Arai, S., Tsai, S. J. and Fujimaki, M., Plastein reaction as a method for enahncing sulfur containing amino acid level of soybean protein. J. agric. Fd Chem., 1977, 19, 1151.
- Arai, S., Yama hita, M. and Fujimaki, M., Enzymatic modification for improving nutritional qualities and acceptability for proteins extracted from photosynthetic micro organism. Spirulina maxima and Rhodopseudomonas capsulatus. J. Nutr. Sci., Vitamirol; 1976, 22, 447.

- Cereal Laboratory Methods, American Association of Cereal Chemists. 7th Ed. 1962, 46, 13.
- Hall, R. J., Trinder, N. and Givens, D. I., Observations on the use of 2-4-6 trinitrobenzene sulfonic acid for the determination of available lysine in anim! protein concentrates. Analyst., Lond., 1973, 98, 673.
- Merrifield, R. B. and Wooley. D. W., The synthesis of Lseryl-L-histidyl-L-Leucy1-L-valyl-L-glutamic acid, a peptide with streptogenin activity. J. Am. chem. Soc., 1956, 78, 4646.
- Borsook, A. in Advances in Protein Chemistry. Anson, M. L., Kenneth Bailey and John, T, Eosal (Ed). Academic Press, Inc., New York, 1953, Vol. 8, 127.
- Fujimaki, M., Utaka, K., Yamashita, M. and Arai, S., Production of high quality plastein from crude single cell protein. Agric. biol. Chem., Tokyo., 1973, 37, 2303.
- Yamashita, M., Tsai, S. J., Arai, S., Kato, H. and Fujimaki, M., Enzymatic modification of proteins in food stuffs. Part V, Plastein yields and their pH dependence. Agric. biol. Chem., Tokyo, 1972, 35, 86.

### Physico-chemical and Respiratory Changes in Dwarf Cavendish Variety of Bananas During Growth and Maturation

PAUL THOMAS<sup>•</sup>, PUSHPA PAUL<sup>\*</sup>, N. NAGARAJA AND V. B. DALAL Central Food Technological Research Institute Mysore-570 013, India

Manuscript received 14 December 1981; revised 26 July 1982

'Dwarf Cavendish' Bananas exhibited a concave growth curve and took 130 days to reach full maturity after the inflorescence emergence. Pulp to skin ratio increased from an initial value of 0.25: 1.0 to 1.9: 1.0 at full maturity. Fruit weight increase was maximum during the final 4 weeks of growth. Dry matter and starch content in the pulp increased consistently up to 100 cays of growth after which there was negligible change in starch whereas dry matter Skin tissues showed less marked changes in dry matter and starch. Soluble sugars in pulp and skin decreased. Titratable acids, ascorbic acid and nitrogen in the pulp tended to increase remained at low level during growth. from 43 day onwards, while in the skin no perceptible changes were noticed throughout the growth period. Total phenols, flavonols and leucoanthocyanidin content showed a continuous fall as the fruit matured. Fruit respiration was maximum at the early stages of development, fell rapidly thereafter and remained steady during the later stages of maturation. Results indicate that the biochemical status of bananas does not change appreciably during the final stages of maturation and, therefore, a combination of chronological age and pulp to skin ratio could be used as an index of maturity at harvest.

Although a considerable amount of work has been done on the physiological and biochemical changes occurring in harvested bananas, report on such changes during growth and maturation while the banana bunch is attached to the plant is rather scanty. Barnell<sup>1</sup> has described changes in carbohydrates in the pulp and peel of 'Gros Michel' bananas during growth, while Steward et al.<sup>2</sup>, have reported the changes in the alcohol solube nitrogen. Buckley<sup>3</sup> studied the synthesis and accumulation of dopamine in the peel of developing banana fruits. Lodh et al.,<sup>4</sup> reported changes occurring in some of the chemical constituents in pulp tissues during growth of 'Dwarf Cavendish' bananas.

The present studies were undertaken with the objective

<sup>\*</sup>Present address: Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Bombay-400 085, India.

of defining the maturity standards for the 'Dwarf Cavendish' bananas, the principal cultivar grown in India for export as well as for internal trade, and to correlate the biochemical status of the fruit with the developmental stages or harvest maturity.

### Materials and Methods

About fifty banana plants were tagged at the time of inflorescence emergence or 'Shooting' in a plantation near Mysore to ensure selection of fruits with uniform chronological age. All observations were made on detached banana fingers harvested at weekly intervals from the time of shooting. Pulp and skin tissues were macerated in a waring blendor and freeze dried. The freeze dried samples were used for the estimation of starch and phenolic constituents. All other chemical analyses were carried out on fresh samples. Two fingers from each of the five bunches were removed at a time, and the values reported are the mean of ten fingers.

Linear measurement and fruit volume: Fruit length was measured to the nearest millimeter by means of callipers and the circumference measured at the middle portion of the fruit. Fruit volume was determined by displacement of water in a measuring cylinder.

*Pulp to skin ratio:* Pulp and skin tissues were separated and the ratio was calculated by dividing the fresh weight of the pulp by fresh weight of the skin of individual fruits.

Fruit respiration: Respiration of individual detached fingers was determined according to the procedure of Loomis and Shull<sup>5</sup>. The cut surface of the finger stalks were smeared with vaseline before placing them in the respiration chamber.  $CO_2$ -free air was passed through the chamber at a rate of 50-60 ml per minute and the  $CO_2$  evolved by the fruit during the two hour experimental set up, was trapped in Petten Koffer tubes containing 0.1 M barium hydroxide. The  $CO_2$  evolved was calculated by titration of the excess alkali against 0.1 N HCl, and values expressed as mg/ $CO_2$ /per kg fresh weight per hour.

Chemical constituents: Starch was determined by diastase method with subsequent acid hydrolysis as outlined in AOAC<sup>6</sup>. Reducing and non-reducing sugars were determined using modified Somogyi's method as described by Hodge and Davis<sup>7</sup>. Ascorbic acid was estimated by the visual titrimetric method using 2,4dichlorophenol indophenol dye<sup>6</sup>. For titratable acids and pH, fresh tissue was homogenized in waring blender with distilled water and the pH of the slurry was determined. An aliquot of the clear supernatant was titrated against 0.1 N NaOH to pH 8.0 and acidity expressed as per cent malic acid. Moisture was determined by drying the finely divided fresh tissues at 75°C to constant weight. Total nitrogen was estimated by the Kjeldal method<sup>6</sup>.

Total phenol content was estimated colorimetrically by the Folin-Denis method<sup>8</sup>. The vanillin- $H_2SO_4$ method for total flavonols and n-butanol-HCl method for total leucoanthocyanins were adopted as described by Swain and Hills<sup>8</sup>. Areca catechin was used as the standard for total phenols and total flavonols, while arecanut monomeric leucocyanidin served as the standard for total leucoanthocyanin estimations.<sup>9</sup> For chlorophyll, peel tissue was repeatedly extracted with cold acetone and the amount calculated according to Maclachlan and Zalik<sup>10</sup> based on the specific absorption coefficients for chlorophyll a and b.

### Results

Changes in fruit weight, and the ratio between pulp to skin from the time of inflorescence emergence upto 130 days of growth are shown in Fig. 1. The fruits exhibited a concave growth curve in terms of whole fruit weight increments and fruit volume. The weight of whole fruit, pulp and skin increased for the entire period over which the bunch remained attached to the plant. A week after inflorescence emergence, the relative proportion of pulp and skin in a single fruit was 21 and 79 per cent respectively which changed to 65 and 35 per cent respectively on the 130th day. The pulp to skin ratio increased from an initial value of 0.25: 1.0 to 1.9: 1.0 when fruits reached maximum size. The angularities



Fig. 1. Changes in fruit weight, and pulp to skin ratio of 'Dwarf Cavendish' banar.<sup>2</sup> fruit during growth and maturation.



Fig. 2. Cross sectional view of 'Dwarf Cavendish' banana fruits at different stages of growth from the time of inflorescence emergence.

of the fruit, which were very prominent in the early stages of development, gradually disappeared as the fruit advanced in maturity. After 108 days of growth, angularities disappeared more markedly and the fruit showed almost a smooth cross sectional view (Fig. 2).

Dry matter and carbohydrates: The dry matter content in the pulp increased consistently from an initial



Fig. 3. Changes in total dry matter, and starch in 'Dwarf Cavendish' banana fruits during growth.

value of 7.4 per cent, a week after inflorescence emergence, to a maximum of 28.6 per cent at 85 days, after which there was no appreciable change until 100 days (Fig. 3). A consistent fall in dry matter level was observed from 100th day till the fruits reached full maturity when it was 22.7 per cent. A somewhat similar trend but of a lesser magnitude was observed in the skin.

Evidence of starch accumulation in the pulp was noticed 8 days after the inflorescence emergence. Starch accumulation occurred in two stages, the first stage of rapid increase from 15 to 50 days and the second stage of less rapid increase from 50 to 100 days after which starch content showed a slight fall until the 130th day (Fig. 3.).

Total soluble sugars remained consistently low in the pulp during the entire growth period, attaining maximal value of 0.3 per cent at full maturity (Table 1). Reducing sugars were present in higher proportions at the initial stages of growth but fell rapidly thereafter and remained at that level while sucrose content increased gradually as the fruit advanced in maturity. A similar pattern in starch and sugar levels was observed in the skin during growth (Table 2).

Titratable acids and pH: Acidity in the pulp remained relatively constant during the first six weeks and thereafter increased gradually as the fruit matured (Table 1). Acid content in the skin remained relatively low and constant throughout the fruit development (Table 2). pH of the pulp which ranged from 6.0—6.1 in the early stages of growth, decreased to 5.6 at full maturity.

Total nitrogen: Nitrogen content of the pulp was low in the early stages of fruit development, ranging from 0.15 to 0.18 per cent (Table 1) which increased to 0.23 per cent at 43 days and remained at that level until about 100 days. A gradual decrease was noticed during the final stages of fruit maturation. The skin had relatively lower amounts of nitrogenous substances and showed a gradual decrease in nitrogen level as the fruit advanced in maturity (Table 2).

Ascorbic acid: The ascorbic acid level in fruit pulp remained low until 36 days from inflorescence emergence (Table 1). Increased accumulation was observed during the next 2 to 3 weeks and remained somewhat constant until fruits attained full size. Skin contained very low levels and did not show any appreciable changes during maturation (Table 2).

Phenolic constituents: Changes in the phenolic constituents in the developing banana fruit are shown in Fig. 4 and 5. On fresh weight basis, immature fruits showed a very high concentration of total phenols which decreased as the fruit advanced in maturity. Pulp contained higher amounts during the first three weeks after the inflorescence emergence, but thereafter the values fell below that of the skin. Both total flavonols

Days from inflorescence	Moisture	Sugars (% mg/100 g)			Ascorbic acid	Titratable acids. (%)	I H	Total N
emergence	(%)	Reducing	Non- reducing	Total	(mg/100 g)	as malic acid)		(mg/100 g)
8	92.62	0.178	0.020	0.198	4.0	0.19	6.0	180
15	89.76	0.093	0.029	0.132	4.0	0.18	6.0	153
22	87.58	0.054	0.066	0.120	6.1	0.19	6.0	160
29	84.06	0.050	0.060	0.110	5.6	0.17	6.0	168
36	80.01	0.054	0.054	0.108	6.8	0.18	6.1	168
43		0.049	0.082	0.131	8.7	0.18	6.1	231
50	75.25	0.040	0.067	0.107	9.5	0.21	6.1	209
57	74.20	0.040	0.075	0.115	10.1	0.20	6.1	203
64	73.60	0.049	0.060	0.109	9.9	0.21	6.1	239
71	73.02	0.040	0.064	0.104	10.7	0.22	6.1	215
78	72.52	0.040	0.060	0.100	9.8	0.21	6.1	209
85	71.41	0.049	0.062	0.111	10.1	0.25	6.1	233
92	72.52	0.068	0.036	0.104	9.3	0.24	6.1	227
99	72.83	0.056	0.050	0.106	10.2	0.25	5.8	245
107	74.50	0.050	0.066	0.116	10.4	0.25	5.7	185
114	76.40	0.048	0.078	0.126	10.7	0.25	5.8	181
123	78.04	0.051	0.089	0.140	10.1	0.29	5.6	176
130	77.30	0.059	0.246	0.305	10.2	0.25	5.6	160

TABLE 1. CHANGES IN SOME CHEMICAL CONSTITUENTS IN 'DWARF CAVENDISH' BANANA PULP DURING GROWTH AND MATURATION

On fresh wt. basis.

TABLE 2. CHANGES IN SOME CHEMICAL CONSTITUENTS IN 'DWARF CAVENDISH' BANANA SKIN DURING GROWTH AND MATURATION

Days from inflorescence	Moisture	Sugars (% mg/100 g)			Ascorbic acid	Titratable acids (% as	рH	Total N (mg/100 g)	Total chlorophyll
emergence	(%)	Reducing	Non- reducing	Total	(mg/100 g)	malic acid)	рп	(iiig/100 g)	(mg/100 g)
8	92.00	0.210	0.062	0.272	1.4	0.13	5.4	194	37
15	91.65	0.090	0.0 0	0.150	1.7	0.09	5.4	131	51
22	90.97	0.054	0.142	0.196	1.2	0.09	5.4	124	53
29	91.09	0.050	0.147	0.197	1.5	0.09	5.6	100	55
36	90.01	0.070	0.135	0.205	0.8	0.09	5.6	94	54
43	90.00	0.067	0.122	0.189	1.0	0.11	۰.6	118	57
50	91.70	0.062	0.147	0.209	1.4	0.13	5.6	106	54
57	89.20	0.055	0.206	0.261	0.9	0.08	5.6	112	51
64	90.42	0.061	0.199	0.260	0.9	0.09	5.6	86	53
71	90.75	0.049	0.209	0.258	0.82	0.09	5.7	99	57
78	91.01	0.078	0.211	0.289	0.82	0.10	5.9	105	55
85	91.40	0.054	0.230	0.284	1.0	0.11	5.9	100	51
92	91.65	0.052	0.206	0.258	1.9	0.08	6.0	105	49
99	91.77	0.049	0.135	0.184	1.4	0.11	5.5	111	50
107	92.49	0.077	0.187	0.244	1.2	0.14	5.6	94	51
114	91.42	0.071	0.167	0.238	1.2	0.05	5.6	111	47
123	92.07	0.057	0.151	0.208	1.6	0.11	5.6	85	43
130	93.07	0.053	0.144	0.197	1.6	0.15	5.7	86	45
On fresh wt. b	asis								



Fig. 4. Changes in total polyphenols in 'Dwarf Cavendish' banana fruits during growth.



Fig. 5. The total flavonol and leucoanthocyanidin content of 'Dwarf Cavendish' banana fruits during growth.



Fig. 6. Respiratory rate of 'Dwarf Cavendish' banana fruits during growth and maturation.

and total leucoanthocyanidins in pulp decreased in concentration as the fruit advanced in maturity.

Chlorophyll: Total chlorophyll content of the skin was rather low immediately after inflorescence emergence but increased as the fruit matured until about 29 days and remained constant till 107 days (Table 2). However, as the fruit volume increased with advancing maturity, the amount of chlorophyll showed a slight decreasing trend towards the final stages of maturation.

Fruit respiration: A very high  $CO_2$  output was observed in immature fruit which fell rapidly until the 50th day and thereafter remained somewhat constant upto 130 days, when the fruits reached full maturity (Fig. 6).

### Discussion

The 'Dwarf Cavendish' banana fruit exhibited a concave growth curve in terms of volume and fruit weight, which agrees with the observation of Simmonds<sup>11</sup> made for several edible banana varieties. The increase in fruit weight was maximum during the last four weeks of the growth, as much as 35 to 40 per cent increase in weight occurring during this period. This shows that as the fruit harvest is delayed, greater will be the weight of whole bunch, and proportion of edible pulp. However, this will greatly influence the post harvest pre-climacetric life of the fruits. Wardlaw et al.,12 reported that 'Gros Michel' bananas attained a pulp to skin ratio of 1.4:1.0 after 80 days of inflorescence emergence. While our results show that the 'Dwarf Cavendish' fruits took nearly 100 days or more to reach the same ratio. These

differences may be attributed to cultivars and variability in agro-climatic conditions.

Present studies however, indicate that the composition of the fruit of different maturities, harvested during the final 8 weeks of maturation, did not show a correlation to their maturity. The content of starch, the major constituent in the pulp, remained rather constant towards the later half of maturation whereas the dry matter showed a gradual decline from 100 days onward. Barnell<sup>1</sup> reported a considerable fall in dry matter and starch content of 'Gros Michel' bananas with concomitant increase in soluble sugars, while Lodh et al.,4 observed a sudden and drastic decrease in the starch content of 'Dwarf Cavendish' bananas after 70 days of fruit growth until fruit maturity, without any increases in sugars. They suggested that the differences in the amount of starch hydrolysed and sugars formed could be due to a high rate of carbon loss in respiration. However, no respiration data were provided to support this. In the developing fruit, the intensity of respiration gives a general indication of the magnitude of metabolism. The low respiration rates observed in our studies even during the final stages of maturation indicate that changes akin to ripening had not taken place while the fruits were still attacked to the plant.

It is concluded that for bananas, harvest maturity must be defined by quantitative and qualitative means other than their chemical composition. Moreover, the tests must be simple and rapid. A number of physical methods like the number of days after inflorescence emergence, pulp to skin ratio, hardness, colour and odour of the pulp are employed by the banana industry as criteria to determine maturity of the fruit at harvest<sup>13,14</sup> Our results show that a combination of the chronological age of the fruit, i.e. the number of days elapsed from inflorescence emergence and determination of the physiological age by tests of dimensional development of the fruits like the pulp to skin ratio and angularities would give a reasonable index of the maturity of the fruit at harvest, when uniform agronomic practices arc followed.

#### References

- 1. Barnell, H. R., Studies in tropical fruits. VIII. Ann. Bot., 1940, 4, 39.
- Steward, F. C., Freiberg, S. R., Humle, A. C., Hegarty, M. P., Barr, R. and Rabion, R., Physiological Investigations on the banana plant, I & II. Ann. Bot., 1960, 24, 117.
- Buckley, E. H., Dopamine in Banana. In Proceedings of a symposium of the Plant Phenolics Group of North America, United Fruit Company, Norwood. Massachusetts, 1964.
- Lodh, S. G., Ravel, P., Selvaraj, Y. and Kohli, R. R., Biochemical changes associated with growth and development of Dwarf Cavendish banana. *Indian J. Hort.*, 1971, 2i, 38.
- Loomis, W. F. and Shull, A. C., *Methods in Plant Physiology*, McGraw Hill Book Co., Inc., New York, 1937.
- 6. Official Methods of Analysis, Association of Official Agricultural Chemists, Washington, D. C., 1960.
- Hodge, J. F. and Davis, H. A., Selected Methods for Determining Reducing Sugars, U.S.D.A., N.R.R.L., Peoria, Illinois, 1952, 13.
- Swain, T. and Hillis, W. F., The phenolic constituents of Prunus domestica. 1. The quantitative analysis of phenolic constituents. J. Sci. Fd Agric., 1959, 10, 63.
- Govindarajan, V. S. and Mathew, A. G., Polyphenolic substances of arecanut. I. Chromatographic analysis of fresh mature nut. *Phytochem.*, 1963, 2, 321.
- Maclachan, S. and Zalik, S., Plastic structure, Chlorophyll concentration and free Amino Acid Composition of a Chlorophyll mutant of Barley. *Canadian J. Bot.* 1963, 41, 1053.
- Simmonds, N. W., The development of the banana fruit, J. exp., Bot., 1953, 4, 87.
- 12. Wardlaw, C. W., Leonard, E. R. and Barnell, H. R., Studies in tropical fruits. VII. Ann. Bot., 1939, 3, 845.
- Deullin, R., Determination of colour of banana pulp in the pre-climateric phase., Bt.II. int. Inst. Refrig., 1963., 61, 73.
- Deullin, R., and Gane, R., Numerical evaluation of the fullness (maturity) of bananas. *Bull int. Inst. Refrig.*, 1963, 61, 77.

### Instrumental Quality Measures: Development, Standardization and Their Correlation to the Sensory Attributes in Apple\*

S. M. ANANTHAKRISHNA, S. DHANARAJ, M. G. RAMAKRISHNARAJAN\*\* AND V. S. GOVINDARAJAN Sensory Evaluation Discipline, Central Food Technological Research Institute, Mysore-570 013, India

Manuscript received 24 December 1981; revised 9 July 1982;

Instrumental measures to assess 'Red Delicious' apple quality have been developed and standardized using Ottawa Texture Measuring System (OTMS). The OTMS yield point reflecting texture and OTMS juice-volume area reflecting juiciness could be obtained simultaneously in one operation and were well defined to assess quality of apples at different stages of ripening during storage and could be used as indices of apple quality. Any abnormal variations in ripening could be detected if both these instrumental parameters are used. A correlation matrix is worked out between all combinations of instrumental and sensory quality factors studied and their significance discussed. The OTMS yield point and juice-volume area showed highly significant correlation with sensory texture and juiciness respectively.

