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## ANNOUNCEMENT

### *JOURNAL OF FOOD SCIENCE AND TECHNOLOGY*

In view of the high increases in production costs, we have been forced to increase the subscription rates from Volume 19, 1982.

The new rates are given on inside front cover and are operative from January 1982. Membership rates are not increased. Subscribers are requested to renew their subscriptions for 1982 at the new rates and cooperate.

Hony. Secretary

## Chemical Constituents of Kokam Fruit Rind

N. KRISHNAMURTHY, Y. S. LEWIS AND B. RAVINDRANATH

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*Manuscript received 17 July 1981; revised 24 September 1981*

**Chemical investigation of the kokam fruit rind revealed the presence of (-) hydroxycitric acid, cyanidin-3-glucoside and cyanidin-3-sambubioside. Their isolation, identification and determination as well as the proximate composition of kokam are described.**

Kokam (*Garcinia indica*: family: Guttiferae) trees are found in the tropical rain forests of western ghats from Konkan southwards in Karnataka, Coorg and Wynaad, and also in southern parts of Maharashtra as well as in West Bengal and Assam. The tree flowers in November–February and fruits ripen in April–May. The kokam of commerce is prepared by sun-drying the rind (skin) of the ripe fruits after repeatedly soaking it in the juice of the pulp<sup>1,2</sup> and sometimes after treatment with salt (salted kokam). The fresh rind is somewhat thick and red (dark purple) in colour and constitutes about 50–55% of the whole fruit. The fruit has an agreeable flavour and a sweetish acid taste.

Kokam has been traditionally used as an acidulant. It is also used to make an attractive red, pleasant-flavoured extract for use as a beverage. The kokam fruit is anthelmintic and cardiogenic, and is used in treatment of piles, dysentery, tumours, pains and heart complaints. A syrup from the fruit juice is given in bilious affections. The root is astringent.<sup>3</sup> Kokam seed is a good source of fat which is known as 'Kokam butter' in commerce. The characteristics and composition of kokam butter are given by Jamieson<sup>4</sup>.

Detailed chemical investigation of kokam fruit has not been carried out earlier. We have recently isolated and determined the structures of two new polyisoprenylated phenolic compounds, garcinol and iso-garcinol present in the hexane extract of the fruit rind<sup>5</sup>. The isolation and identification of the acid and anthocyanin pigments of kokam are reported here.

### Materials and Methods

Red ripe fruits obtained from an orchard near Mangalore are used in these studies. The rind was separated from the rest of the fruit and dried in a cross-flow drier at 60°C. This dried material was

used for analysis of the proximate principles by standard A.O.A.C. methods.<sup>6</sup> The acid in the fruit was estimated by titrating against 0.1 N sodium hydroxide using phenolphthalein as indicator and calculated as hydroxycitric acid. Ascorbic acid was determined by using the xylene extraction method<sup>7</sup>. Pectin was estimated as calcium pectate<sup>8</sup>.

*Isolation of hydroxycitric acid:* The dried kokam was cut into small bits and subjected to solvent extraction for 16 hr with acetone. The dark brown extract was concentrated to remove the solvent and the residue was treated with water and centrifuged. The clear upper layer was decanted from the dark tarry deposit and concentrated under vacuum. The concentrate was repeatedly extracted with ethyl ether and the combined ether extract treated with activated carbon to remove the colouring matter. The ether layer was dried over anhydrous sodium sulphate and concentrated. The crude crystalline material thus obtained was purified by recrystallisation from dry ether-chloroform mixture (1 : 3). After cooling, clusters of small needle shaped crystals of the lactone were obtained; the acid itself could not be obtained as it underwent lactonisation during concentration.

The lactone was converted into acid by dissolving it in water and then neutralising with alkali. This material was passed through cation-exchange resin (Zeocarb-215) for recovery of acid.

The melting point of the lactone was determined by using Gallenkamp melting point apparatus and the optical rotation by Perkin-Elmer Autopolarimeter 243. Infra-red spectrum was recorded on Hilger-Watts Spectrometer as KBr pellet. Ascending paper chromatography on Whatman No. 1 paper using n-butanol: acetic acid: water (BAW) 4 : 1 : 5 and n-butanol: formic acid: water (BFW) 4 : 1 : 5 solvents was carried out. Bromophenol blue spray was used for the detection of spots. In all these experiments,

the acid obtained from *Garcinia cambogia*<sup>9</sup> was used as standard reference.

**Isolation of pigment:** Fresh red ripe fruits were used for isolation, identification and estimation of the pigment. The rind portion was separated from the rest of the fruit and was macerated in a blender using methanol containing 1% HCl. The maceration and extraction procedures were repeated three times. The extracts were combined, filtered and concentrated *in vacuo* at 30°C.

The concentrated pigment extract was streaked on Whatman No. 3 paper and developed by descending chromatography using acetic acid : HCl : water (15:3:82) solvent system. The solvent was allowed to over-run to achieve good separation in 6 hrs. Two clear bands designated as B<sub>1</sub> and B<sub>2</sub>, were obtained which were air dried, cut and eluted with methanol containing 0.01% HCl. The solvent was evaporated *in vacuo* at 30°C. Each band was further purified by paper chromatography using 15% acetic acid. The pigments from the dried, cut strips were conveniently eluted by Gunter Zweig method<sup>10</sup>.

UV and visible spectra were recorded on a Varian Superscan-3 Recording Spectrometer, in methanol containing 0.01% HCl. *R<sub>f</sub>* determinations were carried out by using Whatman No. 1 paper in specified solvent systems.<sup>11</sup>

Hydrolysis of anthocyanin pigments were carried out by heating with 2N HCl in boiling water bath for 1 hr. After cooling, the hydrolysate was first shaken with solvent ether (to remove any carboxylic acid) and then with iso-amyl alcohol to extract anthocyanidins. The aqueous layer containing only free sugars was freed from acid by passing through Dowex-1-hydroxyl form. The amyl-alcohol layer containing the aglycones was treated with slight excess of petroleum ether to precipitate the pigments and left overnight in the refrigerator. The petroleum ether layer was decanted and the pigment dissolved in methanol for use in further studies. The aglycones and the sugars were then identified by direct comparison with authentic samples on paper chromatography in various solvent systems<sup>11,12</sup>.

Removal of sugars attached to the 3-position of the anthocyanin B<sub>2</sub> by hydrogen peroxide oxidation, and subsequent hydrolysis were carried out according to the method of Chandler and Harper.<sup>13</sup> The sample after hydrolysis was then spotted on paper along with glucose and the disaccharide (sambubiose) from cyanidin-3-sambubioside (obtained from *Hibiscus sabdariffa*) in different solvent systems. The *R<sub>G</sub>* values for the disaccharide as well as sambubiose were determined in various solvent systems.<sup>14</sup> The sugar spots were detected by spraying the paper with

aniline hydrogen phthalate and heating at 105°C for 5 min.

The total anthocyanin content in kokam rind was estimated by the method of Fuleki *et al.*<sup>15</sup> The extinction co-efficient of cyanidin-3-sambubioside was determined as follows. The fast moving band B<sub>2</sub> obtained from the paper chromatograms was collected and concentrated *in vacuo* at 30°C to a small volume. This material was kept at 4°C for 72 hrs, when the crystalline pigment got separated. This crystalline material was filtered and dried over phosphorous pentoxide. The *E* value of this for 1% solution (in ethanol containing 0.1% HCl) at 536 nm was found to be 383. Total anthocyanins were determined using 50g of fresh kokam by thoroughly extracting with ethanol containing 0.1% HCl, suitably diluting with the same solvent and determining O.D. at 536 nm.