Quality of apples is influenced by harvesting maturity, transportation and storage conditions. Attempts have been made by earlier workers to develop instrumental methods for apple quality assessment. Hand operated puncture tests have been widely used for measuring apple firmness<sup>1</sup>. This was developed into a mechanical thumb which does not damage the fruit<sup>2</sup>. Bourne<sup>3</sup> studied the puncture test on apples by mounting Magness-Taylor (MT) punches in an Instron and has reported that this test is frequently used as apple quality index, but is not as positive as would be desirable.

A non-destructive method for apple firmness has been suggested by Finney<sup>4</sup> but he used a small number of five untrained panelists to make separate judgements on texture, firmness and toughness. Brennan et al.5 compared puncture tests on whole apples with compression test on cylinders of apple tissue and found that the two techniques gave almost equally high correlation with sensory data on firmness, crispness, coarseness and juiciness using an untrained and uninstructed assessors. Bowman et al.<sup>6</sup> reported significant correlation between shear force to tenderness and crispness and between total moisture content to juiciness. Zaehringer and Hard<sup>7</sup> confirmed the finding of Bowman et al.<sup>6</sup> that shear force values correlated to crispness and tenderness and also to juiciness. Both these workers have used small number of trained panelists but not given any detail of panel training and performance. Against these, Eccher Zerbini<sup>8</sup> in his very recent paper on compression tests of whole 'Golden Delicious' apples in an Instron testing machine has reported that compression parameters are significantly correlated to sensory sweetness and sourness but not with sensory firmness.

Dhanaraj et al.<sup>9</sup> have developed and reported recently an objective sensory evaluation procedure by a descriptive quality profile (DQP) method to assess individual quality attributes in apple. Panel participation in the development of proforma, magnitude of difference discernible and the selection of descriptors for each attribute to clearly define different points on the scale and the training of panelists and their performance for objective and uniform evaluations have been stressed in this paper which are generally not given by other workers mentioned earlier.

Texture profile analysis by Bourne<sup>10,11</sup> using General Foods Texturometer and Instron only define various parameters such as hardness, cohesiveness, adhesiveness, springiness, gumminess, chewiness from force-distance curves showing the complexity of texture property and stresses that these are extremely useful in evaluating the textural quality if correlated with sensory assessments. The paper by Tijskens<sup>12</sup> on texture of 'Golden Delicious' apples using Instron reports that plate compression follows most ideally the textural changes during storage without giving the sensory texture data.

All these instrumental methods have not been com-

<sup>\*</sup>Presented in part at the Second Indian Convention of Food Scientists and Technologists, Central Food Technological Research Institute, Mysore-570013, India (1981).

<sup>\*\*</sup> Present address: Radiation Medicine Centre, Tata Memorial Centre, Parel, Bombay-400 012, India.

prehensively validated by the sensory evaluation study at the same time. Instrumental measurements of these parameters reflecting sensory qualities which have greatest relevance to consumer acceptance is useful and become meaningful when correlated to sensory evaluation data. With this objective, a study was undertaken to develop the instrumental methods which will define the sensorily perceived quality changes in apples for determining its quality and shelf-life during cold storage. The details of the development and standardization of the instrumental methodology, their correlation with sensory data and the range of instrumental values defining quality of apples as objective indices are reported in this paper.

### Materials and Methods

Fresh and cold stored  $(32\pm1^{\circ}F \text{ and } 85-90 \text{ per cent} RH)$  'Red Delicious' apples of three different maturity harvests for four seasons from Himachal Pradesh and Jammu and Kashmir regions, were used.

Physico-chemical methods: Each sample for instrumental analysis consisted of five representative fruits from each treatment lot. Seven millimeter diameter probe MT puncture tester values were recorded for the sample (two readings per fruit) after peeling at either end of the lateral side on the equatorial region. The fruit was cut into two halves at the plane connecting central core and MT tested punctured areas on the fruit. Using a sampling devise (Fig. 1) consisting of a corer and slicer designed for this investigation, standard size apple discs, 2.5 cm diameter  $\times 1$  cm thick, were obtained with both the halves. The disc was placed in between two filter sheets (Whatman No. 4, 15 sq.cm.) which was in turn placed between the flat plates of the compression assembly in Ottawa Texture Measuring System (OTMS)<sup>13</sup>. The apple disc was compressed at a constant rate to a preadjusted clearance between the plates to get the force-distance curve. Fig. 2 gives the operating



Fig. 1. Sampling Devise: Disc size 2.5 cm diam/1 cm thick



Fig. 2. Ottawa Texture Measuring System (OTMS)

Full scale deflection 40 kg. Crosshead speed 50 mm/min Chart paper speed 240 mm/min Plate and plunger clearance 2 mm

conditions of OTMS and sample disc being pressed between the flat plates. The OTMS yield point and maximum force at the first significant break in curve, was computed from force-distance curves. The expressed juice from the apple disc during the deformation in OTMS was absorbed on both the filter paper sheets and the area of spread, OTMS juice-volume area, was immediately marked after the compression cycle. The total area from both the sheets was subsequently measured using Planimeter. Thus OTMS yield point and juicevolume area reflecting texture and juiciness respectively, were obtained simultaneously for the sample (two readings for each fruit). The remaining pieces of each fruit in the sample was pressed out for juice and analysed for brix and titratable acidity (one reading for each fruit). In all, five instrumental measures viz. MT value, OTMS yield point, OTMS juice-volume area, brix and acidity were taken for each sample under each treatment.

Sensory evaluation method: Sensory evaluation of apples under each treatment was carried out by a trained panel of 20 staff members following the DQP procedure.<sup>9</sup> Statistical analysis: The instrumental and sensory evaluation data were analysed by analysis of variance followed by Duncan's new multiple range test<sup>14</sup>. Linear correlation coefficients were worked out between the instrumental measures and the sensory evaluation data.

### **Results and Discussion**

Physico-chemical parameters: Samples were preliminarily tested by MT puncture tester and a small panel to determine the stage of ripeness—fruits with MT values of 9 lb and above were unripe, <9-7 lb semiripe, <7-6lb ripe, <6->4 lb slightly overripe and  $\leq 4$  lb overripe.

Clearly unripe, ripe and overripe samples were selected to standardize instrumental quality measures. Fig. 3 gives typical force-distance curves when these samples were deformed in OTMS. The shape and characteristics of these curves are clearly different for these three stages of ripeness indicating differences in textural characteristics. It may be seen from the curve that in unripe sample, there is an initial high increase in force with steeper slope till yield point, indicating high resistance of the sample to the force applied with very little compression. Afterwards a sudden sharp break in the curve due to a few fractures in the sample and a rise again and a broad plateau reflecting high resistance to disintegration were



Fig. 3. Typical OTMS Force Distance Curves

observed. Further, a sharp and high increase in force due to resistance for packing the turgid structure of the sample is observed in the curve till the end of the compression cycle. In the curve for ripe sample, the initial increase in force and slope are less compared to the unripe sample. Then a gradual decrease of force is seen reflecting possibly multiple fractures of the less firmer tissues of the sample, followed by a gradual rise till the end of the compression cycle which was lesser than the maximum for unripe sample. In overripe sample curve, the force and slope till the yield point are very much less compared to the other two samples. The force dropped suddenly forming a broad 'U' shaped trough indicating a collpase of the structure of the sample and the maximum force at the end of the compression cycle was also very less with little resistance for packing, indicating softness of the tissues. Thus, the shape of the force distance curves clearly showed a distinguishing pattern of plateau, 'V' shaped trough and broad 'U' shaped trough after first significant break for unripe, ripe and overripe samples respectively. OTMS yield point computed from forcedistance curves showed a high value around 23 kg for unripe, 14 kg for ripe and 6 kg for overripe samples respectively.

The OTMS juice-volume area values obtained simultaneously during force-distance curve development for these samples clearly showed a decreasing trend like OTMS yield point, the values being around 170 sq.cm. for unripe, 110 sq.cm. for ripe and 45 sq.cm. for overripe samples. Acidity values also followed a similar pattern, but brix showed a reverse pattern for these three quality stages-values being around 0.45, 0.35 and 0.30 for acidity and 10, 13.5 and 15 for brix respectively.

Sensory scores vs. instrumental values for quality *definition:* A number of samples ranging from unripe to overripe were analysed by instrumental and sensory methods. When the values of the instrumental measurements vs. sensory data were plotted, it was observed that as the instrumental values, except °brix, progressively decreased from unripe to overripe stages the corresponding sensory scores increased. The middle values of the instrumental measures corresponded with the optimal quality description assigned in the descriptive sensory scale. In the physiological changes during ripening, the apple from the initial harvest (unripe) stage has undesirable sensory qualities; and progressively changes during storage to optimal ripe stage perceived as having desirable sensory qualities and finally reaches the overripe stage perceived as having again undesirable sensory qualities. Hence for purpose of graphical representation, unripe (scores 1, 2 and 3) and overripe (scores 7, 6 and 5) qualities were plotted as overlapping points with the optimally ripe quality (score 4) as the turning point. Fig. 4a and 4b show typical curves of OTMS yield point



Texture score Juiciness Score

a) Optimal quality; b) Limits for most desirable quality; c) Limit for cold storage life

vs. sensory texture and OTMS juice-volume area vs. sensory juiciness. The sensory texture evaluated as very good with mean score 3.5-4.5 corresponded to the OTMS yield point range 16-12 kg which defines the most desirable quality in texture. Similarly the OTMS juice-volume area range 125-95 sq.cm. defines the most desirable sensory juiciness. The sensory texture and juiciness mean score of 5.5 reflecting the limit for cold storage life corresponded to the OTMS yield point 8 kg and juice-volume area 65 sq.cm. respectively.

The results obtained by instrumental measurements and sensory evaluation on representative set of samples from the same lot at five stages of ripeness are given in Table 1. It may be seen that OTMS yield point and juice-

IABLE I. SENSORY QUALITY	ATTRIBUTES A	ND PHYSICO-CHEMI	CAL PARAMETER	S-MEAN SCORES	FOR DIFFERENT S	STAGES OF RIPENESS
	Unripe	Semiripe	Optimum ripe	Slight overripe	Overripe	SE <sub>m</sub>
		Sensory q	uality attributes			
Texture	1.8ª	3.26	4.4 <sup>c</sup>	5.6 <sup>d</sup>	6.5 <sup>e</sup>	±0.13 (76)
Juiciness	1.8ª	3.36	4.5°	5.4 <sup>d</sup>	6.5e	±0.13 (76)
Aroma	1.5ª	3.2 <sup>b</sup>	4.1 <sup>c</sup>	5.3 <sup>d</sup>	6.1 <sup>e</sup>	<u></u>
Taste	1.4ª	3.36	4.3¢	$5.2^{d}$	6.2 <sup>e</sup>	±0.12 (76)
Overall quality	1.2ª	2.6 <sup>b</sup>	4.2¢	2.5 <sup>b</sup>	1.3ª	±0.15 (76)
		Physico-che	mical parameter	<b>'</b> 9		
OTMS yield point (kg)	24.2 <sup>e</sup>	18.0 <sup>d</sup>	13.4 <sup>c</sup>	8.1 <sup>b</sup>	5.2ª	±0.79 (36)
MT value (lb)	9.3 <sup>d</sup>	7.6¢	6.2 <sup>b</sup>	5.6 <sup>b</sup>	4.0 <sup>a</sup>	$\pm 0.16$ (36)
OTMS juice-volume (sq. cm.)	174e	128 <sup>d</sup>	102¢	68 <b>b</b>	40ª	±3.21 (36)
°Brix	9.8ª	11.86	13.40	14.0°	15.0 <sup>d</sup>	$\pm 0.19$ (16)
Acidity (% malic acid w/v)	0.45 <sup>d</sup>	0.41 <sup>c</sup>	0.36 <sup>b</sup>	0.34 <i>b</i>	0.30ª	±0.007 (16)

TABLE 1. SENSORY QUALITY ATTRIBUTES AND PHYSICO-CHEMICAL PARAMETERS-MEAN SCORES FOR DIFFERENT STAGES OF RIPENESS

 $SE_m$ —Standard error of means; figures in parenthesis indicate degrees of freedom.

Figures with different superscripts (a to e) in the same row are significantly different (P < 0.05)

							•	•	
Quality factors	MT value	OTMS juice-volume	°Brix	Acidity	Texture	Juiciness	Aroma	Taste	Overall quality
OTMS yield point	τ <b>+0.66**</b>	+ 0.82**	-0.71**	+0.65**	-0.82**	-0.82**	-0.66**	-0.78**	-0.79**
MT value		+0.54*	-0.52*	+0.49*	-0.56*	-0.43NS	-0.52*	-0.45*	-0.42 <sup>NS</sup>
OTMS juice-volur	ne		0.74**	+ 0.67**	-0.82**	-0.82**	-0.63**	0.76**	-0.77**
°Brix				-0.72**	+0.47*	+ 0.67**	+0.42 <i>NS</i>	+0.75**	+0.41 <sup>NS</sup>
Acidity					-0.45*	-0.56*	-0.41 <sup>NS</sup>	-0.53*	-0.39**
Texture						+0.92**	+0.87**	+0.92**	+0.92**
Juiciness							+0.87**	+ 0.91**	+0.89**
Aroma								+0.92**	+0.92**
Taste									+ 0.89**
DF-Degrees of	of freedom			NSNot sig	gnificant				
*P<0.05				** P<0.01					

TAPLE 2. CORRELATION COEFFICIENT BETWEEN PHYSICO-CHEMICAL AND SENSORY QUALITY FACTORS, 18 DF

volume area show significant differences between all the five stages, whereas MT puncture tester, brix and acidity values do not show differences significantly between ripe and slightly overripe stages. In our experience, MT puncture tester values are also subject to high variation as it is manually operated.

*Correlations:* Correlation coefficients between physico-chemical and sensory quality parameters were worked out. Since both unripe and overripe fruits scored lower than the optimally ripe fruits for overall quality, the overall quality mean scores on the 5-point scale were unfolded as 9-point scale for correlation purpose. The points 1 to 5 overall quality mean scores reflecting unripe to optimally ripe stages were kept unaltered when the individual quality mean scores were less than or equal to 4. When the individual quality mean scores were less than or equal to 4. When the individual quality mean scores were above 4 (indicating overripe quality), the corresponding overall quality mean scores were converted to 5 to 9 instead of 5 to 1.

Linear correlation coefficients between the physicochemical and sensory quality parameters are given in Table 2. Sensory quality attributes and overall quality showed highly significant positive correlation between one another, while each one of them showed highly significant negative correlation to OTMS yield point and juice-volume area. MT values also showed negative correlation with sensory attributes. The negative correlations of MT values to sensory juiciness and overall quality were not significant. OTMS yield point and MT values showed significant positive correlation with OTMS juice-volume area. °Brix showed negative correlation with instrumental texture measures and OTMS juice-volume area but positive correlation with each of sensory attributes. The positive correlations of °brix with aroma and overall quality were not significant. Acidity showed positive correlation with instrumental texture measures and OTMS juice-volume area, but negative correlation with each of sensory attributes. The negative correlations of acidity with aroma and overall quality did not attain significance. °Brix and acidity showed significant negative correlations. Since MT puncture tester is manually operated, it is less sensitive and its correlation coefficients with OTMS yield point and texture are low, though significant. In general, high correlations, either positive or negative, have been established between all the quality parameters as measured by both physico-chemical and sensory methods indicating that these quality parameters change together.

conclusion: Based on this study, OTMS yield point ranging from 25 to 5 kg clearly brought out the textural changes and OTMS juice-volume area ranging from 175 to 35 sq.cm juiciness changes in apple during cold storage. There is no adequate knowledge about the aroma components to assess the aroma changes reliably. <sup>°</sup>Brix and acidity reflecting taste ranged too narrowly show clear differences in quality of apples. to OTMS yield point in the range of 18-10 kg and OTMS juice-volume area 140-80 sq.cms corresponding to the sensory score 3-5 could define fruits with good to acceptable range in texture and juiciness attributes. The overall quality of the fruits in this range of instrumental values was also found to score between excellent to good. Therefore, the OTMS yield point and OTMS juicevolume area which showed highly significant correlations to individual quality attributes and overall quality were found to be very good indices of apple quality as validated by the sensory quality and sensitive to assess the effect of treatments clearly.

### Acknowledgement

The authors are indebted to Dr. P. Narasimham, for supply of samples used in this study. The authors

wish to thank Dr B. L. Amla and Mr C. P. Natarajan, Directors; Drs. M.V. Patwardhan and K.G. Raghuveer for their keen interest and encouragement during the course of this investigation

### References

- 1. Haller, M. H., Fruit pressure testers and their practical applications, USDA Circular No. 627, 1971, 17.
- Schomer, H. A. and Olson, K. L., A mechanical thumb for determining firmness of apples. *Proc. Amer. Soc. hort. Sci.*, 1962, 81, 61.
- 3. Bourne, M. C., Studies on punch testing of apples. *Fd Technol.*, 1965, **19**, 413.
- 4. Finney, E. E., Dynamic elastic properties and sensory quality of apple fruit. J. Texture Stud., 1971, 2, 62.
- Brennan, J. G., Jowitt, R. and Mohamed, A.M.A., Instrumental measurement of fruit texture: a study on apples. *Ann. Appl. Biol.*, 1977, 87, 121.
- Bowman, F., Kylen, A. M., and Adam, S. F., Relationship between certain physical-chemical measurements and sensory appraisal of apple texture. J. J. Texture Stud., 1972, 3, 478.

- Zaehringer, M. V. and Hard, M. M., Evaluation of methods for measuring texture using fresh apples as test material. J. Can. Inst. Fd Sci. Technol., 1974, 7, 125.
- Eccher Zerbini, P., Comparison tests on intact Golden delicious apples and correlation with other objective measures for quality evaluation. *Lebensm. Wiss. Technol.*, 1981, 14, 12.
- Dhanaraj, S., Ananthakrishna, S. M. and Govindarajan, V. S., Apple quality: Development of descriptive quality profile for objective sensory evaluation. J. Fd Quality, 1980, 4, 83.
- Bourne, M. C., Texture profile of ripening pears. J. Fd Sci., 1968, 33, 223.
- Bourne, M. C., Texture profile analysis. Fd Technol., 1978, 7, 62.
- Tijskens, L.M.M., Texture of Golden delicious apples during storage. Lebensm. Wiss. Technol., 1979, 12, 138.
- Ottwa Texture Measuring System, An operation manual, Engineering Specification No. 7024, 1972, Engineering Research Services, Agriculture, Canada.
- Harter, L. N., Critical values for Duncan's new multiple range test. *Biometrics*, 1960, 16, 676.

### Lipid Composition of Salted Sun-dried Indian Mackerel (Rastrelliger Kanagurta)

### B. Y. KRISHNOJI RAO AND C. BANDYOPADHYAY

Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085

Manuscript received 21 December 1981; revised 2 August 1982

Lipid composition of two commercial samples of salted sun-dried Indian mackerel (*Rastrelliger kanagurta*) has been investigated. As compared to fresh mackerel there was considerable reduction in glyceride content and iodine value with concomitant increase in free fatty acid content and peroxide value of muscle lipids. The fatty acid composition of fish lipid as determined by gas liquid chromatography indicated that salting and sun-drying of mackerel caused considerable loss in higher polyunsaturated fatty acids attributable to lipid oxidation.

Indian mackerel (*Rastrelliger kanagurta*), a fatty fish is normally consumed as fresh by coastal inhabitants. However, during seasonal glut, a substantial quantity of mackerel is cured by salting and drying. Sun-drying is usually employed to obtain a product for internal consumption as well as for export<sup>1</sup>, for which specifications have been laid down<sup>2</sup>. However, precise control of final moisture content becomes difficult resulting in variability in the keeping quality of the final products. In mackerel, the depot fats stored in muscle are reported to be rich in polyunsaturated fatty acids<sup>3</sup>, which are likely to undergo oxidation during prolonged exposure to air and sunlight. Reports on the lipid composition of salt-cured, sun-dried Indian mackerel are scanty. Sodium chloride in salted, dried fish such as tuna, is reported to possess pro-oxidant effect on lipids<sup>4</sup>, whereas Nambudiry<sup>5</sup> had observed that higher levels of sodium chloride in sardine meat inhibited lipid hydrolysis. The present paper relates to the lipid composition of commercial samples of salted sun-dried mackerel.

### Materials and Methods

Two different batches, designated as I and II of freshly processed, salted sun-dred mackerel (average lengthi

63

	Fresh**	Batch I	Batch II
Moisture content (% wt.)	68.5 ±1.5	35.2 ± 1.4	$21.6 \pm 2.1$
Total lipid (g/100 g flesh)	8.6 ±2.0	5.4 ± 0.4	9.5 ± 1.5
Iodine value (IV) (Wij's method)	173.4 ±1.9	133.0 ± 2.0	128.5 ± 2.0
Glyceride content (g/100 g lipid)	86.4 ±1.9	70.0 ± 2.0	68.8 ± 1.8
Free fatty acid (FFA) content ( $\mu$ mole of oleic acid/g lipid)	40.0 ±2.16	500.0 ±20.4	475.6 ±35.5
Peroxide value (PV) (meq/kg lipid)	14.1 ±2.2	184.6 $\pm$ 14.4	112.6 ± 9.5
Thiobarbituric acid (TBA) value (mg of malonaldehyde/kg flesh)	0.73±0.03	$0.73\pm~0.07$	0.93 ± 0.66
*Average of three determinations $\pm$ standard deviation. **See ref. 14.			