### Results and Discussion

Table 1 gives the composition of the rind. Though the moisture content in the rind is as high as 30 per cent, it did not get spoiled probably due to the high acid content in the skin. The rind is a poor source of protein and vitamin C.

The major acid present in kokam rind has been identified as (-) hydroxycitric acid based on melting point, optical rotation, infrared spectroscopy and paper chromatographic studies. Results are given in Table 2. Hydroxycitric acid obtained earlier from *Garcinia cambogia* by Lewis *et al.*<sup>9</sup> was used as reference material. The IR spectra of kokam lactone and (-) hydroxycitric acid lactone were superimposable. Malic acid, citric acid and tartaric acid reported to be present in kokam<sup>1</sup> could not be traced.

TABLE 1. ANALYSIS OF KOKAM RIND\*

Moisture (%)	..	..	..	..	..	30
Protein (N×6.25) (%)	..	..	..	..	..	1.92
Crude fibre (%)	..	..	..	..	..	14.28
Total ash (%)	..	..	..	..	..	2.57
Tannins (%)	..	..	..	..	..	2.85
Pectin (%)	..	..	..	..	..	5.71
Starch (%)	..	..	..	..	..	1.00
Crude fat (%) (hexane extract)	..	..	..	..	..	10.00
Acid (as hydroxycitric acid) (%)	..	..	..	..	..	22.30
Pigment (total anthocyanins) (%)	..	..	..	..	..	2.40
Ascorbic acid (%)	..	..	..	..	..	0.06
Carbohydrates by diff. (%) (excluding starch and pectin)	..	..	..	..	..	36.40

\*All the values are on moisture free basis and each is the mean of two analyses.

TABLE 2. COMPARISON OF KOKAM ACID WITH HYDROXYCITRIC ACID

Property	Kokam acid	(-)-hydroxycitric acid
M. P. (Lactone)	176°C	178°C
( $\alpha$ ) <sub>D</sub> <sup>20</sup> (1% H <sub>2</sub> O) (Lactone)	+100°	+98°
( $\alpha$ ) <sub>D</sub> <sup>20</sup> (1% H <sub>2</sub> O) (Free acid)	-20°	-21°
Paper chromatography:		
R <sub>f</sub> values in		
BAW—acid	0.30	0.32
lactone	0.48	0.46
BFW—acid	0.22	0.22
lactone	0.41	0.40

Paper chromatography of the kokam pigment extract showed two anthocyanin bands. The slower moving band was designated as B<sub>1</sub> and the other B<sub>2</sub>.

*Anthocyanin B<sub>1</sub>*: This compound on hydrolysis gave cyanidin and glucose. The ether extract of the hydrolysate did not contain any carboxylic acid. The UV spectral maximum (527 m $\mu$ ) of the glycoside shifted to 567 nm with aluminium chloride indicating that the 3'- and 4'-hydroxyl groups of the cyanidin are free. The *E* 440/*E* max. value (0.26) showed that the 5-hydroxyl is also free. Of the other two possibilities, namely 3- and 7-glycosylations, the former is indicated by the stability and R<sub>f</sub> values of

the anthocyanin<sup>12</sup> (Table 3). This was confirmed by direct comparison with a sample of cyanidin-3-glucoside obtained from mulberry.<sup>16</sup> Thus the pigment is identified as cyanidin-3-glucoside.

*Anthocyanin B<sub>2</sub>*: This anthocyanin on complete hydrolysis gave cyanidin, glucose and xylose. The ether extract of the hydrolysate did not contain any carboxylic acid. The spectral data as discussed above suggested that B<sub>2</sub> is a 3-substituted glycoside of cyanidin. Hydrogen peroxide hydrolysis removed the disaccharide from the pigment which on further acid hydrolysis gave glucose and xylose. The R<sub>G</sub> values for the disaccharide as well as sambubiose were 0.76 in n-butanol: acetic acid: water (4:1:5), 0.79 in n-butanol: ethanol: water (4:2:2:2) and 0.70 in n-butanol: benzene: pyridine: water (5:1:3:3). Anthocyanin B<sub>2</sub> was therefore, considered to be cyanidin-3-sambubioside. Direct comparison of R<sub>f</sub> values and spectral data of the compound B<sub>2</sub> with authentic cyanidin-3-sambubioside isolated from Roselle<sup>16</sup>, confirmed that the pigment is cyanidin-3-sambubioside (Table 3).

The total anthocyanin concentration has been found to be 2.4% (calculated as cyanidin-3-sambubioside) on dry weight basis. The ratio of cyanidin-3-sambubioside to cyanidin-3-glucoside is 4:1. In view of the high pigment content, kokam could be a good source of the water soluble red colour as well as the fat soluble yellow colour (garcinol) for use in various foods. Preliminary studies in this direction are promising and further work is in progress.

TABLE 3. R<sub>f</sub> ( $\times 100$ ) VALUES AND ABSORPTION MAXIMA OF ANTHOCYANINS AND THEIR AGLYCONES

Pigment	R <sub>f</sub> values in different solvent systems ( $\times 100$ )							Absorption max. in methanol with 0.01% HCl (nm)
	n-butanol: acetic acid: water: (upper layer) (4:1:5 v/v)	n-butanol: 2 N HCl water (1:1 v/v)	HCl: water (3:97 v/v)	Acetic acid: HCl: water (15:3:82 v/v)	n-butanol: formic acid: + water R <sub>Cy 3G</sub> $\times 100^*$ (100:25:60)	Acetic acid: HCl: water (30:3:10 v/v)	Formic acid: HCl: water (5:2:3 v/v)	
Anthocyanin-B <sub>1</sub>	42	33	7	27	101	—	—	527,330,280
Cyanidin-3-glucoside	42	33	8	28	100	—	—	526,328,281
Anthocyanin-B <sub>2</sub>	41	39	25	52	82	—	—	527,330,281
Cy-3-sambubioside	40	41	24	52	81	—	—	527,330(sh), 280
Aglycone-B <sub>1</sub>	72	—	—	—	—	49	21	537,272
Aglycone-B <sub>2</sub>	74	—	—	—	—	50	24	537,275
Cyanidin	76	—	—	—	—	52	24	537,278

\*R<sub>Cy 3G</sub> is chromatographic mobility relative to cyanidin-3-glucoside and is used with BFW.

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## Biochemical Changes Associated with the Pod Development of French Bean (*Phaseolus vulgaris* L.) Under Kumaon Hill Conditions

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*Manuscript received 19 March 1981; revised 19 October 1981*

**Biochemical changes with pod development in two varieties of bean viz. 'V. L. Bauni-1' and 'Contender' were studied. It was observed that moisture, total soluble solids, crude fibre and crude fat showed significant positive or negative correlation with pod development. Eight amino acids, namely, lysine, glycine, threonine,  $\alpha$ -alanine, cysteine, methionine, valine and leucine were identified and found in varying concentrations at different stages of pod development. Concentration of lysine,  $\alpha$ -alanine and methionine were found to increase with pod development. The period between 18 to 22 days after fruit set was found suitable for picking the pods for vegetable purpose.**

Maturity plays an important role in effecting biochemical changes during the development of fruit. Systematic growth studies, coupled with changes in the contents of important chemical constituents, could lead to better understanding of the ripening process and enable to harvest at the optimum time. The biochemical constituents vary with variety and also depend upon the soil and environmental conditions.<sup>1-5</sup>

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no systematic literature is available on the changing pattern of chemical constituents with pod development in French bean.

The present work is concerned with the changes in biochemical constituents associated with the pod development in two cultivars of French beans, grown under Kumaon hill conditions.