 TABLE 1. PHYSICO-CHEMICAL CHARACTERISRICS\* OF MUSCLE LIPIDS OF FRESH AND 1WO BATCHES OF COMMERCIAL, SALTED

 SUN-DRIED INDIAN MACKEREL

20 cm) were procured from a local market. Each batch of fish was evaluated by a panel of three judges with reference to appearance, texture, colour and odour<sup>6</sup>. The fish was beheaded after removing the central bone and chopped into small pieces. The moisture content was determined by a Sauter apparatus equipped with an infrared heating arrangement. The fish muscle (10 g) was dried to a constant weight and the moisture content in percentage was read directly on the scale.

Lipid analysis: Lipid was extracted from the chooped fish muscle (100 g) of the respective batches with a mixture of chloroform, methanol and water according to the method described by Bligh and Dyer<sup>7</sup>, and the lipid content of each batch was determined after removal of the solvent in a flash evaporator at room temperature.

Iodine value (IV) and peroxide value (PV) of each lipid extract was determined according to standard procedures<sup>8</sup>. Thiobarbituric acid (TBA) value was measured according to the method of Sinhuber and Yu<sup>9</sup> using 1:5 aqueous extract of fish muscle centrifuged at  $3000 \times g$  for 10 min. Glycerides and free fatty acid (FFA) content of each lipid extract was estimated according to the method described by Van Handel and Zilbersmit<sup>10</sup> and Duncombe<sup>11</sup> respectively.

Gas liquid chromatography (GLC): The methyl esters of fatty acids prepared according to the method described earlier<sup>12</sup> were analysed in a BARC model gas chromatograph equipped with a flame ionisation detector. A stainless steel column (0.625 cm  $O.D \times 180$  cm) packed with 20 per cent ethylene glycol succinate on 60/80 mesh Chromosorb W was used. The column and detector temperature was maintained at 192°C with a carrier gas, N<sub>2</sub> flow of 30 ml/min. The fatty acids were identified by comparing the retention time of authentic reference samples as well as by equivalent chain length determination. Gas chromatographic peak areas were calculated by multiplying peak height by peak width at half height.

### **Results and Discussion**

A difference in the organoleptic attributes between the two commercial samples was observed. The batch I was

TABLE 2. FATTY ACID COMPOSITION<sup>®</sup> OF MUSCLE LIPIDS OF FRESH AND TWO BATCHES OF COMMERCIAL, SALTED SUN-DRIED INDIAN MACKEREL

		Relative %	
Fatty acid	Fresh**	Batch I	Batch II
12:0	0.25±0.02	_	—
14:0	6.35±0.75	13.5±1.5	12.5±1.1
15:0	$1.50 \pm 0.20$	1.5±0.1	1.0±0.1
16:0	13.10±1.20	$32.0 \pm 3.5$	30.0±1.5
16:1	10.80±1.32	21.5±1.0	21.5±0.3
17:1	3.80±0.25		
18:0	9.65±1.55	14.0±2.2	14.0±1.7
18:1	10.65±2.56	14.0±0.5	16.5±1.0
18:2	$4.45 \pm 0.74$	$2.0 \pm 0.3$	$3.0\pm0.2$
18:3	5.50±0.50	1.5±0.2	1.5 <u>±</u> 0.4
20:1	4.60 <u>±</u> 0.35	_	-
18:4	$3.50\pm0.40$		
20:3	3.80±0.15		
22:1 (?)	3.55±0.80		-
20:4	4.30±0.57		—
22:2 (?)	5.30 <u>+</u> 0.50		
22:3	$0.50 \pm 0.03$		-
22:4 (?)	4.00±0.75		-
22:5	$1.55 \pm 0.05$	-	
22:6	2.75±0.04	_	_
*Average of		ations $\pm$ standard	deviation

\*\*See ref. No. 14.

vellowish in colour and slightly soft in texture, whereas batch II was pale brown in colour and had relatively harder texture. Besides cooked odour, both the samples did not exhibit any unusual off-odour. The average moisture content of the former was found to be around 35 per cent, while that of the latter was 21 per cent. The physico-chemical characteristics of muscle lipids of the two commercial samples are shown in Table 1. The variation observed in the lipid content between the two samples in the present case could be due to the difference in moisture content<sup>13,14</sup>. As compared to fresh fish, both the commercial samples exhibited a decrease in glyceride content concomitant with the increase in FFA content indicating hydrolytic cleavage of the glycerides. Table 2 gives the fatty acid composition of fish samples. In both the samples, the polyunsturated fatty acids (PUFA) higher than  $C_{18-3}$ , which are characteristics of marine fish3, were absent indicating their breakdown. The oxidative breakdown of lipids is also evidenced by the incidence of low IV, high PV and TBA values in the respective samples (Table 1). The foregoing results suggest that salting and sun-drying of mackerel causes considerable loss in higher polyunsaturated fatty acids attributable to lipid oxidation.

### References

- 1. Valsan, A. P., A comparitive yield and biochemical evaluation of the existing fish curing methods in India. *Proc. Symp. Fish Processing Industry in India*, AFST, Mysore, Feb., 13-14, 1975, 77.
- 2. Specification for dry salted mackerel, IS: 4302-1967 Indian Standards Institution, New Delhi.
- 3. Ackman. R. G. and Eaton, C. A., Mackerel lipids and fatty acids. Can. Inst. Fd Technol., J., 1971, 4, 169.

- Koizumi, C., Terashima, H., Waɗa, S. and Nonaka, J., Lipid oxidation of salted freeze-dried fish meats at different equilibrium relative humidities. J. Bull. Jap. Sec. Sci., Fish., 1980, 46, 871.
- Nambudiry, D. D., Lipid oxidation in fatty fish: The effect of salt content in the meat. J. Fd Sci. Technol., 1980, 17, 176.
- Venugopal, V., Savagaon, K. A., Kumta, U. S. and Sreenivasan, A., Extension of shelf-life of Indian mackerel (*Rastrelliger konagurta*) by irradiation. J. Fish. Res. Bd Can., 1973, 30, 305.
- Bligh, E. G. and Dyer, W. J., A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physico.*, 1959, 37, 911.
- Official and Tentative Methods, American Oil Chemist's Society, ILL, U.S.A. 1974.
- Sinnhuber, R. O., and Yu, T. C., 2-thiobarbituric acid method for the measurement of rancidity in fishery prooucts, II. The quantitative determination of malonaldehyde. *Fd. Technol.*, 1958, 12, 9.
- Van Handel, E. and Zilbersmit, D. B., Suggested Modification of the microdetermination of triglycerides. *Clin. Chem.*, 1961, 7, 249.
- 11. DunCombe, W. G., The colorimetric microdetermination of long chain fatty acids. *Biochem. J.*, 1963, 88, 7.
- Bandyopadhyaya, C. and Gholap, A. S., Relationship of aroma and flavour characteristics of mango (*Mangifera indica* L.) to fatty acid composition. J. Sci. Fd Agric., 1973, 24, 479.
- Leu, S. S., Jhaveri, S. N., Karakoltsidis, P. A. and Constantinides, S. M., Atlantic mackerels (Scombes scombrus, L.) Seasonal variation in proximate composition of chemical nutrients. J. Fd Sci., 1981, 46, 1635.
- Rao, B. Y. K. and Bandyopadhyaya, C., Changes in lipid composition of radurized Indian mackerel (*Rastrelliger* kanagurta) during = efrigerated storage. Die Fleischwirtschaft, 1982, 62, 193.

### Studies on the Extraction of Caffeine from Coffee Beans

K. UDAYA SANKAR, C. V. RAGHAVAN, P. N. SRINIVASA RAO, K. LAKSHMINARAYANA RAO, S. KUPPUSWAMY AND P. K. RAMANATHAN

Central Food Technological Research Institute, Mysore-570013, India

Manuscript received 14 November 1981; revised 9 August 1982

Studies were carried out on the extraction of caffeine from coffee beans using dichloromethane on a pilot soxhlet extraction unit of capacity 10 kg per batch. Coffee beans were conditioned to moisture content of 40 per cent and extracted till 97 per cent of the initial caffeine was removed. Apparent coefficient of diffusion was calculated using Fick's Law of Diffusion.

There has been a steady increase in the demand of decaffinated coffee in U.S.A. and Europe due to growing consumer awareness to personal health care. About 10

per cent of the coffee consumed in U.S.A. is reported to be decaffinated.

Extraction of caffeine from coffee beans using a solvent

is a diffusion controlled rate process. Solutions for liquid diffusion equations for several systems have been published<sup>1-3</sup>. A knowledge of diffusion coefficient will be useful in the design of caffeine extraction plants and such data currently are not available. The present study was undertaken to apply the unidirectional diffusion equation to caffeine extraction systems and calculate the diffusion coefficient using the pilot plant data of the present work along with published data on laboratory scale<sup>4</sup> and industrial<sup>5</sup> extraction units.

### Materials and Methods

Coffee beans were moistened by soaking in water and autoclaving with atmospheric steam. The beans were extracted in a pilot plant soxhlet extraction unit of capacity 10 kg beans per batch. A solvent to material ratio of 1:4 was found optimum. The solvent was evaporated in a kettle and the vapours were condensed at a temperature of 5°C. The cycle time of 20-30 min, was maintained. The beans were analysed for solvent content, moisture content and caffeine content by AOAC<sup>6</sup> methods at regular intervals. The extraction was stopped after 97 per cent of the initial caffeine present in the beans were removed. The caffeine content is expressed as a percentage on dry weight solvent free basis. The average radius of the beans was determined by volumetric measurements.

In the solvent extraction of caffeine from the coffee beans, the rate of extraction depends on the rate of caffeine movement from the centre of the coffee beans. Diffusion is the primary mechanism involved in the movement of caffeine to the surface of the beans.

The governing equation that expresses liquid diffusion in a solid can be written for spherical geometry as

$$\frac{\partial c}{\partial t} = D \left[ \frac{\partial^2 c}{\partial r^2} + \frac{2}{r} \frac{\partial c}{\partial r} \right] \qquad \qquad -(1)$$

This equation can be solved for the present case with the following boundary conditions and initial conditions.

$$\frac{\partial c}{\partial r} = 0 \quad r=0 \quad t \ge 0$$

$$C = C_f \quad r = r_o, \quad t \to \infty$$

$$C = C_i \quad 0 < r < r_o, \quad t = 0$$

The first boundary condition specifies a finite concentration at the centre of the sphere. The second boundary condition means that the surface is at the final concentration. According to the initial condition, the initial concentration is uniform throughout the object.

Using separation of variables equation, (1) can be solved to obtain the following relationship for caffeine extraction rate as a function of time.

$$\frac{C-C_f}{C_i-C_f} = \frac{6}{\pi^2} \frac{n}{n=1}^{n=\infty} \frac{1}{n^2} \exp\left(\frac{-Dn^2\pi^{2t}}{r_o^2}\right) \qquad ...(2)$$

When diffusion of caffeine has been taken over a long period of time, the second and higher terms of the series can be neglected, thus

$$\frac{\mathbf{C}-\mathbf{C}_f}{\mathbf{C}_f-\mathbf{C}_f} = \frac{6}{\pi^2} \exp\left(\frac{-\mathbf{D}^2\mathbf{n}^2\pi^2\mathbf{t}}{\mathbf{r_0}^2}\right) \qquad (...(3)$$

Equation 3 can be solved for time of extraction

$$\ln \frac{\pi^2}{6} \left( \frac{C - C_f}{C_i - C_f} \right) = -t / \gamma \qquad ...(4)$$

Where  $\gamma = \frac{1}{\sigma^2 D}$ 

From a plot of equation 4 which gives a straight line, the diffusion coefficient can be evaluated.

### **Results and Discussion**

Fig. 1, 2, and 3, represent the caffeine content of beans at different time intervals for the laboratory (0.45 kg), pilot plant (10 kg), and industrial decaffination plant (20t/day) using dichloromethane as solvent. It can be seen from the figures that there is a steady rate of extraction of caffeine till it reduces to 0.4 per cent. The rate becomes too low on further removal of caffeine. Fig.

4, 5 and 6 represent the plot of  $\ln \frac{\pi^2}{6} \left( \frac{\mathbf{C} - \mathbf{C}_f}{\mathbf{C}_i - \mathbf{C}_f} \right)$  as a function of time for the pilot plant, industrial unit and



Fig. 1. Caffeine content of beans Vs. time (Pilot Plant 10kg unit.)



Fig. 2. Caffeine content of beans vs. time (20t/day)

laboratory data. The regression equation for the straight line in Fig. 4 & 5 is found to be  $\ln \frac{\pi^2}{6} \left( \frac{\mathbf{C} - \mathbf{C}_f}{\mathbf{C}_i - \mathbf{C}_f} \right) = -0.187t.$ 

Based on the measured equivalent radius of 3.91 mm, the apparent diffusion coefficient was found to be  $0.81 \times 10^{-6}$  cm<sup>2</sup>/sec. Bichsell *et al.*<sup>4</sup> estimated the diffusion coefficients of caffeine from coffee beans under similar conditions on a laboratory scale and have reported the apparent diffusion coefficients to be in the



Fig. 3. Caffeine content of beans vs time (0.5 kg)



DICHLOROMETHANE					
Type of caffeine	Solvent	D. $10^{6} \text{ cm}^{2}/\text{sec}^{-1}$ .			
Pure caffeine	Water	5.3			
Pure caffeine	Dichloromethane	13.7			
Caffeine from beans	Dichloromethane (reported data <sup>3</sup> )	0.2-1.1			
Caffeine from beans	Dichloromethane (present work)	0.81			

TABLE 1. DIFFUSION COEFFICIENT OF CAFFEINE IN WATER AND

range of  $0.2-1.10^{-6}$  cm<sup>2</sup>/sec<sup>-1</sup>. Table 1 shows the diffusion coefficients of pure caffeine and the data reported in literature in comparison with the present value. It can be seen that there is good agreement between them.

### Nomenclature

C=caffeine content of beans at time t hr;  $C_i$ =initial caffeine content of beans;  $C_f$ =final caffeine content of

beans; D=diffusion coefficient cm<sup>2</sup>/sec;  $r_0$  radius of the beans in cm.

### Acknowledgement

The authors wish to thank Dr. S. Bhargava, Prof. & Head of Dept. of Mathematics, Manasagangothri and Dr. N. Mohan Madhyastha, Reader in Statistics, Manasagangothri, for their useful discussions.

### References

- 1. Crank, J., The Mathematics of Diffusion, Clavendon Press, Oxford, 1970.
- 2. Jost, W., Diffusion in Solids, Liquids and Gases. Academic Press, New York, 1952.
- Heldman, D. R. and Paul Singh, R., Food Processing Engineering, AVI, Connecticut, 1980.
- Bichsell, B., Gal, S. and Singer, R., Diffusion phenomena during the decaffination of coffee beans. J. Fd Technol. 1976, 11, 637.
- Sivetz, M. and Desrosin, N. W., Coffee Technology. AVI, Connecticut, 1979.
- 6. Official Methods of Analysis, Association of Official Agricultural Chemists, 10th Ed, 1961.

### Investigations on Large Scale Preparation and Preservation of Milk Burfi

B. R. RAMANNA, K. K. BHAT, B. MAHADEVAIAH, C. T. DWARAKANATH, S. DHANARAJ, V. H. POTTY AND D. P. SEN

Central Food Technological Research Institute, Mysore-570013, India

Manuscript received 23 November 1981: revised 31 August 1982

Khoa of good quality with proper consistency could be prepared when steam was used to concentrate milk instead of coal or gas fired hearths and stainless steel jacketted open kettle was used in place of traditional hearths. In large scale (50 litres), Khoa was prepared with preliminary concentration ( $30-40^{\circ}$  Brix) in vacuum (620mm Hg) and 0.2-0.3 kg/cm<sup>2</sup> steam pressure) in forced circulation evaporator with further final concentration being achieved in open kettle. Burfi was prepared by adding sugar to khoa in 1:2 proportion and 0.15% sorbic acid and replacing 25% of sugar by adding liquid glucose. They were packed in different packaging materials. Adding sorbic acid and packing in an inner polycel and outer polythene pouch kept the Burfi for 90 days instead of 6-8 days as in traditional methods. Addition of liquid glucose is not necessary for this packaging material.

Burfi is the most popular sweet dish prepared from Khoa. Khoa is a milk concentrate with about 28-30 per cent moisture. Preparation and storage of Khoa have been extensively studied<sup>1-4</sup>. In the conventional method, Khoa is prepared by concentrating milk in open karahis (pans) over coal fired ovens with continuous stirring for preventing charring. The product is ready when it forms into one lump and does not stick to the sides. This

*Khoa* is used as a base for the preparation of *Burfi* and other sweet meats.

A continuous *Khoa* making machine has been developed at NDRI in which milk is first concentrated in a roller drier and further processed in open keettles fitted with spring loaded, reciproacting typescrapers<sup>2</sup>.

The conventional method of preparing *Burfi* is to mix *Khoa* and sugar in the proportion of 2:1 and concentrate in the open *Karahis* as in the case of *Khoa* preparation. Heating is stopped when it forms into a butter-like mass and it is afterwards spread to a thickness of 1.25 cm in a stainless steel plate and is allowed to cool overnight before being cut into pieces. Data on Proximate composition<sup>5</sup> and microbiological quality<sup>6-9</sup> of the market samples of *Burfi* have been reported.

The present study deals with the large-scale preparation of *Khoa* for the manufacture of *Burfi* by using a vacuum evaporator for pre-concentration and also to standardise processing conditions. Different preservatives and packagings have also been tried to increase the storage life of *Burfi*.

### Materials and Methods

**Preparation of burfi:** Five to ten litres of cow's milk were concentrated in a stainless steel steam jacketted open kettle. Steam was used for heating instead of coal fired ovens as in the traditional practice. Continuous stirring and scraping of the sides of the kettle was done to prevent scale formation and subsequent charring. Sugar was added when the °brix was between 66 and 68° and concentration continued. Maximum temperature of **Burfi** attained during processing was 90-95°C. The processing was monitored by the use of hand refractometer. At the optimum concentration, **Burfi** was removed from the Kettle and spread evenly on a stainless steel plate, cooled overnight and cut into bits.

Large-scale preparation: Fifty litres of cow's milk was concentrated to a brix of  $40^{\circ}$  in a forced circulation evaporator under a 620 mg Hg vacuum. Steam pressure was maintained at 0.2-0.3 kg/cm<sup>2</sup>. Different levels of vacuum and different levels of concentration were tried to standardize the conditions for pre-concentration. Final concentration was effected in open steam jacketted kettles with continuous stirring and scraping. Burfi was also prepared from this Khoa.

Addition of preservative: Sorbic acid was added at 0.15 per cent level just before the product is being removed from the kettle.

Addition of liquid glucose: Liquid glucose was used to replace 25 per cent of the sugar added to overcome surface hardening of *Burfi* on storage.

Sensory evaluation: Sensory quality assessment of fresh and stored products were carried out by ranking method for individual quality attributes and by a 5-point aptitude rating for overall quality by a 20-discriminative communicative panel. During a few panel orientation sessions, a quality description describing desirable and undesirable aspects was finalised (Table 1), and used in further regular evaluations. The samples evaluated comprised an experimental sample with 0.15 per cent sorbic acid which was compared with two fresh market samples. The stored samples evaluated comprised

TABLE 1. D	ESCRIPTION OF QUALITY	ATTRIBUTES-MILK BURFI
Quality attributes	Desirable	Undesirable
Colour (Natural)	Shades of pale brown/ yellowish brown/creamy/ tan uniform; fresh ap- pearance	Dark shades; spotty, not uniform; dull appearance
Appearance (Structure)	Regular, uniform shape Soft / smooth surface Broken surface, partially particulate inside	Irregular, not uniform surface crusty/dry/oily/ spots of extraneous mat- ter, grossly granular due to drying
Texture (Mouthfeel)	Moist/soft/granular easi- ly melting, little residual particles	Dry / sticky / gritty, not easily melting residual/ oily/fatty particles
Flavour	Aroma: characteristic, heated milk, slightly caramalised, creamy, no off aroma; fresh	Bland/too caramalised/ burnt, stale / oxidised / rancid, not fresh
	Taste: moderately sweet slightly fatty; fresh	Slight acidity / slight bitterness, foreign taste, not fresh.

sample with 0.15 per cent sorbic acid and stored at -20°C (control) (A), sample with 0.15 per cent sorbic acid + 25 per cent liquid glucose and stored at 27°C (B) and sample with 0.15 per cent sorbic acid and stored at 27°C (C). These were compared with fresh market sample.

The ranks for individual quality attributes were analysed by Kramer's rank sum method<sup>10</sup> for difference between any two treatments by re-ranking. The overall quality scores were analysed by analysis of variance followed by Duncan's new multiple range test<sup>11</sup>.

Packaging studies: Flexible packaging materials such as (i) MSAT cellophane, (ii) Low Density Polyethylene (LDPE) (400 g), (iii) High Density Polyethylene (HDPE) (300 g), (iv) MSAT+HDPE, (v) Glassine/PE (150 g) laminate, (vi) Saran/Cello saran/PE laminate, (vii) Paper/Al. foil (150 gauge), and (viii) Polycel inside and Polythene pouch outside, were used for packing Burfi and their efficiency in preserving the product was studied.

*Microbiological analysis:* Samples of *Burfi* were taken for standard plate counts and total yeast and mould counts following the methods of APHA<sup>12</sup>. Sample was prepared as follows-Fifty grams of sample were blended for 2 min. in a sterile buffered phosphate buffer dilutent to obtain 1/10 dilution. Decimal solutions were prepared upto 1/1000. Appropriate aliquots of dilutions were plated with Tryptone Glucose Yeast Extract Agar for standard plate count, and Acidified Potato Dextrose Agar for yeast, and mould counts.

Chemical analysis: During the concentration of milk on the open kettle, samples were taken out at regular intervals and were analysed for moisture, lactose, -amino nitrogen, soluble protein and pH<sup>13,14</sup>

### **Results and Discussion**

Burfi was prepared in the conventional way except by changing the heating method. Steam was used to concentrate milk instead of coal or gas fired hearths and stainless steel, jacketted open kettle was used in place of iron Karahi. The optimum processing was arrived at It was observed that when by noting the <sup>o</sup>Brix. the product attained 66-68 °Brix, Khoa of proper consistency was obtained. Further processing of Khoa was continued by adding the required quantity of sugar to prepare Burfi. Burfi of desirable texture and taste was obtained when the refractometer reading was between 75 and 80 °Brix (Table 2). In large scale preparation of Khoa, preliminary concentration was done under vacuum in a forced circulation evaporator by continuous feeding. After concentrating upto a Brix of 35-40°, further concentration was achieved in stainless steel jacketted open kettle. It was found that the concentration at 620 mm Hg and 0.2-0.3 kg/cm<sup>2</sup> steam pressure was the most suitable. The resultant product had raw milk taste. To overcome this raw milk taste, milk was boiled for a few minutes and cooled before feeding into the evaporator. Preboiled milk removed the raw taste in Burfi. Use of the evaporator for preliminary concentration reduces the time needed for open pan concentration followed in the traditional method.

Burfi has a very short storage life of 6-8 days, because of mould infection and drying of the surface. To over-

come this problem, 0.15 per cent sorbic acid was incorporated as a preservative. In the fresh sample evaluation, the addition of preservative did not affect texture, flavour and overall quality and at the same time gave significantly better colour and appearance (Table 3*a*). Humectants

TABLE 2.	RELATIONSHIP	BETWEEN	HAND REFRA	CTOMETER	READING
	( <sup>o</sup> brix) AND	MOISTURE	CONTENT	IN BURF	r

°Brix	Moist	Texture*	
BLIX	Batch I	Batch II	1exture*
70	18.5	18.1	Very soft
75	16.6	16.1	Soft
80	12.9	12.8	Firm
85	9.4	9.2	Hard

\*Applies to both the batches

 
 TABLE 3a.
 Sensory quality evaluation of fresh samples of BURFI by rank sum analysis

Type of sample	Colour	Appea- rance	Texture	Flavour	Overall quality*
Sample with sorbic					
acid	23 <i>ª</i>	23.5ª	36ª	41 <i>ª</i>	3.5ª
Market I	50 <b>b</b>	47 <sup>b</sup>	42.5ª	38ª	3.3ª
Market II	47 <sup>b</sup>	49.5 <sup>6</sup>	41.5ª	41 <i>ª</i>	3.8ª
S.E <sub>m</sub>					±0.24
					(40 df)

Figures carrying different superscripts for

each attribute along the column are significantly different ( $P \leq 0.05$ )

S.E<sub>m</sub>—Standard error of mean df-Degrees of freedom \*Mean score=(maximum 5).

Type of sample	Storage temp. (°C)	Colour	Appearance	Texture	Flavour	Overall* quality
Fresh market sample	-	39ab	42.5ªb	37 <i>ab</i>	37ª	3.7 <i>ab</i>
0.15% sorbic acid	-20	29.5ª	29a	27.5ª	330	<b>4</b> .0 <sup><i>a</i></sup>
0.15% sorbic acid + 25% liq. glucose	27	43.5 <sup>b</sup>	51.5 <b>b</b>	58.5 <b>b</b>	56 <sup>6</sup>	2.9 <b>b</b>
0.15% sorbic acid	27	60¢	776	7 <b>7</b> ¢	76 <sup>c</sup>	1.60
S.E <sub>m</sub>						$\pm 0.30$ (57 df)

TABLE 3b. SENSORY QUALITY EVALUATION OF 50 DAYS' STORED SAMPLES OF BURFI BY RANK SUM ANALYSIS

S.E<sub>m</sub>-Standard error of mean df-Degrees of freedom

\*Mean score—maximum 5.

TAI

Storage period	Standard plate count/g		Total yeast count/g		Total mould count/g	
(days)	Control	<i>Burfi</i> + liq.gl.	Control	<i>Burfi</i> + liq.gl.	Control	<i>Burfi</i> + liq.gl.
Initial	1500	_	650	_	10	_
30	4200	1100	230	100	10	10
60	2500	2200	220	20	40	50
90	500	30	70	Nil	50	80
All samples contained 0.15 per cent sorbic acid						

TABLE 4. MICROBIOLOGICAL ANALYSIS OF STORED SAMPLES OF BURFI

like liquid glucose was added to overcome surface hardening of *Burfi* on storage. In the stored sample evaluation, the sample with 0.15 per cent sorbic acid, stored at  $-20^{\circ}$ C (control) and the sample with 0.15 per cent sorbic acid +25 per cent liquid glucose, stored at 27°C showed quality comparable to fresh market sample even after 50 days of storage. The sample with 0.15 per cent sorbic acid, stored at 27°C showed significantly poor quality after 50 days of storage. With the addition of sorbic acid and liquid glucose, *Burfi* could be stored upto 50 days without surface hardening even when kept in carbdoard cartons without any wrappings (Table 3b).

Of the different packaging materials tried, only the product containing sorbic acid and packed with inside polycel and outside polythene pouch, did not show mould growth and surface hardening. This was acceptable even when stored for 90 days.

The results of microbiological analysis of *Burfi* stored at room temperature are shown in Table 4. Standard plate counts of the control sample where no liquid glucose was added was gradually reduced as the period of storage increased. This might be due to the loss of moisture resulting in reduced water activity during the storage period. Heavier load of aerobic mesophiles is suggestive of contamination in the manufacturing premises. Control sample shows higher yeast count. The increase in mould counts during storage period (Table 4) may be partly due to contamination from packaging materials.

Changes in chemical composition of milk during open pan concentration are presented in Table 5. There was considerable decrease in soluble protein and  $\ll$  -amino nitrogen and lactose content during heating. It could be concluded that refractometer reading could be an useful indicator to monitor the optimum processing in the preparation of *Burfi*. Sorbic acid at 0.15 per cent level is effective in extending the storage life of milk *Burfi* to 90 days when packed in polycell and then sealed in polythene

BLE 5.	CHANGES IN THE CHEMICAL COMPOSITION OF MILK DURING
	KHOA AND BURFI PREPARATION

°Brix	Moisture (%)	Lactose (%)	≪-Amino N (mg %)	Soluble protein (%)	pН
		F	resh Milk		
10	85.7	30.0	294.0	23.2	6.90
		B	oiled Milk		
10	85.6	28.7	260.4	23.8	6.85
15	82.5	29.4	252.0	22.9	6.70
20	74.2	28.1	250.9	21.1	6.70
25	68.4	25.5	250.0	17.7	6.50
30	61.5	25.5	220.0	16.4	6.40
35	54.6	25.5	184.8	15.9	6.35
40	49.4	25.5	184.0	15.6	6.25
45	44.1	25.2	184.0	14.9	6.25
50	37.4	25.2	184.0	13.1	1.20
55	33.9	24.9	176.0	12.7	6.20
60	28.2	24.9	169.0	10.5	_
65	24.8	24.6	168.0	9.8	-
			Burfi		
70	16.8	15.9	109.0	3.7	
75	13.6	15.9	109.0	3.5	
80	11.5	15.9	109.0	3.4	-

pouch. The addition of liquid glucose to overcome surface hardening may not be a necessity as the packaging itself keeps the product soft.

#### References

- 1. Annual Report, National Dairy Research Institute, Karnal, India, 1948.
- Bonerjee, A. K., Verma, I. S. and Bagchi, B., Pilot Plant for continuous manufacture of Khoa. *Indian Duiry.*, 1968, 20, 81.
- 3. Abhoy Kumar, Rajhoria, G. S. and Srinivasan, M. R., Effect of Modern packaging materials on the keeping quality of Khoa. J. Fd Sci. Technol., 1975, 12, 172.
- Rao, O. V., Singh, S and Surjan Singh, Effect of packaging materials on the keeping quality of Khoa. J. Fd Sci. Technol 1977, 14, 152.
- Hemavathy, J., Ramanna, B. R. and Potty, V. H., Studies on commercial Burfi preparations—some preliminary observations. *Indian Fd Packr.*, 1974, 28, 25.
- Dwarakanath, C. T. and Srikanta, S., Studies on the microbiological quality of traditional Incian sweetmeat products. J. Fd Sci. Technol., 1977, 14, 201.

- 7. Kamat, M. Y. and Sulebele, G A., Microbiological quality of pedha. J. Fd Sci. Technol., 1974, 11, 50.
- Gatlewar, W.N., Fernandes, Y. and Sant, M.V., Bacteria from sweetmetas of Bombay. *Indian J. Microbiol.*, 1970, 10, 65.
- Ajab Singh, Singh, R. B. and Edward, J. C., Survey of the microbiological quality of *Burfi* and *Peda* in Allahabad market. *Indian J. Dairy Sci.*, 1975, 28, 219.
- Kramer, A and Twig<sub>b</sub>, B. A., Quality control for the food industry, AVI Publishing Co. Inc., Westport, Connecticut, 1970. 3rd Edn., 143.
- 11. Leon Harter, H-Critical values for Duncan's new multiple range test. *Biometrics*, 1960, 16, 671.

71

- Speck, M. L., (Ed), Compendium of Methods for Microbiological Examination of Foods, APHA Intersociety-Agency Committee on microbiological methods for foods, 1976.
- 13. Official Methods of Analysis, Association, of Official Agricultural Chemists, 12th Edn., 1975, 254.
- 14. Pearson, D., Chemical Analysis of Foods, Chemical Publishing Co. Inc., New York, VII Edn., 1976.

Statement about ownership and other particulars about the periodical entitled JOURNAL OF FOOD SCIENCE AND TECHNOLOGY as required to be published under Rule 8 of the Registration of Newspapers (Central) Rules 1956. FORM IV Place of Publication Mysore City 1. 2. Periodicity of the Publication **B**imonthly 3. Printer's Name Dr. L. V. Venkataraman (For and on behalf of AFST (I) Nationality Indian CFTRI, Mysore-570 013 Address Dr. L. V. Venkataraman 4 Publisher's Name (For and on behalf of AFST (1) Nationality Indian Address CFTRI, Mysore-570 013 Dr. K. R. Sreekantiah 5. Editor's Name Indian Nationality Address CFTRI, Mysore-570 013 I, Dr. L. V. Venkataraman, hereby declare that the particulars given above are true to the best of my knowledge and belief.

> **L. V. Venkataraman** Signature of the Publisher

### SOLVENT EXTRACTION OF WHOLE GROUND-NUTS

Oil was extracted from whole groundnuts using petroleum ether. Roasting for a short time at temp. between 160 and  $250^{\circ}$ C facilitated skin removal and also aided in extraction. Variors extraction methods were tested. A combination of overnight soaking and a short extraction of 2 hr by refluxing was found to be ideal for maximum oil removal without cracking of the seeds. Solvent was removed from seeds by heating at 70-90°C for a few hr. Such grourdnuts were whole, without cracks and possessed good colour, odour and texture.

Groundnuts have been used mainly as source of groundnut oil. The oil content of the kernels range from 35 to 55 per cent<sup>1</sup>. The traditional methods include mechanical expression and solvent extraction of the press cake. These yield a groundnut cake which has been mainly used as live stock feed. For preparing flour or protein isolates, cake from pre-selected groundnuts is used because of the risk of mycotoxins in commercial cake. The commercial value of the deoiled groundnuts, however, will be fairly high if the oil could be removed from the kernels without their breakage. Willich and Feuge<sup>2</sup> used solvent extraction for deoiling whole They soaked dry blanched and water groundnuts. blanched groundnuts in various solvents like petroleum ether, hexane, trichlorofluoromethane, acetone and isopentane. About 70 to 80 per cent oil was extracted when soaked for 50 to 120 hr. in hexane or petroleum ether. Bhuchar et al.<sup>3</sup> have developed a modified version of soxhlet extractor which removes about 70 per cent oil from roasted whole groundnuts in 6-7 hr. Pressing was used by a number of workers to get oil without appreciably damaging the shape of the groundnuts $4^{-6}$ . In the present study, attempts have been made for solvent extraction using petroleum ether.

Spanish variety of groundnut was procured from the local market. Petroleum ether tried was of two grades: one having boiling point between 40 and 60 and the second between 60 and 80. For extraction, refluxing apparatus with water condenser was used. Moisture, fat, etc. were determined as per the standard AOCS methods.<sup>7</sup>

**Pretreatment:** Groundnut kernels may contain moisture as high as 13 per cent. This interferes with the solvent extraction of whole kernels. To overcome this, roasting was carried out at different temperatures to reduce the moisture to about 2 per cent. The temperatures between 130 and 250°C were used. Roasting also facilitates easy removal of skin manually.

*Extraction:* Extraction of each batch of 25 g was done with 200 ml portions of petroleum ether. In case of multiple extractions, each extraction was done with fresh 200 ml solvent. Extraction times ranged from 4 to 26 hr. Both soaking as well as refluxing were studied.

Solvent removal: After extraction, groundnuts were removed from the solvent and kept in open atmosphere for 10-15 min and then dried in oven for 4-5 hr at, 70-90 °C.

Oil was recovered by distilling off the solvent.

Table 1 gives the various treatments and conditions that were used. Table 2 gives the results obtained in each case.

From the results, it is clear that only refluxing does not remove sufficient oil from the groundnuts. Longer refluxing gives more oil as is shown by treatments A and B. However, the longer refluxing develops cracks and shorter time leaves oil in the centre. The removal of oil was found to be greatly improved by repeated extractions using fresh solvent. This was shown by treatment C. Higher temperatures during roasting facilitate extraction giving better removal of oil. This was seen in treatments  $D_s$ , E and F. In these treatments,

reatment	Pretrea	tment	Extraction	
	Temp °C	Time min		
А	160	30	8 hr reflux	
В	160	25	4 hr reflux	
С	160	25	2 hr reflux	
			(a) 2 hr reflux	
			(a) 2 hr reflux	
			(a) 2 hr reflux	
$D^+$	210	13	Same as in C	
E+	250	6	Same as in C	
F	210	13	Same as in C	
G	130	30	Same as in C	
			(a) 2 hr reflux	
н	160	25	24 hr soak	
			2 hr reflux	
I	160	25	20 hr soak	
			(c) 2 hr reflux	
J	160	25	20 hr soak	
			(a) 2 hr reflu	
			(b) 2 hr reflux	

<sup>+</sup>Petroleum ether of 60-80 fraction was used.

Treat- ment	Moisture after Pre- treatment (%)	Oil content (%)	Oil removed Appearance of the (% of product <sup>+</sup> total oil)
Α	2.60	47.7	59.3 Extensive cracking
В	2.45	49.4	40.0 No cracks, central portion oily.
С	2.45	49.4	70 0 Cracks developed, good in colour
D	2.10	42.6	76.0 Many cracks
Е	1.56	49.9	70.0 Extensive cracking
F	2.10	49.6	79.2 Some cracking
G	3.20	<b>49</b> .0	73.2 Some cracking
Н	2.45	49.4	57.0 No cracks. Oil spots at centre
T	2.45	49.4	87.2 Extensive cracking; chalky and brittle
J	2.45	49.4	74.1 No cracks; excellent product.
+ ATLAN	tractmente	walded wh	

TABLE 2. YIELD OF OIL AND THE APPEARANCE OF DEFATTED, GROUNDNUTS

<sup>+</sup> All the treatments yielded whole groundnuts without splitting.

higher the temperature, shorter is the time needed for roasting; longer duration caused discolouration of the product. The temperature of 210°C gave the best removal of oil. Hewever, in these experiments, oil oozes out before the skin was removed and the oil is lost along with the skin. The high temperature also causes darkening of the kernels if time is not short enough. Cracking was also observed in these kernels. In treatments D and E, since higher boiling solvent was used, its removal from seeds was found to be difficult.

Refluxing alone causes cracking problems. Hence, combination of soaking and refluxing was tried; soaking alone takes very long time. In treatment H, soaking for 24 hr after 2 hr refluxing was done. This removed only 57 per cent oil and still some oil spots were noticeable in the centre. There was no cracking of kernels. In treatment I, after refluxing and soaking additional refluxing was done to extract the residual oil from the central portions. As the results show, this gave the best removal of oil i.e. 87.2 per cent. However, extensive cracking developed and the groundnuts were chalky and very brittle. They did not have any natural flavour. Finally, soaking followed by short refluxing in treatment J, gave good removal of oil i.e. 74.1 per cent. There were also no cracks. The product was excellent with adequate colour, flavour and taste. This showed that soaking with refluxing gave good product with high commercial

value in a reasonable time of 26 hr instead of 50-120 hr extraction done by earlier workers<sup>2</sup>. Such deoiled groundnuts may not find difficulties for acceptance by the Indian population in their normal dietaries and particularly in snacks.

Dept of Chemical Technology, R. C. BELANI\* University of Bombay, Matunga Bombay-400 019, India Received 3 December 1981 Revised 5 July 1982.

#### References

- 1. Hoffpauir, C. L., Peanut composition: relation to processing and utilization. J. Agric. Fd Chem., 1953, 1, 668.
- 2. Willich, R. K. and Feuge, R. O., De-Oiling of peanuts to yield a potentially useful food product. Food Technol., 1957, 11, 332.
- 3. Bhuchar, V. M., Agrawal, A. K. and Sharma, S. K., A Study of solvent extraction of 'ipids from whole peanut kernels using all-purpose automatic solvent extractor. J. Fd Sci., 1981, 46, 25%.
- 4. Hennessey, G. R., Stansbury, M. F. and Persell, R. M., USDA creates nutritive functional products. Food Engng, 1971, 43, 71.
- 5. Ramachar, D., Thirumala Rao, S. D. and Reddy, B. R., Laboratory prepared high protein, low oil groundnuts. Indian Fd Pckr., 1971, 28, 79.
- 6. Bongirwar, D. R., Padwal-Desai, S. R. and Sreenivasan A., Studies on defatting of peanuts and soyabeans for developing ready-to-eat snack items. Indian Fd Pckr. 1977, 31. 61.
- 7. Official and Tentative Methods of Analysis, American Oil Chemists' Society, Chicago, 1966.

\*Present address: Yojana Chemicals, 304 Sujata Chambers, 1/3, Abech and Gandhi Marg, Bombay-400 009.

### **BULK DENSITIES OF OILSEEDS**

Data on bulk densities of different oilseeds and their products are given. Wide variations observed in the values are due to varietal and structural Characteristics.

A knowledge of bulk densities of oilseeds is useful in storage and transport.

In the course of work at the institute over years, data on the bulk densities of several oilseeds and their products have been accumulated.

The method used for determining bulk densities is as follows:

A representative bulk sample of the material is packed in a natural condition in a tare metallic container (one foot cube) specially constructed for the purpose and

J. S. PAI

TABLE 1. BULK DENSITIES OF OILSEEDS

			Bulk density
Common	Scientific name	Part used	kg/cubic
name			metre)
		Seed	645
Ambadi	Hibiscus sabdariffa	Seed	864
Babul	Acucia arabica	seed	605
Castor	Ricinus communis	seed	005
(Arun var)	<i>a</i> :	Sand	512
Chilli	Capsicum annum	Seed Cake	512
Coffee	Coffea robusta	Coarse particles	305
		Flakes	66
<b>C</b>	Cocos nucifera	Cup	375
Copra	Cocos macijera	Ball	329
0	Commission himseline	Wholeseed	337
Cotton J.S.	Gossypium hirsutum	Delinted seed	478
34 var			627
(Medium-		kernel	
lintered)		Hulls (delinted)	245
Cotton	-do-	Wholeseed	305
Varalakshmi		Delinted seed	420
var (High-			
lintered)		C	328
Deodar	Cedrus deodara	Seed	528 72
Gokru	Xanthium strumarium		
		Kernel	580
		Hulls	264
Groundnut	Arachis hypogea	Pods (Peanut)	255
		Pods (Coromanda)	
		Kernel "	640
		Shells ,,	110
		Cake ,,	455
Kapok	Ceiba pentandra	Seed	509
Mango	Mangifera indica	Stones	250
		Kernel	625
Mustard	Brassica juncea	Seed	685
Oil palm	Elacis quineensis	Nuts	522
		Kernel	586
Rice bran	Oryza sativa	Sheller bran	424
		Huller bran	402
		Deoiled bran	216
		(Sheller)	
		Hulls (Fine powde	
Safflower	Carthamus tinctorius		564
Salseed	Shorea robusta	Kernel	760
6	<b>C 1</b>	Hulls	250
Sesame	Sesamum indicum	Seed	630
(Brown var)		C f	7/0
Soybean	Glycine max	Seed	760
(Indigenous, black seed		Hulls	230
coat)			
Sunflower	Helianthus annus	Seed	415
Juniowei		Hulls	145
Tapioca	Manihot esculenta	Seed	145 545
Teak	Tectona grandis	Seed	200
Tobacco	Nicotina tabacum	Seed	200 540
Tamarind	Tamarindus indica	Kernel	
i amai mu	ramurmuns muica	Reinei	793

weighed at ambient room temperature. An average of replicate values is taken and the values are metricised.

The data are summarized in Table 1 so as to form a ready reference. The authors thank Mr. R. Prasada Rao Junior Scientific Assistant of the Institute for his assistance and Indian Council of Agricultural Research, New Delhi for financing shcemes on post-harvest technology of oilseeds.

Oil Technological Research Institute, Anantapur-515 001, India. Received 5 October 1981 Revised 2 June 1982 Y. VENKATESWARA RAO G. Azeemoddin D. Atchuta Ramayya S. D. Thirumala Rao

### POST HARVEST CONTROL OF SPOILAGE IN MANGO (MANGIFERA INDICA L.) WITH HOT WATER AND FUNGICIDES

Four fungicides, (Benlate, Thiabendazole, Captan and Rovral) each at 500 ppm concentration in cold or hot water were used singly to control fungal spoilage. Post harvest dip treatment of Alphonso mango with Benlate (500 ppm) in cold water (28°C) reduced the fungal spoilage significantly during storage for 12 days. It also retarded the ripening of fruits. Benlate combined with hot water dip treatment did not show further reduction in spoilage. Captan (500 ppm) and Thiabendazole (500 ppm) were found to be the next best fungicides for controlling spoilage in Alphonso mango. In 'Totapuri' cultivar, no fungicide was effective in controlling fungal spoilage. The edible quality of the fruit was not altered due to these treatments.

During ripening and storage, mangoes become susceptible to microbial spoilage<sup>1</sup> like soft rot, anthracnose<sup>2</sup> and stem-end rot<sup>3</sup>. Hot water dip treatment<sup>2</sup>, use of Benomyl<sup>3</sup>,<sup>4</sup> and fumigants<sup>5</sup> are reported to control some of the spoilages in mango.

Stem-end rot and anthracnose are the most important post harvest diseases of 2 commercial cultivars of mango namely, 'Alphonso' and 'Totapuri' in South India. It is essential to reduce this spoilage for better utilization of the fruit. In this report, an attempt has been made to examine the efficacy of four fungicides individually/ along with hot water treatment, in controlling the fungal spoilage in 'Alphonso' and 'Totapuri' mangoes.

Mature mango fruits of 'Alphonso' and 'Totapuri' grown in our experimental farm at Hessaraghatta were used. One thousand 'Alphonso' fruits were divided into lots of 25 fruits each. Four lots were used for each
treatment. Four hundred fruits of 'Totapuri' were divided into lots of 25 fruits each. Triplicates lots were used for each treatment.

The fungicides used were Benlate (500 ppm), Thiabendazole (500 ppm) Captan (500 ppm) and Rovral (500 ppm) (obtained from May and Baker India Ltd). Solutions of the fungicides were made in water containing 0.1% Tween 80. Fruits were dipped in individual fungicide solutions for 5 min, air dried and stored in ventilated wooden boxes for ripening at ambient conditions (28°C, 40-60 per cent RH). In case of hot water dip treatment, the temperature of the bath was maintained at 52°C. Fruits were treated within 24 hr after harvest.

Ripening and fungal spoilage of fruits were assessed on the 8th and 12th days after treatment. Ripening was judged by changes in colour, texture (softening) and flavour development. Fungal spoilage was detected by the development of external symptoms of the disease and further confirmed by microscopic examination. Chemical analyses for acidity and sugar content were made by following established procedures reported earlier<sup>2</sup>, using flesh homogenate of 4 fruits in duplicate samples. Vitamin C was estimated titrimetrically using 2-6, dichlorophenol indophenol dye. Experiments were carried out during 2 seasons and the results are presented here.

Ripening and spoilage: In 'Alphonso' mango, application of cold aqueous Benlate solution retarded ripening and reduced spoilage significantly (at 1 per cent level) as seen on the 8th and 12th days of storage (Table 1). Thiabendazole and Captan treatments had no effect on ripening, but reduced the spoilage (at 5 per cent level). Rovral was not useful in controlling the fungal spoilage. Hot water dip treatment at 52°C for 5 min enhanced ripening as seen on the 8th day of storage and reduced the fungal spoilage (at 1 per cent level). Combination of Benlate and hot water was not advantageous in further control of spoilage. However, the enhanced ripening due to hot water treatment was counteracted by Benlate. The extent of control of spoilage in cold water + Benlate, was on par with hot water treatment.

In 'Totapuri' mango, where the fungicide treatments were used only in cold water, there was neither change in rate of ripening nor reduction in spoilage (Table 2).

Chemical composition with respect to acidity, vitamin C and sugar content did not alter due to the fungicide treatments, except in Benlate where the total sugars/ acidity ratio was less in 'Alphonso' mango (Table 3 & 4). Firmness of the ripe fruit measured as pressure in kgs was around 2.6-3.2 in 'Alphonso' and 4.0-4.8 in 'Totapuri'.

Study on the effect of fungicides in cold and hot water in 'Alphonso' and 'Totapuri' mangoes has shown that Benlate (500 ppm) in cold water is effective in controlling the fungal spoilage in 'Alphonso' mango but not in 'Totapuri'. This difference could be attributed to structural differences, like the thickness of the peel in

		Afte	er 8 days	After 12 days		
Type of water	Fungicides	Ripe (%)	Fungal spoilage (%)	Ripe (%)	Fungal spoilage (%)	
Cold	Nii	48 (44)	23 (30)	97 (83)	34 (36)	
Cold	Benlate	18 (25)	5 (15)	62 (52)	13 (21)	
Cold	Thiabenoazole	40 (39)	12 (22)	100 (90)	20 (26)	
Cold	Captan	47 (43)	11 (21)	94 (78)	20 (26)	
Cold	Rovral	48 (44)	25 (31)	96 (85)	28 (32)	
Hot	Nil	59 ( <b>50</b> )	2 (11)	99 (87)	13 (21)	
Hot	Benlate	43 (41)	6 (16)	100 (90)	10 (16)	
Hot	Thiabendazole	55 (48)	7 (16)	94 (78)	10 (18)	
Hot	Captan	62 (52)	4 (13)	94 (78)	16 (23)	
Hot	Rovral	64 (53)	2 ( 8)	93 (79)	10 (18)	
C.D. at 5%		5.7	8.0	12.7	9.2	
C.D. at 1%		7.7	14.3	19.9	12.4	

Table 1. Effect of fungicides on Ripening behaviour and fungal spoilage in 'alphonso' mango held at  $28^{\circ}$ C rh 40-60 %

Tween 80 is used in all treatments including control.

All fungicides were used at 500 ppm level.

Transformed figure in parenthesis are used for comparison (note in the text).

T	Provide de	After	6 days	After	9 days
Type of water	Fungicide	Ripe	Spoilage	Ripe	Spoilage
Cold	Nil	25 (33)	4 (9)	83 (66)	8 (16)
Cold	Benlate	41 (40)	4 ( \$)	77 (62)	5 (13)
Cold	Thiabendazole	47 (43)	8 (15)	89 (72)	12 (20)
Cold	Captan	41(40)	9 (15)	88(70)	16 (24)

# TABLE 2. THE EFFECT OF POST HARVEST TREATMENT WITH FUNGICIDES ON RIPENING BEHAVIOUR AND FUNGAL Spoilage in 'totapuri' mango

Tween 80 is used in all treatments including control.

All fungicides were used at 500 ppm level.

Transformed figure in parenthesis is used for comparison.

None of the treatments showed significant difference.

TABLE 3. EFFECT OF POST HARVEST TREATMENT OF FUNGICIDES ON CHEMICAL COMPOSITION OF RIPE "ALPHONSO" MANGO HELD AT 28°C (40-60%RH)									
Type of water	Fungicides used	Acidity (as citric)	Vitamin C	Reducing sugars	Total reducing sugars	Sugar acid ratio	Pressure (kg)		
Cold	Nil	0.47	23	4.25	7.5	37.2	3.2		
Cold	Benlate	0.59	25	4.00	13.4	22.7	3.0		
Cold	Thiabendazole	0.46	23	3.25	16.0	28.6	2.9		
Cold	Captan	0.44	19	4.25	14.0	31.8	2.6		
Cold	Rovral	0.27	20	4.00	17.0	37.0	2.9		
Hot	Nil	0.51	28	4.75	17.0	33.0	3.1		
Hot	Benlate	0.71	23	5.00	16.0	22.5	2.9		
Hot	Thiabendazole	0.42	22	4.20	14.5	34.8	2.9		
Hot	Captan	0.40	18	3.00	14.0	35.0	2.7		
Hot	Rovral	0.42	20	3.75	14.0	33.3	2.7		
Initial (at harvest	)	2.3	98	2.7	5.2	-	12		

Tween 80 was used in all treatments including control.

All fungicides were used at 500 ppm level

Values are on % fresh weight basis.

Data are the average of 2 replicates each one respresenting 4 fruits.

TABLE 4.	EFFECT OF POST HARVEST TREATMENT WITH FUNGICIDES ON CHEMICAL COMPOSITION OF RIPP.
	'totapuri' mango held at $28^{\circ}$ c (40-60% rh)

Type of water	Fungicides used	Acidity (as citric)	Vitamin C (mg)	Reducing sugars	Total reducing sugars	Pressure (kg)
Cold	Nil	0.16	20.2	4.5	11.0	4.0
Cold	Benlate	0.15	14.8	3.8	11.0	4.7
Cold	Thiabandazole	0.12	16.7	4.0	11.5	4.4
Cold	Captan	0.12	20.0	3.2	11.2	4.8
Initial (at harvest)		1.43	30.0	3.0	5.6	12

Tween 80 was used in all treatments.

All fungicides were used at 500 ppm level

Values are on % fresh weight basis.

Data are the average of 2 replicates each of 4 fruits.

'Totapuri' resulting in non penetration of the fungicide in cold water. Similar cases of ineffectiveness of Benomyl in cold water are reported by Spalding and Reeder<sup>3</sup>, and Muirhead<sup>3</sup> in mangoes of 'Miami' and 'Queensland' respectively.

Retardation of ripening by Benlate as seen in 'Alphonso' mango could be attributed to its growth regulatory effect<sup>6</sup>. This retardation was counteracted when Benlate was used in hot water and it is known that hot water treatment enhances ripening<sup>2</sup>. Similar retardation of ripening has been recorded<sup>7</sup> when Zineb was used to control spoilage in 'Alphonso' mango. Efficacy of the fungicides in controlling fungal spoilage was not enhanced when they were used in hot water. There were no differences in consumer's acceptance of the fruit in different treatments.

Authors thank Dr. G. S. Randhawa and Dr. K. L. Chadha, Directors of the Institute for the encouragement during this investigation and United States Department of Agriculture for providing PL-480 funds for this work. Authors also thank Mr. G. S. Karibasappa, Technician for the chemical analysis and Mr. V. R. Srinivasan, Junior Statistician for the statistical analysis of the data.

Indian Institute of Horticultural Research (ICAR) 255, Upper Palace Orchards, Bangalore-560 080 (India). Received 9 November 1981 Revised 29 July 1982 SHANTHA KRISHNA MURTHY K. P. GOPALAKRISHNA RAO

#### References

- Srivastava, M. P., Tandon, R. N., Bhargava, S. N., and Ghosh A. K., Studies on fungal diseases of some tropical fruits. III. Some Post harvest diseases of mango. (Mangifera indica L.). Proc. Nat. Acad. Sci. India, Sect. B. 1965, 35, 69.
- Shantha Krishnamurthy and Subramanyam, H., Effect of maleic hydrazide and 2, 4-5, trichlorophenoxy propionic acid on ripening and quality of mango fruit. *Pestic. Sci.*, 1970, 1, 63.
- Spalding, D. H. and Reeder, W. F., Post harvest disorders of mangoes as affected by fungicides and heat treatments. *Plant Disease Reporter*, 1972, 56, 751.
- Muirhead, I. F., Post harvest control of mango anthracnose with Benomyl and hot water. Australian J. exp. Agric. Animal Husbandry, 1976, 16, 600.
- Subramanyam, H., Murthy N.V.N., Subhadra, N. V. and Muthu, M., Control of spoilage and inhibition of ripening in *Alphonso mangoes* by fumigation. *Trop. Sci.*, 1972, 11, 120.
- Skene, K.C.M., Cytokinin-like properties of the systemic fungicide benomyl. J. lort. Sci., 1972, 47, 179.
- Subramanyam, H. and Murthy, N.V.M., Control of spoilage in mango fruit by Zineb and Sodium diethyldithiocarbamate. *Pestic. Sci.*, 1973, 4, 25.

#### STEEPING PRESERVATION OF FRUITS

Fruits like, peach, plum, nectrine, grape, guava, banana, pear and pineapple were preserved in steeping solutions having 30 per cent sugar, 0.4 per cent acidity and 400 ppm of  $SO_3$ , in glass jars. All the fruits except plum and nectrine were found acceptable after four months steeping.

Fruit production in Punjab State has increased during the past few years<sup>1</sup>. During the seasonal glut, prices are fairly low, but in the off season, there is a great increase in the prices. Canned fruits are very costly, and some of their desirable qualities like texture and flavour are lost. It is, therefore, important to develop simpler techniques of preservation, which can be used by the house-wives in the urban as well as in the rural areas. The preservation of fruits by steeping, does not require costly equip ment or packaging materials. It is of practical interest to the fruit industry, by way of extending the seasons's availability of the fruits.

Varieties of Peach, plum, nectrine (Nectrine is a type of peach, which is fuzzless. The cultivar used is 'Sun Red' introduced from Florida, U.S.A.), grape, guava, banana, pear and pineapple were procured from the orchard of Punjab Agricultural University or from the local market (Table 1). After washing thoroughly in tap water, pineapple and pear were cut into slices and halves respectively. Banana was peeled, while other fruits were kept as such. Except grape and banana all the fruits were blanched for 4 min in boiling water. They were filled in glass jars and covered with 30 per cent sugar syrup having 0.4 per cent acidity and 400 ppm. of  $SO_2$ . The ratio of fruit to steeping solution was 1:1. The jars were kept at room temperature from June to November.

The fruits were analysed for total soluble solids (TSS), acidity, ascorbic acid when fresh, as well as after four months steeping preservation (Table 1). The syrup also was tested for TSS., acidity, ascorbic acid and SO<sub>2</sub> content and change in colour after storage. (Table 2). The organoleptic evaluation was carried out by ten semitrained panelists, by a 7-point scale with extremely desirable=7, very desirable=6, slightly desirable=5, neither desirable nor undesirable = 4, slightly undesirable =3, very undesirable =2 and extremely undesirable =1. Average scores of these attributes are given in Table 3. The TSS was determined by hand refractometer and acidity by titration method<sup>2</sup>. Ascorbic acid was determined using 2,6 dichlorophenol indophenol dye for titration<sup>2</sup>. The residual  $SO_2$  was determined by Monior Williams method<sup>2</sup>.

It is evident from Table 1, that the increase in TSS of fruits is related to the texture of the fruit, the relation is more in peach (var. Flordasun) and less in pineapple

Fruit Variety			Fresh fruit		Drained fruit					
rruit	Variety -	TSS (°Brix)	Acidity (% citric acid)	Ascorbic acid (mg/100 g)	TSS (°Brix)	Acidity (% citric acid)	Ascorbic acid (mg/100 g)	SO <sub>2</sub> (ppm)		
Plum	Alucha Black	9.0	1.92	1.0	25.0	1.80	0.0	35		
Nectrine	Sun Red	8.0	0.82	5.1	23.0	0.80	3.2	40		
Peach	Shan-e-Punjab	7.0	0.96	6.0	25.0	0.92	3.5	52		
Grape	Perlette	23.0	0.57	1.5	26.0	0.56	1.0	40		
Grape	Anab-e-Shahi	15.0	0.43	2.0	24.0	0.43	1.5	40		
Grape	Beauty Seedless	19.0	0.45	1.0	25.0	0.45	0.0	40		
Peach	Flordasun	7.0	1.00	6.0	25.0	0.95	3.5	52		
Guava	Allahabad Safeda	11.0	0.44	186.0	17.8	0.43	56.0	60		
Pear	Patharnakh	8.5	0.42	2.0	21.0	0.42	0.0	60		
Banana		28.4	0.14	5.1	29.0	0.36	2.1	<b>6</b> 0		
Pineapple	_	11.5	0.87	8.0	18.0	0.62	4 0	65		

#### TABLE 1. CHEMICAL COMPOSITION OF THE FRESH AND DRAINED FRUITS

TABLE 2. COMPOSITION OF SYRUP AFTER FOUR MONTHS

Fruit	Variety	SO <sub>2</sub> (ppm)	Acidity (% citric acid)	TSS (°Brix)	Syrup colour
Plum	Alucha Black	210	0.45	28.5	Light red
Nectrine	Sun red	220	0.43	29.0	Slight red
Peach	Shan-e-Punjab	220	0.43	29.0	No change
Peach	Flordasun	220	0.44	28.8	No change
Grape	Perlette	200	0.42	28.0	Light pale green
Grape	Anab-e-shahi	200	0.41	28.5	Slight green
Grape	Beauty Seedless	180	0.40	28.0	Light reddish
Guava	Allahabad Safeda	220	0.40	29.0	No change
Pear	Patharnakh	240	0.42	28.5	No change
Banana		210	0.35	29.8	Slight whitish
Pineapple		220	0.43	28.5	No change

Original TSS of syrup was 30°Brix; acidity was 0.4% citric acid and; SO<sub>2</sub> content was 400 ppm.

TABLE 3. ORGANOLEPTIC SCORE OF DRAINED FRUITS AFTER FOUR MONTHS OF STEFPING

Fruit		Variety		Colour and appearance	Texture	Flavou
Plum		Alucha Black		3.5	3.5	3.0
Nectrine		Sun red		3.0	3.6	3.2
Peach		Shan-e-Punjab		6.6	6.2	6.5
Peach		Flordasun	- · ·	6.0	5.0	6.6
Grape		Perlette		6.5	6.1	6.3
Grape	10 420	Anab-e-Shahi		6.3	6.3	6,5
Grape		Beauty Seedless		6.4	6.0	6.0
Guava		Allahabad Safeda		6.2	5.5	6.8
Pear		Patharnakh		6.5	6.0	5,9
Banana		_		6.4	5.5	6.1
Pineapple		_		6.8	6.0	6.4

guava and pear. The acidity decreased in all those fruits which had acidity higher than that of syrup, but in banana, it increased to the level of acidity of the syrup. Ascorbic acid decreased in all the fruits. Fresh guava had 186 mg/100 g of ascorbic acid, which decreased to 56 mg/100 g after storage. The residual SO<sub>2</sub> in the fruits ranged from 35 to 65 ppm.

There was slight change in the composition of the steeping syrup (Table 2). A slight decrease in TSS was noticed in all the solutions. In banana, acidity decreased, whereas it increased slightly in other fruits. The  $SO_2$  content decreased in all the solutions and it ranged from 180 to 200 ppm

The red colour of plum, nectrine and grapes (Beauty Seedlus var.) was reduced appreciably (Table 3). There was slight leaching of the green pigment in grapes (var. 'Perlette' and 'Anab-e-Shahi). There was no marked change in the colour of peach, guava, pear and pineapple. The skin of plum, nectrine and peach (var. Flordasun) loosened slightly, resulting in soft texture of the fruit. Plum and nectrine lost their natural flavour and white film was found on the surface of the solution. These two fruits were found unacceptable organoleptically, while other fruits like peach, guava, grape, banana, pineapple and pear, which had their normal texture and flavour were acceptable.

Department of Food Sci. & Tech., Punjab Agricultural University, Ludhiana-141 004, India. Received 8 January 1982 Revised 29 July 1982

#### References

- 1. Statistical Abstract of Punjab, Govt. of Punjab, Chandigarh, India 1979.
- 2. Official Methods of Analysis, Association of Official Analytical chemists, Washington, D. C., 11th Ed. 1970.

### USE OF TOMATO SEED POWDER AS AN ANTI-OXIDANT IN BUTTER AND GHEE

Tomato seed powder at 5% level added to fats, inhibits rancidity and ensures their stability practically to the same extert as 0.01% of Butylated hydroxytoluene (BHT) or Butylated hydroxyanisole (BHA).

In the processing of tomatoes for different products such as juice, puree, sauce, ketchup, etc. the wastes which include skin, core, trimmings, culls and seeds, comprise nearly 20 per cent of the tomatoes. These wastes can be utilized for the preparation of several useful products<sup>1</sup>. The seeds have been reported to posses antioxidant property<sup>2</sup>, and as such, it was of interest to study its effectiveness as an antioxidant in butter and ghee.

Different methods have been reported for the separation of seeds from tomato pomace, namely, gravity separation<sup>3</sup>, water or dilute hydrochloric acid or washing soda treatment prior to drying of pomace<sup>4</sup> in the sun or rotary drying of press cake<sup>5</sup> etc. In the present studies, sun drying of the cannery waste obtained from NAFED factory, followed by separation of the seeds using 50



Fig. 1. Oxidation experiments on white butter

A. Control sample of white butter; B. White butter + 1% tomato seed; C. White butter + 3% tomato seed; D. White butter + 5% tomato seed; E. White butter + 0.01% BHT; F. White butter + 0.01% BHA; G. Ghee from white butter + 5% tomato seed;





H: Control sample of U.P. ghee; I: U.P. ghee+1% tomato seed powder; J: U.P. ghee+3% tomato seed powder; K: U.P. ghee+5% tomato seed powder; L: U.P. ghee+0.01% BHT; M: U.P. ghee+0.01% BHA:

per cent hydrochloric acid was found satisfactory and also economical.

The scparated seeds were powdered in a small grinder and mixed with white butter and ghee at 1, 3 and 5 g per cent levels. BHT and BHA at 0.01 per cent concentration were used for comparison. The treated samples were dried in an oven at 100°C (373K) and the peroxide values determined by standard ISI method<sup>6</sup>. The analytical data are shown graphically in Fig. 1 & 2, for butter and ghee respectively.

The peroxide values were reproducible within 5 per cent variation. It will be seen that with increasing level of tomato seed powder, the peroxide value and hence the degree of oxidation decreases in a given interval of time. Addition of powder at 5 per cent had practically the same effect as 0.01 per cent BHT or BHA. These results are in agreement with those reported in the case of sun flower and rapeseed oils<sup>2</sup>. The tomato seeds are non-toxic and also rich in essential amino acids and oil can serve as a useful natural antioxidant instead of BHT or BHA. Work is in progress to identify the component or components in the seeds which are responsible for the antioxidant property. Thanks are due to NAFED for providing the fruit waste.

Centre for R.D. and A.T.	S. P. S. GULERIA
I.I.T., Delhi	P. VASUDEVAN
·	K. L. Madhok
Received 28 October 1981	S. V. PATWARDHAN
Revised 30 August 1982	

#### References

- 1. Guleria, S. P. S., Vasudevan, P. and Patwardhan, S. V., Utilization of Tomato Seeds and Waste, (Communicated).
- Szanto-Nemeth, E., Inhibition of rancidity of fats by paprika and tomato seeds. Acta Alimentaria, 1980, 9, 173.
- 3. Eggers, L. K. and Geisman, J. R., Studies concerning the protein of tomato seed recovered from tomato cannery waste. *Ohio Agric. Res. and Dev. Centre Res. Cir. Wooster*, Ohio, 1980, 213, 18.
- Brodowskii, D. and Geisman, J. R., Protein content and amino-acid composition of protein of seeds from tomatoes at various stages of ripening. J. Fd Sci., 1980, 45, 228.
- Edwards, E. W., Eskew, R. K., Hoersch, A. N., Aceto, N. C. and Redfield, C. S., Recovery of tomato processing wastes. *Fd Technol.*, 1952, 6, 383.
- 6. Indian Standard Methods of Sampling and Test for Ghee IS: 3508-1966, Indian Standards Institution, New Delhi.

### IS POTASSIUM SORBATE NECESSARY FOR PRESERVING CANNED BUTTER?

Four types of canned butter, namely, (a) normal butter, (b) normal butter + 0.1% potassium sorbate, (c) normal butter + 10% whole milk powder and (d) normal butter + 0.1% potassium sorbate + 10% whole milk powder were studied for their microbiological keeping quality over a period of 3 months stored at-10°C, 27°C and 37°C. Addition of whole milk powder prolonged the stability of butter under higher ambient storage temperatures. The presence of air prolongs the survival of moulds in butter. Although potassium sorbate effects a reduction in the microbial status, its addition appears to be unnecessary and may lead to substandard materials being marketed.

Butter (canned) normally requires low temperature storage for protection from curdling, deemulsification and mould growth<sup>1</sup>. However, due to limited refrigeration facilities in the field areas, butter is stored at ambient temperatures only by the defence personnel. Under high temperature conditions prevalent in many parts of India during summer, the butter gets separated into fat and curd with the product no longer looking smelling or tasting like butter, making it unacceptable. A process using 10 per cent whole milk powder was found highly successful to counteract this<sup>2</sup>. However, since the addition of milk powder increases the microflora and nutrient content, the manufacturers made a plea for the addition of potassium sorbate. It was therefore, investigated whether the incorporation of potassium sorbate in normal or modified canned butter is necessary.

Four types of treatments were given namely,

- (a) normal butter;
- (b) butter containing 0.1 per cent potassium sorbate,
- (c) butter containing 10 per cent whole milk powder, (d) butter containing 0.1 per cent potassium sorbate
- and 10 per cent whole milk powder.

These were manufactured by one of the Defence suppliers as per ASC Specifications<sup>3</sup>. It contained milk solids 1 per cent, milk fat 80 per cent and sodium chloride 3 per cent. Initial analysis was carried out for moisture, total viable count, proteolytic and lipolytic organisms, coliforms, yeasts and moulds as per standard methods4.5. There was a time lapse of nearly one month between the manufacture of canned butter and their receipt at the laboratory. Each treatment was divided into 3 lots and were stored at -10°C, 27°C and 37°C. and periodically analysed up to a period of 3 months. Butter of a commercial brand was procured, from local market, mixed with 10 per cent whole milk powder and inoculated with spores of a mould earlier isolated from butter and similar studies were carried out. Butter without milk powder served as control. These were studied under two different conditions. One was kept in a flask with cotton plug to give aerobic condition and the other set was canned.

The initial viable count ranged from 3.5 to 5 logs (Table 1). Coliforms were present in negligible numbers (less than 10/g) in the initial samples. None of these were found to be of the faecal type. Staphylococci were present in substantial numbers, many of which were positive for coagulase, D Nase and phosphatase. A few of them were found to be enterotoxigenic producing enterotoxins A, B, C and E. Caseolytic and lipolytic organisms were present in large numbers. As butter is chiefly composed of butter fat and milk casein, they are important in bringing about spoilage.

The control sample (normal butter) showed the highest bacterial load. Addition of potassium sorbate reduced the total microflora (Table 1). This could be expected, as potassium sorbate is bacteriostatic against catalase positive bacteria<sup>6</sup>. However, its addition does not seem to be necessary, as there is no increase in any of the microbial groups throughout the storage period in samples not containing potassium sorbate. The increase in coliforms and mould count observed by Kaul et al.7.8 in control samples may be due to higher moisture content and lack of NaCl in butter.

In the samples containing milk powder, the bacterial population is lower than that of control. Since addition of milk powder increases the milk solids content, it was

Storage period	Moisture		Total	viable	count	Staphylococci		C	laseolyti	с	Lipolytic				
(days)	-10°C	27°C	37°C	-10°C	27°C	37°C	-10°C	27°C	37°C	-10°C	27°C	37°C	-10°C	27°C	37°C
					1	Normal	butter (	Control	)		*				
Initial	16.1	16.1	16.1	5.2	5.2	5.2	3.8	3.8	3.8	4.2	4.2	4.2	3.3	3.3	3.3
45	15.6	16.0	16.4	_	3.8	3.6	_	2.6	1.6	_		2.6		2.8	1.8
90	15.5	14.4	-	5.3	1.8	2.2	3.5	1.8	nil	4.5	2.1	1.6	3.9	1.6	1.6
					В	utter + 0	.1% Pot	. sorbat	e						
Initial	16.3	16.3	16.3	3.7	3.7	3.7	2.5	2.5	2.5	2.7	2.7	2.7	2.6	2.6	2.6
45	16.8	16.9	16.8		3.5	2.3	_	3.3	3.9		3.5	3.8	_	2.9	3.8
90	16.1	15.2		3.5	3.3	1.6	2.1	2.1	1.5	2.4	2.9	2.5	2.4	1.3	2.5
					Butte	r + 10%	whole r	nilk po	wder						
Initial	14.7	14.7	14.7	4.6	4.6	4.6	3.7	3.7	3.7	3.6	3.6	3.6	3.6	3.6	3.6
45	15.0	14.9	15.0	_	3.7	4.8	—	3.2	3.3	_	3.4	3.2		2.9	2.1
90	15.0	14.7		3.6	3.1	2.3	3.0	2.2	1.3	3.5	2.2	2.0	2.6	2.3	2.0
				Butter -	- 0.1 %	Pot sort	oate + 10	% whol	e milk	powder					
Initial	15.0	15.0	15.0	4.3	4.3	4.3	3.1	3.1	3.1	3.8	3.8	3.8	3.7	3.7	3.7
45	15.0	15.0	15.0	_	3.4	4.3		3.1	4.1	_	3.4	4.0	—	3.3	3.8
90	15.0	14.9	—	4.2	3.3	2.9	3.1	1.8	_	3.4	2.6	2.6	3.2	2.7	1.8

reasonable to expect a higher load. Perhaps the intrinsic nature of butter might have restricted the survival of many of the micro-organisms present in the milk powder. Besides, the addition of milk powder, lowers the water activity  $(a_w)$  of the modified butter and might have reduced the microbial flora. Model experiments carried out by introducing mould spores in all the four types of butter and studying the mould count on storage showed drastic reduction in their survival.

On storage, there was a gradual reduction in all the groups of microflora. Presence of potassium sorbate showed higher reduction as this is active against catalase positive bacteria also. Samples stored at -10°C showed very slow decrease in the microbial profile even at the end of 3 months due to the low biological and chemical activity at this temperature. However, in the study on the use of potassium sorbate for improving the quality of butter, Kaul et al.7,8 observed an increase in the coliform count of control at -18°C during the early part of storage. This is rather unusual as the minimum temperature of growth of all bacteria and particularly the coliforms fall much above this. The coliforms will decrease in number drastically on shifting to -18°C as they are among the most sensitive to freezing and frozen storage. They further reported that the mould count and coliform count of initial butter as  $0.1 \times 10^4$ /g and  $1.6 \times 10^{5}$ /g respectively. It could be presumed that in this case the original butter samples themselves might have been heavily contaminated and unacceptable. Since the purpose is not to make unacceptable butter sample acceptable, the incorporation of potassium sorbate is not only unnecessary, but may lead to substandard material being marketed.

Inoculation studies showed that the mould spores introduced were unable to grow and in course of time died off (Fig. 1.). Moulds being aerophilic require oxygen for their growth and hence in the hermetically sealed atmosphere, death was faster. In flasks with cotton plugs they survived longer. However, surviving numbers slowly reduced on longer storage due to the very intrinsic nature of the substrate. It is therefore, concluded that addition of potassium sorbate is unnecessary in canned butter and maintaining the moisture content within specifications and addition of 2 per cent sodium chloride are sufficient for its preservation.

As the present day trend is to reduce the use of additives and preservatives as far as possible<sup>10</sup> and to use them only when absolutely essential, we suggest that the use of sorbic acid and its salts in butter need not be encouraged.

We are deeply indebted to Dr P.K. Vijayaraghavan,



Fig. 1. Effect of air on the survival of inoculated mould spores.

1. Butter+milk powder (cotton plug); 2. Butter+Milk powder (sealed); 3. Butter (cotton plug); 4. Butter (sealed).

Director, Defence Food Research Laboratory, Mysore for his interest in the work and encouragement.

Defence Food Research Laboratory	R. Sankaran
Mysore-570 011. India,	M. S. MOHAN
Reseived 7 April 1981	R. K. LEELA
Revised 6 May 1982	

#### References

- Hammer, B. W. and Babel, F. J. Dairy Bacteriology, John Wiley & Sons, Inc. New York, 4th Ed. 1957, 455.
- 2. Defence Food Research Laboratory, unpublished data.
- 3. Methods of Sampling and Test for Butter, ASC Specification No. 22. 1973.
- Harrigan, W. F. and McCance, M. E., Laboratory Methods in Food and Dairy Microbiology, Academic Press, 1976, 208.
- 5. Methods of sampling and test for butter, IS 3507-1966., Indian Standards Institution. New Delhi.
- Furia, T. E., Hand Book of the Food Additives, Chemical Rubber Co., Cleveland, Ohio, 1968.
- Kaul, A., Singh, J. and Kuila, R. K. Effect of sorbate on the microbiological quality of butter. J. Fd Prot., 1979, 42, 656.
- Kaul, A., Singh, J. and Kuila, R. K. Potassium sorbate as preservative of butter. J. Fd Prot., 1981, 44, 33.
- Michener, H. D. and Elliot, R.P., Microbiological conditions affecting frozen food quality. in *Quality and Stability of Frozen foods*, Ed. W. B. Van Arsdel, M. K. Copley and R. L. Olson, Wiley InterScience. 1969, 68.
- Baird-Parker, A. C., Food Microbiology in the 1980s. Fd Technol. Austr. 1980, 32, 70.

## AEROBIC MESOPHILIC COUNT OF FRESH AND REFRIGERATED GROUND MUTTON: EFFECT OF PLATING AND INCUBATION TEMPERATURE

Pour and spread plate methods were compared at incubation temperature of 30 and  $37^{\circ}C$  for enumeration of aerobic mesophiles in fresh and refrigerated ground mutton. Spread plating resulted in significantly higher recovery of microbial contamination than pour plating in both fresh and refrigerated meats. Incubation temperature of 30 or  $37^{\circ}C$  may be used for screening retail market meats and  $30^{\circ}C$  for refrigerated meats.

Aerobic mesophilic count has been one of the most useful indicators of the microbiological status of foods<sup>1</sup>. The three commonly used procedures for enumerating aerobic mesophilic microorganisms are pour plate<sup>2</sup>,<sup>3</sup> spread plate<sup>4</sup> and drop plate<sup>5</sup> methods. Pour plate or Standard Plate Count (SPC) is the most widely used method in India<sup>2</sup>, North America<sup>6</sup>,<sup>7</sup> and is recommended by International Standard Organisation (ISO)<sup>3</sup>. Spread plate method is used in Europe<sup>8</sup> and is recommended by British Standards Institution<sup>9</sup>. The International Commission on Microbiological Specifications for Foods (ICMSF), however, recommended retention of pour plate method, inspite of its limitations, since alternate methods are not studied extensively<sup>10</sup>.

Microorganisms have diverse temperature limits for their growth. Incubation temperature between 30 and 37°C is used in different laboratories<sup>1</sup>. Indian Standards Institution (ISI)<sup>2</sup> and ISO<sup>3</sup> recommended incubation temperature is 30°C.

The purpose of the present study was to compare pour plate and spread plate methods at incubation temperature of 30 and 37°C for the enumeration of aerobic mesophiles in fresh and refrigerated ground mutton.

Fourteen samples of retail ground mutton and sixteen samples of refrigerated ground mutton were used in the present study. Twenty-five gram sample of meat was blended in 225 ml of 0.1 per cent peptone-water for 1 min in a pre-sterilized blendor. Serial decimal dilutions were prepared in 9 ml volumes of 0.1 per cent peptonewater. All dilutions were plated in Standard Plate Count Agar<sup>2</sup>,<sup>3</sup>. For each dilution, pour plates<sup>2</sup>,<sup>3</sup> and spread plates<sup>4</sup> were prepared in duplicate for each incubation temperature of 30 and 37°C. Pour plates were incubated at 30°C for 72 hr and at 37°C for 48 hr. Spread plates were incubated at 30°C and 37°C for 48 hr.

Spread plating resulted in significantly higher recovery of microbial contamination than pour plating at the same incubation temperature of 37 or 30°C from both fresh and refrigerated ground mutton (Table 1). A highly significant difference (P < 0.01) was observed TABLE 1. MEAN AEROBIC MESOPHILIC COUNT ( $\log_{10}/g$ ) of fresh and refrigerated ground mutton by two plating methols at two incubation temperatures

Incubation	Ground mutton			
(°C)	Fresh	Refrigerated <sup>+</sup>		
37	6.13ª	6.09 <sup>a</sup>		
	(11.25)	(8.99)		
30	6.24ac	6.44 <sup>b</sup>		
	(11.24)	(7.28)		
37	6.30bc	6.28¢		
	(11.25)	(8.08)		
30	6.41 <sup>b</sup>	6.64 <sup>d</sup>		
	(8.92)	(7.38)		
	temp (°C) 37 30 37	temp (°C)         Fresh           37         6.13 <sup>a</sup> (11.25)           30         6.24 <sup>ac</sup> (11.24)           37         6.30 <sup>bc</sup> (11.25)           30         6.41 <sup>b</sup>		

Figures with the same superscript in a column do not differ significantly.

<sup>+</sup>Period of storage  $4 \pm 1$  days.

<sup>++</sup>Figures in parenthes indicate coefficient of variation.

between the colony counts obtained by the two plating methods at 30°C incubation for fresh meat and at 37°C for refrigerated meat. Higher estimates of bacterial populations by spread plating was reported by Clark<sup>11</sup> in processed poultry and also in ISO studies on meat<sup>1</sup>. The 95 per cent confidence limits of the mean differences between the two plating methods at the same incubation temperatures was less than 0.35 log<sub>10</sub> cycles in fresh and refrigerated samples. Krammer and Gilbert<sup>12</sup> showed variation in counts of less than  $0.5 \log_{10}$  cycles in 98 per cent of the samples with no significant difference between the plating methods from a wide range of foods. The differences in the colony counts may be due to oxygen requirements of the bacteria predominating in the food<sup>1</sup> and the heat sensitivity of the organisms particularly, psychrotrophs, to molten agar medium in pour plating<sup>13</sup>.

Plate incubation temperature differs depending upon the nature of the food and the type of microflora to be enumerated. In microbial evaluation of fresh ground mutton, no significant difference was found in the mesophilic counts between the incubation temperatures by using pour or spread plating (Table 1) probably due to higher proportion of mesophiles in retail market meat. Gill and Newton<sup>14</sup> showed that the microflora was predominantly mesophilic during early stages of holding meat at 30°C, although the final aerobic spoilage flora was composed of approximately equal numbers of psychrotrophic and mesophilic bacteria. A highly significant difference (P<0.01) was found in the colony counts of refrigerated samples between the incubation temperatures since many psychrotrophic organisms in food have maximal growth temperatures between 30 and  $32^{\circ}C.^{15,16}$ . The 95 per cent confidence limits of the mean difference was 0.8 log<sub>10</sub> Cycles between pour plate method at  $37^{\circ}C$  and spread plating at  $30^{\circ}C$ .

The present study suggests the use of spread plating in the microbial evaluation of fresh and refrigerated ground mutton. Spread plating requires less medium, fewer petri dishes, less expensive pipettes and the prepoured plates can be moved from laboratory to the field<sup>10</sup>. Plate incubation temperature of 30 or 37°C may be used for screening retail market meats and 30°C for refrigerated meats. The main disadvantage of spread plates when compared with pour plates is their inadequacy when bacterial populations are very low (less than 100/g) as 0.1 ml, is usually the maximum inoculum that can be applied per plate<sup>10</sup>. However, microbiological criteria for mesophiles in foods are likely to be well above this figure.

Thanks are due to Director, I.V.R.I. for providing facilities.

Division of Livestock Products Technology Indian Veterinary Research Institute, Izatnagar-243 122. India. Received 12 October 1981 Revised 1 July 1982 T. R. K. MURTHY

#### References

- 1. Christian, J.H.B., Methodology in Food Microbiology The Present Position in the Microbiological specifications for Foods, F.A.O., Rome, 1975, 27.
- 2. Method for Standard Plate Count of Bacteria in Food Stuffs, 15:5402. Indian Standards Institution, New Delhi, 1969.
- 3. General guidance for enumeration of microorganisms-Colony count technique at 30 C, Draft International Standard ISO/DIS 4833 Geneva, 1976.
- Gilliard, S. E., Busta, F. F., Brinda, J. J. and Campbell, J.E., in the Compendium of Methods for the Microbiological Examination of Foods., Speck, M. L., (Ed) American Public Health Association, Washington, 1976, 120.
- 5. Miles, A. A. and Misra, S. S., The estimation of the bactericidal power of the blood. *Hygiene*, 1938, 38, 732.
- 6. Official Methods of Analysis, Association of Official Analytical Chemists, Washinton, 11th ed., 1970.
- 7. Recommended Methods for the Microbiological Examination of Foods. American Public Helath Association, 1966.
- Barraud, C., Kitchell, A. G., Labotes, H., Reuter, G. and Simonsen, B., Standardisation of total aerobic count in meat and meat products. *Fleischwirtschaft*, 1967, 47, 1313.
- Methods for microbiological examination of meat and meat products, Part I. Enumeration of microorganisms—Colony Count at 30 C, BS:5393, British Standard Institution, London, 1976.

- Eliott, R. P., Clark, D. S., Lewis, K. H., Lundbeck, H., Elson, J. C. and Simonsen, J. C., *Microorganisms in Foods Their significance and methods of enumeration*, University of Toronto Press, 1978, 114.
- Clark, D. S., Comparison of pour and surface plate methods for determination of bacterial counts. *Can. J. Microbiol.*, 167, 13, 1409.
- Krammer, J. M. and Gilbert, R. J., Enumeration of microorganisms in food a comparative study of five methods. J. Hyg. Camb., 1978, 81, 151.
- Vanderzant, C. and Matthys, A. W., Effect of temperature of plating medium on the viable count of psychrophilic bacteria. J. Milk Fd Technol., 1965, 28, 383.
- 14. Gill, C. O. and Newton, K. G., Growth of bacteria on meat at room temperature. J. aprl. Bact., 1980, 49, 315.
- Eliott, R. P., Temperature gradient incubator for determining the temperature range of growth of microorganisms J. Bacteriol., 1963, 85, 889.
- Ingraham, J. L. and Stokes, J. L., Psychrophilic bacteria. Bact. Rev., 1959, 23, 97.

## MICROBIAL DEGRADATION OF CELLULOSIC MATERIALS: SCREENING OF FUNGAL ISOLATES

Several fungal isolates obtained from air, decaying plant debris and other sources were screened for their ability to produce cellulases both in liquid cultures and by solid state fermentations. Of the the twenty three isolates which showed significant activity, only *Pencicillium* sp. and *Aspergillus oryzae* gave consistently high enzyme titres. A strain of *Sporotricihum pulverulentum* degraded cellulose and released large quantity (1720/g/ml) of reducing sugars into the medium.

Interest in the hydrolysis of cellulose has increased in recent years for its conversion to glucose. Studies were undertaken to identify and isolate cellulolytic fungi which arc capable of sccreting significant quantity of cellulolytic enzymes. It was observed that some fungal strains secrete cellulolytic enzymes into the medium, while few other strains attack cellulosic substrates and release significant quantities of reducing sugars. The results are presented in this communication.

Microorganisms: Strains of fungi, isolated from air, decaying plant debris and other sources, along with a few selected strains being maintained at the Microbiology and Fermentation Technology Discipline of the Central Food Technological Research Institute, Mysore, were used in these studics. Purification was done by hyphal-tip method. All the cultures were maintained on potato-dextrose-agar slants. Liquid medium: The mineral solution of Reese and Mandels<sup>1</sup> containing 1 per cent alkali treated rice straw (ATS) as the carbon source, was used in the experiments. Erlenmeyer flasks (250 ml) containing 50 ml medium were autoclaved at 1.1 kg/cm<sup>2</sup> for 20 min. cooled to room temperature and inoculated with fungal spore suspension. The flasks were incubated at ambient temperature (25-30°C) on a rotary shaker having a stroke of 5 cm and revolving at 230 r.p.m.

Delignification of rice straw was carried out by autoclaving 100g air-dried straw in 1.8 litre of 1 per cent NaOH solution fcr 1 hr at 120°C followed by thorough washing in tap water till free of alkali, followed by drying.

Solid medium: Wheat bran was mixed with the mineral solution<sup>1</sup> in the ratio of 1:1 and 4 g of this medium was dispensed into 50 ml Erlenmeyer flasks, autoclaved at 1.1 kg/cm<sup>2</sup> for 45 min and cooled to ambient temperature. The medium was inoculated with fungal spore suspension and incubated at ambient temperature (25-30°C).

Cellulase activity: Filter paper degrading activity (FPD) which gives an overall assessment of cellulose attack by the fungus, was determined by the procedure described by Mandels *et al.*<sup>2</sup>. This assay was carried out with the culture filtrate and aqueous extract of the solid medium. From each gram of air dried mouldy bran, 10 ml water extract was obtained.

FPD activity was determined by adding 0.5 ml enzyme solution to 1 ml 0.05 M citrate buffer (pH 4.8) with a strip of filter paper (Whatman No. 1) measuring 1  $cm \times 6$  cm weighing 50 mg. The reaction was carried out in  $1.5 \times 15$  cm test tubes at 50°C for 60 min. After completion of the reaction, 3 ml of dinitrosalicylic acid (DNS) reagent was added and the amount of reducing sugar released was measured. One micromole of glucosc (0.18 mg) released per minute was taken as a unit of activity. Cellulase activity was calculated by making use of the formula:

 $\frac{\text{mg glucose released}}{\text{assay time in min} \times 0.18}$ 

Screening trials: Out of the 108 fungal strains tested for cellulase activity and cellulose hydrolysis, only 23 showed a fair amount of activity. The cellulase activity and the reducing sugars accumulated in the cuture filtrate, after 96 hr of growth of selected fungal strains, are presented in Table 1.

The results incicate that 23 strains of fungi secrete significant quantities of cellulase. The culture broth of *Sporotrichum pulverulentum* contained maximum quantity of reducing sugars. One strain of *Penicillium* sp. gave the highest enzyme activity. TABLE 1. CELLULASE ACTIVITIES AND REDUCING SUGAR PRODUCED BY A FEW SELECTED FUNGAL STRAINS IN LIQUIL SHAKE CULTURE USING ATS AS SUB<sup>C</sup>TRATE AFTER 96 HR

Strain	Reducing sugar in the filtrate (mg/1000ml)	FPD activity in the filtrate I.U./ml x 10 <sup>3</sup> )
Aspergillus niger (1)*	60	53
A. niger (1t)	32	39
A. niger (17)	41	45
A. niger (18)	22	46
A. niger (35)	30	56
A. oryzae (55)	56	57
A. oryaze (61)	10	39
A. oryaze (63)	27	32
A. oryaze (64)	80	57
A. wentii (52)	27	48
A. wentii (59)	253	_
A. carbonarius (60)	92	51
A. carbonarius (67)	40	42
A. carbonarius (68)	30	58
A. terreus (23)	113	31
A. flavus (24)		24
A. japanicus (66)	42	65
Aspergillus sp. (2)	106	46
Aspergillus sp. (36)	65	35
Aspergillus (7)	46	24
Penicillium sp. (6)	66	86
Penicillium sp. (32)	20	52
Sporotrichum pulverulentum (	(39) 1720	40

\*Figures in parenthesis indicate the serial number of fungal strains used in the trial.

Cultivation on solid substrates: It is the common experience that, fungi produce large quantities of cellulase when grown on solid substrates. Toyama and Ogawa<sup>3</sup> found that strains of *Trichoderma viride* produce more FPD. cell separating enzyme activity, avicellase, carboxymethyl cellulase, 3, glucosidase, chitinase and cellobiase activity in solid state fermentation than in liquid cultures. Fifteen strains of fungi, which had given high enzyme titres on liquid shake cultures were selected and grown on wheat bran solid medium. The cellulase activities of the mouldy bran were determined. The results are presented in Table 2.

The mouldy bran obtained from *Aspergillus* strains contained large amounts of reducing sugars. Cellulase production after 96 hr of growth was the highest in

TABLE 2.	CELLULASE ACTIVITIES AND REDUCING SUGAR PRODUCED	
BY A FE	W SELECTED FUNGAL SPECIES ON SOLID CULTURE USING	
	WHEAT BRAN AS SUBSTRATE AFTER 96 HR	

	Reducing sugar	FPD activity in
Strain	in the filtrate	the filtrate
	(mg/1000 ml)	I.U./ml×10 <sup>3</sup> )
Aspergillus niger (1)*	1650	12
A. niger (16)	1240	120
A. niger (18)	860	87
A. niger (35)	780	124
A. oryzae (55)	1950	397
A. oryzae (61)	860	—
A. carbonarius (60)	1175	115
A. carbonarius (67)	1175	130
A. carbonarius (68)	300	490
A. japanicus (66)	2700	259
Aspergillus sp. (2)	2620	98
Aspergillus sp. (36)	2580	40
Penicillium sp. (6)		240
Penicillium sp. (32)	690	137
Sporotrichum pulverulentum (39)	1000	314

\*Numerals in parenthesis indicate the serial number of fungal strains used in the trial.

Aspergillus carbonarius followed by Penicillium sp. which gave higher activity in liquid medium also.

This preliminary study shows that several strains of *Aspergillus, Penicillium* and *Sportotrichum* can break down modified cellulose material or utilize wheat bran, both in broth or solid cultures. The application of selected organisms in degrading various modified, cellulosic waste materials for practical utilization is, therefore, under study.

Central Food Technological	K. Theja
Research Institute	T. R. SHAMALA
Mysore-570 013, India.	K. R. Sreekantiah
Received 20 April 1982	V. Sreenivasa Murthy
Revised 6 July 1982	

#### References

- 1. Reese, E. T. and Mandels, M., Induction of cellulases in *Trichoderma viride* as influenced by carbon sources and metals. J. Bacteriol., 1957, 73, 269.
- Mandels, M. R., Andreotti R. and Roche, C., Measurement of saccharidying cellulase. *Botach. Bioengng, Symp.*, 1976, 6, 21.
- Toyama, N. and Ogawa, K., Cellulase production by Trichoderma viride in solid and submerged culture methods. International Course on Biochemical Engineering and Bioconversion, February 7-19, 1977, BERC, Indian Institute of Technology, New Delhi.

## A NOTE ON ANTIBIOTICS SENSITIVITY OF E. COLI ISOLATED FROM MARKET MILK OF LUDHIANA CITY

Sensitivity to antibiotics of *E. coli* isolated from market milk of Ludhiana city was studied. The results showed that the isolates were most sensitive to kanamycin and least sensitive to oxytetracyclia. Sensitivity to antibiotics like erythromycin, Kanamycin, Oxytetracyclin, streptomycin, ledramycin, cloxacillin, amplcillin, polymyxin-B and chloramphenicol was shown by 39.62, 84.90, 30.16, 73.58, 43.58, 52.83, 40.14, 79.24 and 62.81 per cent of the isolates respectively. Isolates from raw milk were highly sensitive to kanamycin and polymyxin-B while those from pasteurized milk were sensitive to kanamycin and streptomycin. Isolates from water and hands were highly sensitive to kanamycin, polymyxin-B and chloramphenicol. However, the enteropathogenic strains showed a variable response to these antibiotics.

Many food poisoning outbreaks have been reported<sup>1-5</sup> incriminating *E. coli* from many countries, due to consumption of contaminated foods including dairy products. The enteropathogenic strains of this organism have also been responsible for idipoathic, acute and infantile diarrhoea in Bangla Desh<sup>6</sup>, India<sup>3</sup> and United Kingdom,<sup>1</sup> while a study of Boston City Hospital<sup>7</sup> has revealed bacteramic infection attributed to *E. coli*. In view of the prevalence of enteropathogenic strains in foods and in hospitals, the role of sensitivity testing of the antibiotics employed for treatment against it, becomes very important. A study was undertaken to investigate the antibiotics sensitivity of *E. coli* isolated from market milk, water and swabs from hands. The results are reported in this communication.

Samples of raw and pasteruized milk, water and swabs from hands were collected from Ludhiana City according to recommended methods<sup>8,9</sup>. These were analysed for total viable bacterial and E. coli counts as per the Standard methods<sup>6,9</sup>. The incidence and distribution of E. coli in various samples is given in Table 1. Out of a total of 55 samples analysed, 39 (70.91 per cent) were found contaminated with E. coli (Table 1). The average E. coli content of raw milk from different sources ranged from 1, 285 to 11,040/ml, while in pasteruized milk E. coli was 32/ml. Water and swabs from the hands gave a count of 18,425 and 4,655/ml. and square cm. respectively. For further investigation, cultures were isolated from the E. coli positive samples. These cultures were characteriszed morphologically and biochemically according to Bergey's Mannual<sup>10</sup>. The biochemical tests were carried out according to standard methods<sup>11</sup>. Enteropathogenicity of 30 isolates was studied by the

Type/source	No. of samples	<i>E. coli</i> conta	minated samples	Av. E. coli	Av. total bacterial
	collected	No.	(°/)	counts/ml	counts/ml
Raw milk					
Milk vendors	5	5	100.00	11,040	1,88,440
Shops	8	7	87.50	13,900	1,06, <b>0</b> 63
Darries	11	8	72.72	7,141	85,556
Canteens	2	2	100.00	1,285	1,56,000
Pasteurised milk	22	10	45.45	32	15,991
Dairy	3	3	100.00	18,425	59,772
Swabs* from hands	4	4	100.00	4,665	22,685

TABLE 1. DISTRIBUTION OF E. COLI AND TOTAL VIABLE BACTERIAL COUNTS IN VARIOUS SAMPLES

rabbit ileal loop method<sup>12</sup> and only 4 of these isolates were found enteropathogenic.

The antibiotics sensitivity of these isolates was determined by dry disc diffusion technique<sup>13</sup>. The antibiotics in the form of paper discs were obtained from Desai Laboratories, Surat. The concentration of the antibiotics and the symbols used for them is as follows:

Antibiotics	Symbols	Concn.
Annoiones	<b>By</b> moons	mcg/disc
Erythromycin	(E)	10
Kanamycin	(K)	30
Oxytetracyclin	(0)	10
Streptomycin	(S)	25
Ledramycin	(L)	10
Cloxacillin	(V)	10
Ampicillin	(I)	10
Polymyxin-B	(X)	250
Chloramphenicol	(C)	50

Criterion for testing the sensitivity of an isolate was the presence or absence of Zone of inhibition formed around the antibiotics disc.

The results of antibiotics sensitivity presented in
 Table 2 showed that kanamycin, polymxin-B, strepto mycin and chloramphenicol are the effective antibiotics since approximately 70 per cent or more of the total isolates were sensitive to these antibiotics, while cloxacillin and ledramycin inhibited the growth of 52.16 and 43.38 per cent of the isolates respectively. Garanin<sup>14</sup> reported the antibiotics sensitivity of chloramphenicol, erythromycin and oxytetracyclin to be 72.00, 21.40 and 31.00 per cent respectively against *E. coli*. Ampicillin and erythromycin inhibited the growth of approximately 40 per cent of the isolates, while oxytetracyclin gave very low sensitivity of 30.16 per cent towards these isolates. Polakava *et al.*<sup>15</sup> has reported

TABLE 2. SENSITIVITY OF E. COLI ISOLATES FROM RAW AND PASTEURIZED MILK, WATER AND HANDS TO DIFFERENT ANTIBIOTICS

Source, type	Total	Number of sensitive isolates to indicated antibiolses								
	isolates	E	К	0	S	L	v	I	x	C
Raw milk	32	12 (37.50)	26 (81.25)	10 (31.25)	22 (68.75)	12 (37.50)	15 (46.75)	14 (43.75)	27 (84.36)	21 (65.62)
Pasteurized milk	9	5 (55.56)	9 (100.00)	4 (44.45)	7 (77.78)	4 (44.45)	5 (55.56)	4 (44.44)	5 (55.56)	6 (66.67)
Water and hands	12	4 (83.33)	10 (83.33)	2 (16.66)	10 (83.33)	7 (58.33)	8 (66.63)	4 (33.33)	10 (83.33)	10 (83.33)

Figures in parentheses indicate percentages.

F, K, O, S, L, V, I, X, C, are antibiotics (See text)

TABLE 3.	ANTIB	IOTICS SENSITIVITY	OF ENTROPA	THOGENIC E. COLI
	lsola	te No.	Antibiotics	o which sensitive
	3	SHI	К,	I
	7	SH2	<b>К, S</b> ,	, V, I, X
	19	DI	K, S	, 1, C
	29	D2	K, S	, L, X

All isolates are from raw milk SH for shops; D for dairies Symbols used for antibiotics see text.

ampicillin while the findings of Milch *et al.*<sup>16</sup> indicated 26.41, 30.16, 69.81 and 59.84 per cent of the isolates resistant to streptomycin, chloramphenicol oxytetra-cyclin and ampicillin, respectively.

The isolates from raw milk, pasteurized milk, water and hands differed in sensitivity to different antibiotics (Table 2). The results showed that more than 16 per cent of the isolates from all the sources were sensitive to all the antibiotics tried, while more than 33 per cent of the isolates were sensitive to all the antibiotics except erythrimycin and oxytetracyclin. Further, it was observed that more than 60 per cent of the total isolates were sensitive to kanamycin, streptomycin and chloramphenicol. Kanamycin was effective against 80 per cent of the isolates from raw milk, water and hands while 100 per cent of the isolates from pasteurized milk were sensitive to this antibiotic. Other antibiotics tested exhibited intermediate sensitivity to these isolates. Isolates from water and hands were found to be more sensitive to kanamycin, streptomycin, polymyxin-B and chloramphenicol. The enteropathogenic strains (Table 3) indicate a variable sensitivity to different antibiotics tested.

It is clear from this study that although the antibiotics like kanamycin, polymyxin-**B**, streptomycin, and chloramphenicol are effective against this organism, yet there is need for carrying out the antibiotics sensitivity testing before starting antibiotic therapy for treatment against this organism.

S. S. KAHLON

V. K. JOSHI

Dept. of Microbiology, Punjab Agricultural University, Ludhiana, India. Received 22 September 1981 Revised 21 June 1982

#### References

- 1. Gross, R. I., Scotlant, S. M. and Rowe, B., Enterotoxin testing *E. coli* causing epidemics infantile enteritis in U.K. *Lancet*, 1976, 1, 629.
- Mehlman, I.J.D., Fishbein, M., Sherwood, L. G., Sanders, A.C., Eide, E. L. and Olson, J. C., Methodology for enteropathogenic *E. coli. J. Ass. off. anal. chem.*, 1976, 59, 283.
- Pande, R., Bacteriology of infantile diarrhoea and gastroenteritis in Allahabad. Indian J. Path Microbiol., 1976, 19, 169.
- Sack, R. S., Sack, A. D., Mehiman, I. J. Crskov, F. and Oskov I., Interotoxigenic *E. coli* isolated from foods. *J. Infect-Dis.* 1977, 135, 313.
- Shiffrin, F. A. and Osttlosvaskays, R. Ya., Outbreak of food poisoning caused by *E. coli. Gig Sanit.*, 1963, 28, 80.
- Nalin, D. R., Mclaughlin, M., Rehman, M. Yanus and Culin, G., Enterotoxigenic *E. coli* and indiopathic diarrhoea in Bangla Desh. *Lancet*, 1976, 2, 1116.
- McGowan, J. E. Jr. and Barnes, M. V., Bacteremia at Boston city Hospital: Occurrence and mortality during 12 selected years (1935-1972) with special reference to hospital acquired cases. J. Infect. Dis., 1975, 132, 316.
- Standard Methods for Examination of Dairy Products., American Public Health Association, Inc. New York, 10th ed. 1960.
- Harrigan, W. F. and McCance, E. M., Laboratory Methods in Microbiology, Academic Press, London and New York, 1966.
- Breed, R. S., Murray, E.G.D. and Smith, N. R., Bergey's Manual of Determinative Bacteriology, Williams and Wilkins, Co., Baltimore, 8th ed. 1957.
- Official Methods of Analysis, Association of Official Analytical Chemists, Washington, Secs. 41.00-41.016, 11th ed., 1970.
- 12. De, S. N., Bhattacharya, K. and Sarkar, J. K., A study of pathogenicity of strains of *Bacterium coli* from acute and Chronic enteritis. J. Path. Bact., 1956, 71, 201.
- Cruickshank, R., A Guide to Medical Microbiology: Laboratory Diagnosis and Control of Infection, E. S., Livingstone, London, 1966.
- Garanin, B. A., Sensitivity of enteropathogenic *E. coli* 0124 to some antibiotics. *Antibiotica*, 1970, 15, 251.
- Polakava, O., Janous, K. J., Hustavova, H., Stankovsky, Iand Krocmery, V., Occurrence of *E. coli* strains with multiresistance and transferable resistance to antibiotics in food stuff. J. Hyg. Epidem. Microbiol. Inumun. 1972, 16, 467.
- Milich, H., Gynes, M. and Harmen, G., Characterisation on the basis of phage restriction of R. Plasmid, derived from *E. coli.* strains, *Proc. 2nd Int. Symp. on Antibiotic Resistance and drug inactivating enzymes*, Castls of Smolenice, Czechoslovakia, 1974, 391.

### Advances in Nutritional Research, Vol. 4: (Edited by Harold H. Draper)—A Review, 1982, pp. 344, Price: \$ 39.50

The fourth volume in this series is devoted to reviews of recent advances in nutritional research related to both human beings and animals. The present volume covers the following chapters: vitamin responsive genetic abnormalities; vitamin D binding proteins; vitamin D compounds in human and bovine milk; dietary protein, metabolic acidosis and calcium balance; the nutritional significance, metabolism and function of myo-inositol and phosphatidyl inositol in health and disease; neurology of pyridoxine; carnitine biosynthesis: nutritional implications; insect nutrition: a comparative perspective; the nutrient requirements of cultured mammalian cells; and fatty acid metabolism in the neonatal ruminant.

The first chapter focusses attention on certain aspects of vitamin responsive genetic disorders. Such conditions have recently attracted much attention from human genetecists and those concerned with inborn error metabolism. The significance of research in this area is, that it can provide a tool for elucidating the normal metabolic pathways.

The second chapter describes the vitamin D binding protein (DBP) and their role in transport of active forms of vitamin D sterols in blood and their binding in tissues. Not much is known about the metabolism of DBP. Its possible role in the development of vitamin D deficiency needs to be studied. The third chapter describes the vitamin D compounds in human and bovine milk and their origin. The mode of transfer of the vitamin from plasma to milk and the transfer of the vitamin from plasma and the relevance of the milk levels of the vitamin in the intestinal transport of calcium in neonates are discussed.

In chapter-4, dietary protein, metabolic acidosis and calcium balance are discussed. Recent observations on the effect of high protein diets on Ca excretion are discussed in terms of acidosis produced by high protein diets. Acidosis appear to be an important cause of osteoporosis and represents an attempt on the part of the body to use of the buffer capacity of the bone to maintain ph homeostasis.

In chapter-5, the nutritional significance, metabolism and function of myo-inositol and phosphatidyl inositol in health and disease are discussed. This chapter brings into focus recent studies on inositol as an essential factor in human nutrition. It is now recognised that many of the functions of inositol can be attributed to cellular level of inositol containing phospholipids and though present in small amounts they may have an important role in membrane function.

The role of pyridoxine in neurobiology is discussed in chapter-6, in terms of the role of vitamin  $B_2$  dependent enzymes in the metabolism of amino acids leading to the formation of biogenic amines.

Chapter-7 describes carnitine biosynthesis and its nutritional implications. There has been increasing interest in the carnitine which plays an important role in the transport of fatty acid in muscle. This chapter describes the current studies on the biosynthesis of carnitine and discusses the nutritional situations under which carnitine levels may alter and their possible biological implications.

In chapter-8, insect nutrition and its possible similarities with mammalian nutrition are discussed. There appear to be sufficient similarities between the two, that data obtained with the insects may be applied to mammals. The need for further research in insect nutrition to evolve a suitable model to study the nutrition and metabolism of mammals is emphasized.

Chapter-9 describes the research developments on the nutritient needs of cultured mammalian cells. There has been considerable interest in the use of cultured cells for experimental purposes, particularly in the studies related to human nutrition and precise information on the nutritional requirement of these cells are important in such studies.

Fatty acid metabolism in the neonates is still a controversial subject and the last chapter describes the metabolism and transport of fat across placenta, digestion, transport of fat in a neonate and metabolism of lipid in foetus of newborn. This chapter also discusses some aspects of brown adipose metabolism in the newborn.

This volume, like its predecessors presents timely review and recent developments on the important aspects of nutrition, placing emphasis on the newer developments like nutrition of cell in culture which has the potential to become an important tool in the future nutrition research. This volume will be of special interest to clinical nutritionists and researchers engaged in experimental nutrition and metabolic studies.

> B. S. NARASINGA RAO NATIONAL INSTITUTE OF NUTRITION, HYDERABAD.

- An introduction to fish handling and processing by I. J. Clucas and P. J. Sutcliffe, Report No. G. 143. Tropical Products Institute, London, 1981, pp. iv+86; Price: £ 2.60
- Fish handling, preservation and processing in the tropics: Part 2. I. J. Clucas (Compiler). Report No. G 145. Tropical Products Institute, London, 1982, pp. vii+144; Price £ 4.05.

The first of the above two reports, as its summary states, "presents notes on twelve lectures, which in conjunction with practical demonstrations and audio visual aids provide the basis for a one-week training course suited to middle-level administrators and managers. These notes range broadly all over post-harvest aspects of handling, preservation, processing and storage of fish. Chilling, freezing, salting, drying and smoking are described in detail with illustrations of the different processing equipment available. The various instruments used in the fish processing industry are also described."

The second report, also presenting notes on lectures to be given to the same type of personnel, covers: detailed discussion of the methods in traditional fish preservation by salting, drying, smoking, fermentation, marination and boiling, description of the processes of canning, freeze-drying and irradiation, description of fisheries products and by-products, and discussion of subjects such as quality assessment, microbiology relating to spoilage and public health, landing and retail facilities, extension services and training.

These two reports should serve as very useful guides for all those engaged in teaching the principles of fish preservation and imparting practical training in the field. Training personnel and trainees will find the reports extremely valuable.

> N. V. SRIPATHY C.F.T.R.I. Fish Technology Experiment Station, Mangalore.

Economic aspects of small-scale fish canning: by D. Edwards, P. Street and I. Clucas Report G 151, Tropical Products Institute, London  $i\nu+36$ , pp. Price: £ 1.40.

The Indian fish canning industry comprises of about 70 canneries, but possibly only a fifth of this number are now operating. As a means of conserving fish for human consumption, their role unfortunately is not of any significance. Canned fish products are too expensive for the average consumer. The few fish canning units which are still surviving marginally, draw sustenance from limited urban markets, some demand during recent years in the N-E region of India and army purchases. The capital blocked up in unused and underutilised fish canneries perhaps has to attract some remedial action, but then in the overall industrial scene, the fish canneries form a drop in the ocean, too small to elicit attention. Lest we forget, it is the little drops of water that make a mighty ocean.

Occasionally, nowadays, we do hear of priorities given to revival of sick industrial units. Perhaps, it would be worthwhile to give it a try whether some unconventional approach might overcome this sickness. For instance, if all the components of direct and indirect duties and taxes that add up to the final consumer price of canned fish products are withdrawn by the magic wand of a policy decision, it is possible, hopefully, that the lowered cost to the consumer would trigger a chain reaction of expanded market, increased output, put idle installed capacity into harness and lead on to the revival of the health of the fish canning industry. The overall revitalisation of the economy of this sector of small scale industry might well result in significant quantities of marine fish being canned, first for the domestic market and later for export as well. On the other hand, by a hide-bound approach, canned fish products which have become so dear with direct and indirect duties and taxes, and due to the high cost of containers, could be dubbed luxury items and taxed further, leading to the extinction of the industry. Technological exercises like introduction of aluminium cans in place of tin cans or the eventual commercialisation of packing in flexible pouches would remain curiosities in the Indian context.

All this points to the pressing need for an economic re-appraisal of the state of Indian fish canning units. The report entitled "Economic aspects of small-scale fish canning" brought out by the Tropical Products Institute, London, purports "to indicate to administrators, planners and potential investors, the technical and economic factors essential to evaluate the establishment and successful operation of a small fish canning enterprise in tropical countries". Three basic cost models based on canning of sardines have been used for the financial analysis. On the basis of 250 day 8-hr shifts worked annually, the models are of production capacity per shift of: 10,000 cans, 20,000 cans and 10,000 cans substituting labour for machinery for beheading and gutting of fish and labelling of cans. The last model is nearest to the Indian situation. The discounted cash flow method has been used to analyse the cost models. A project life of 10 years has been assumed. Sensitivity analysis to a marked seasonality of fish resulting in reducing of shifts worked to 150 per year shows an adverse effect on profitability. Difficulty and expense of obtaining cans and other inputs tend to shift the project towards becoming nonviable. These indeed seem to be the very constraints under which the Indian fish canneries are now operating.

This report by the Tropical Products Institute should serve as the basis, for all those with real concern, for a study of the present malaise and the bleak future of the Indian fish canning industry.

> N. V. SRIPATHY C.F.T.R.I. FISH TECHNOLOGY EXPERIMENT STATION MANGALORE.

Psychrotrophic micro-organisms in spoilage and pathogencity. (Ed. by J. H. Roberts, G. Hobbs, J.G.B. Christian and N. Skovgaaed) Academic Press, London New York, Toronto, Sydney, 1981, pp. 526, £ 24.00

This book is based on the proceedings of the XI International Symposium on food microbiology organised by the Committee on Food Microbiology and Hygiene of the International Union of Microbiological Societies held at Aalbory, Denmark on 6-11 July 1980. The papers presented at the Symposia are grouped into three parts. The first part includes papers dealing with the fundamentals of microbial activity at low temperature. Subjects covered in this part refer to a comparative study of the physiology of psychrotrophic and psychrophilic bacteria, the properties by gelfiltration of extra cellular lipase enzymes of psychrotrophic bacteria, growth potential of most common salmonellae and arrhizona between 3-17°C and phenetic affliation of psychrotrophilic bacillus. The last paper in this part tries to show that dissemination of R-plasmids by conjugation in food stuff during normal storage continues particularly at high temperatures.

The second part referring to low temperature spoilage of foods, includes a larger number of papers which are sub-divided again into 5 subsidiary groups based on the type of food material examined. In the first subgroup-A the papers deal with some enzyme systems in milk stored at low temperature, taxonomy of psychrophiles and on evaluation of different methods of analysis. In the 2nd subgroup—B, papers deal with the organisms involved in the spoilage of meat at low temperature with particular reference to Microbacterium thermosphactum. The subgroup C and D deal with the psychrotrophic bacterial flora of fish and those of agricultural products respectively. The fifth subgroup in this part is mainly concerned with the influence of the microclimate on psychrotrophic microbial activity in food.

The third part includes papers dealing with the ecology of psychrotrophic pathogens. Under two subgroups in this part, Yersinia enterocolitic, Clostridium botulimum and Leptospires are examined with respect to their taxonomy, ecology and pathogeniocity.

This is indeed a very valuable compilation of papers written by competent people. At a time when refrigeration storage is becoming the choice for storage, these papers vividly present the advantages and disadvantages of cold storage. For any research on psychrophiles or psychrotrophies, this book provides the required guidelines for future work. Hence, this will be a very valuable addition to the library used by research workers on psychrophiles.

> V. SREENIVASA MURTHY C.F.T.R.I., Mysore.

Food Carbohydrates: (edited by David R. Lineback and George E. Inglett), IFT Basic symposium series; AVI Publishing Company, U.S.A. 1982, pp. 494, Price: £ 49.50.

This book represents the proceedings of a symposium held on June 5-6 1981, by the Institute of Food Technologists and the International Union of Food Science and Technology, U.S.A. The 23 papers presented at the symposium deal with (i) simple sugars-fructose, polyols, sucrose and lactose (ii) honey, corn and maple syrups (iii) food polysaccharides (iv) dietary fiber (v) Maillard reaction (vi) lectins and covered aspects of chemistry, structure, analysis, metabolism, food applications of derived and new carbohydrates, taste, consumption patterns, health implications and regulatory status. Each author reviewed his area of work and emphasized the future course of research.

The progress in the field of analysis related to the online application of modern analytical methods have been reviewed using micro processor-aided digital read out refractometers, densitometers, HPLC and atomic adsorption spectrophotometers in the determination of carbohydrates and ash content and development of analytical schemes for detailed fiber analysis.

The need to understand (i) the structure of native and modified starches, (ii) the interaction of starch with hydrocolloids and proteins, (iii) the processes of gelatinization and retrogradation of starch and (iv) the conformation of polysaccharides in pure and combined systems of solutions and gels was pointed out, in order to construct models for interpreting structures in the natural context and for guiding fabrication of new foods.

The food application of lactose and its derived products, polyols, high fructose corn syrups in bakery, beverages, canned fruits and vegetables, confectionery, dairy products and dietetic foods have been described. The health implication of carbohydrates has received due attention. The reported adverse effects of excessive sucrose consumption in causing dental caries, elevation of serum triglycerides and impairment of the insulinproducing system depend on a number of factors, necessitating caution in drawing conclusions. More knowledge about the physiological effects of fiber in the human diet was felt necessary for evaluating its role. The advocacy of special dietic foods, promotion of new foods and labelling of marketed foods require restraint and care.

The book with its good coverage of the subject, useful bibliography and index is a valuable addition to the libraries of research and teaching institutions devoted to nutrition, food science and technology.

> M. N. SATYANARAYANA C.F.T.R.I., Mysore.

# NATIONAL SYMPOSIUM ON SURFACTANTS,

# EMULSIONS AND BIOCOLLOIDS

### (Fundamental and Industrial Aspects)

October 27-29, 1983

Department of Chemistry and Department of Food Technology and Biochemical Engineering Jadhavpur University is holding the above Symposium from October 27-29, 1983 at Calcutta.

The technical sessions are intended to comprise of invited lectures, presentation of papers and recommendation for future work through panel discussion on the following areas:

1. Fundamental and applied aspects of surfactants, micells, emulsions and microemulsions.

2. Physical chemistry of biopolymers, biosurfactants, biogels and interaction of biopolymers.

Interested persons may contact the Conveners, Prof. S. K. Aditya and Prof. D. K. Chattoraj, National Symposium on Surfactants, Emulsions and Biocolloids, Jadhavpur University, Calcutta-700 032 for further details.

### Delhi Chapter

The Annual General Body Meeting was held on 1st February 1983 and the following Office bearers were elected: *President*—Mr. Laljeet Singh, *Vice-President* — Dr. J. S. Pruthi, Dr. (Mrs) K. K. Sharma, *Secretary*— Mr. O. P. Grover, *Jt. Secretary*—Mr. N. K. Dadlani, *Treasurer*—Mr. Y. K. Kapoor and *Editor*—Dr. Susanta K. Roy.

#### Hyderabad Chapter

The Annual General Body Meeting was held on 19th March, 1983 and the following Office bearers were elected: *President*—Mr. P. V. Surya Prakasa Rao, *Vice*-

President—Mrs. Yamuna Ranga Rao, Secretary— Mr. Surendra Kumar Sood, Jt. Secretary—Mr. V. V. L. Narasimham, Treasurer—Mr. B. D. Tripathi.

#### **Trivandrum Chapter**

A seminar on "Utilization of cassava for production of alcohol" was jointly sponsored by the Trivandrum chapter of AFST(I), State Committee on Science & Technology, Govt. of Kerala and RRL, Trivandrum on 2nd December 1982. The seminar was inaugurated by Dr. K. Gopalan, Vice-Chancellor, Cochin University and Presided by Dr. Vasudev, Chairman, Kerala State Committee on Science and Technology.

## NATIONAL SYMPOSIUM ON QUICK FROZEN FOODS (Present Status and Prospects)

November 12-13, 1983

The Delhi Chapter of the Association of Food Scientists and Technologists (India) is conducting the above Symposium from November 12-13, 1983 at New Delhi.

The main object of the Symposium is to assess the present status, recent trends and future scope for development and manufacture of frozen foods in India. The subject areas proposed to be covered in the Symposium are:

- 1. Frozen Meat and Poultry Products
- 2. Frozen Fish and Crustaceans
- 3. Frozen Dairy and Bakery Products
- 4. Frozen Fruits, Vegetables and Products
- 5. Food Machinery for Freezing of Foods
- 6. Quality Control and Standardization of Frozen Foods
- 7. Packaging, Storage, Transportation & Distribution of Frozen Foods.

For further information regarding Symposium, interested persons may please contact:

Mr. O. T. Grover Hony. Secretary AFST(I) Delhi Chapter C/o Gardners' Corporation 6, Doctor's Lane New Delhi-110 001

## 

# **INSTRUCTIONS TO AUTHORS**

- 1. Manuscripts of papers (*in triplicate*) should be typewritten in double space on one side of bond paper. The manuscripts should be complete and in final form, since only minor corrections are allowed at the proof stage. The paper submitted should not have been published or data communicated for publication anywhere else. Review papers are also accepted.
- 2. Short communications in the nature of Research Notes should clearly indicate the scope of the investigation and the salient features of the results.
- 3. Names of chemical compounds and not their formulae should be used in the text. Superscripts and subscripts should be legibly and carefully placed. Foot notes especially for text should be avoided as far as possible.
- 4. Abstract: The abstract should indicate the principal findings of the paper. It should be about 200 words. It should be in such a form that abstracting periodicals can readily use it.
- 5. Tables: Tables as well as graphs, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. They should be typed on separate paper. 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.

٢

٢

- 6. Illustrations: Graphs and other line drawings should be drawn in *Indian ink* on tracing paper or white drawing paper preferably art paper. The lettering should be in double the size of printed letters. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size 16cms (ox axis) × 20cms (oy axis); photographs must be on glossy paper and must have good contrast; *three copies* should be sent.
- 7. Abbreviations of the titles of all scientific periodicals should strictly conform to those cited in the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.
- 8. References: Names of all the authors along with title of the paper should be cited completely in each reference. Abbreviations such as *et al.*, *ibid. idem*, should be avoided.

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

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

- (a) Research Paper: Jadhav, S. S. and Kulkarni, P. R., Presser amines in foods, J. Fd Sci. Technol., 1981, 18, 156.
- (b) Bock: 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 Verkataraman, K., Academic Press, Inc., New York, 1952, Vol. II, 966.
- (d) Proceedings, Conferences and Symposia Papers: Nambudiri, E. S. and Lewis, Y. S., Cocoa in confectionery, Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India, Mysore, May 1979, 27.
- (e) Thesis: Sathyanarayan, Y., Phytosociological Studies on the Calcicolous Plants of Bombay, 1953, Ph.D. Thesis, Bombay University.
- (f) Unpublished Work: Rao, G., unpublished, Central Food Technological Research Institute Mysore, India.

9. Consult the latest copy of the Journal for guidance.

# JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Vol. 20 No. 3 May/June 1983 Contents of forthcoming issue **Research** Papers EFFECT OF HEAT PROCESSING ON THE PASTE VISCOSITY OF CEREAL FLOURS S. N. Raghavendra Rao, S. Sreedhara Murthy and H. S. R. Desikachar STUDIES ON BEBONTOT-A TRADIONAL BALINESE PORK PRODUCT M. B. Arihantana and K. A. Buckle STUDIES ON THE PROCESSING OF CULLED APPLES Ghulam Hassan Shah and B. S. Bhatia INSECTICIDAL POTENTIAL OF INDIGENOUS PLANTS: COMPARATIVE EFFICACY OF SOME INDIGENOUS PLANT PRODUCTS AGAINST MUSA DOMESTICA L. S. M. Ahmed and Harish Chander OPTIMISATION OF CONDITIONS FOR MALTING OF SORGHUM R. A. Pathirana, K. Sivayogasundaram and P. M. Jayatissa A MECHANISM FOR BREAKING EGG AND SEPARATING ALBUMEN S. K. Mahaptra and H. Das **Research Notes** EFFECT OF SURFACTANTS, FATTY ACIDS AND GLYCERIDES ON THE GELATINIZATION VISCOSITY OF ATTA (WHEAT FLOUR) S. S. Arya and M. C. Narasimha Murthy CHEMICAL AND MICROBIOLOGICAL EVALUATION OF STORED GUAVA PULP IN PVC **CONTAINERS** D. K. Tandon, S. K. Kalra, J. H. Kulkarni and K. L. Chadha SYNERGISTIC ACTION OF GIBBERELLIN AND ETHREL ON THE INDUCEMENT OF SPROUT-ING IN POTATOES M. N. Shashi Rekha, M. V. Rama and P. Narasimham EFFECT OF SCOURING AND CONDITIONING VARIABLES ON MILLING, RHEOLOGICAL AND BAKING PROPERTIES OF INDIAN WHEATS H. P. S. Nagi and G. S. Bains CARBOHYDRATE COMPOSITION OF MUSTARD (BRASSICA JUNCEA) SEED MEAL T. C. Sindhu Kanya and M. Kantharaj Urs GELATINIZATION OF WEANING FOOD INGREDIENTS BY DIFFERENT PROCESSING CONDITIONS H. N. Chandrasekhara and G. Ramanathan SED'MENTATION AND EXTENSOGRAPH CHARACTERISTICS OF SOME WHEATS IN RELA-TION TO GLUTEN COMPOSITION **B.** P. Ram and S. N. Nigam INCORPORATION OF TEXTURIZED SOY PROTEINS IN FRESH PORK SAUSAGES G. S. Padda and N. Kondaiah GAS CHROMATOGRAPHIC DETERMINATION OF MENTHOL IN MENTHOLATED SWEETS AND PANMASALA M. Veerabhadra Rao, M. N. Krishnamurthy, K. V. Nagaraja and O. P. Kapur INHIBITION OF GROWTH AND AFLATOXIN B<sub>1</sub> PRODUCTION OF ASPERGILLUS PARA-SITICUS BY SPICE-OILS R. Tiwari, R. P. Dixit, N. C. Chandan, A. Saxena, K. G. Gupta and D. E. Vadehra COUNTRACTION CONTRACTOR CONTRACTOR CONTRACTOR

Printed and Published by Dr. L. V. Venkataraman, Secretary, AFST (India), CFTRI, Mysore-570013, at Sharada Press, Mangalore-575 001.