#### Materials and Methods

Two varieties of dwarf French beans—'V. L. Bauni-1' and 'Contender' were grown in randomized block design with three replications at the experimental fields of Defence Agricultural Research Unit, Almora. The mean temperature and relative humidity during the growth was 25.34°C and 75% respectively. The soil was sandy loam, well drained, high to medium in nitrogen, low in phosphorus and high in potassium.

For chemical analysis, pods were picked at random from all three replications and a composite sample was made for each variety. The pickings were done 6, 12, 18, 22 and 25 days after fruit set. Samples from different pickings were analysed for various biochemical constituents.

Protein, crude fibre, crude fat and ascorbic acid were estimated as per A.O.A.C. procedure.<sup>6</sup> Fehling solution titrimetric method, described by Kanwar *et al.*<sup>7</sup> was used for estimating reducing and total sugars. Total soluble solids were determined by hand refractometer. Total calories were calculated by adding the calories contributed by crude protein, crude fat and total carbohydrate.<sup>8</sup> Sucrose was

calculated by multiplying the difference between total and reducing sugars by 0.95. The carbohydrate percentage was calculated by difference.

The free amino acids were determined on the 95% ethanol extract of fruits. For the determination of amino acids, the ethanolic extract was passed through a column of Dowex 50W-X8 (H-form), 200-400 mesh and then eluted with 2N NH<sub>4</sub>OH. The qualitative identification of amino acids was done by paper chromatography.<sup>9,10</sup>

Amino acid spots were visualized by ninhydrin reagent<sup>11</sup> and identified by alloxan<sup>12</sup>, isatin<sup>13</sup>, ophthalaldehyde<sup>14</sup>, Folins reagent<sup>15</sup> and by specific chemical methods.<sup>16</sup> The amino acid spots were cut, sprayed with ninhydrin, and eluted with 50% ethanol. The optical density was measured at 570 mμ. The concentration of amino acids were determined by comparing with standard curves prepared from the known concentration of amino acids.

#### Results and Discussion

From the data in Table 1, it is clear that the moisture content increases upto 12 days after fruit set followed by decreases upto the last picking stage. This finding is similar to the finding of Reddy *et al.*<sup>17</sup> Ascorbic acid content increases upto 22 days after fruit set and then decreases. On the other hand, a reverse trend was found with the sugars. This may be due to the inter-conversion of sugar to ascorbic acid.<sup>18</sup>

In both the varieties, total soluble solids, crude

TABLE 1. CHANGES IN BIOCHEMICAL CONSTITUENTS DURING POD DEVELOPMENT IN FRENCH BEAN "ON FRESH WEIGHT BASIS"

Days after fruit set	Variety	Moisture (%)	T.S.S. (%)	Total minerals (%)	Ascorbic acid (mg/100g)	Crude fibre (%)	Crude fat (%)	Total sugar (%)	Reducing sugar (%)	Sucrose (%)	Protein (%)	Carbohydrate	Calories 100 g
6	A	92.4	4.33	0.64	14.70	0.76	0.16	4.08	2.70	1.31	1.88	4.16	25.60
	B	90.5	4.00	0.58	13.72	0.73	0.18	3.98	2.40	1.50	1.90	6.09	33.58
12	A	93.0	4.47	0.68	14.92	0.84	0.20	3.96	2.60	1.29	1.76	3.56	23.56
	B	90.8	4.28	0.64	13.92	0.89	0.22	3.84	2.52	1.25	1.84	5.57	31.62
18	A	90.4	4.96	0.78	15.42	0.97	0.27	3.99	2.73	1.20	1.68	5.93	32.87
	B	89.8	4.70	0.73	14.57	0.92	0.25	3.86	2.68	1.22	1.76	6.56	35.53
22	A	89.5	5.50	0.82	15.87	1.23	0.29	4.04	2.86	1.12	1.57	6.62	35.42
	B	89.1	4.90	0.78	15.32	1.40	0.28	4.31	2.74	1.49	1.63	6.77	36.12
25	A	88.7	5.80	0.88	15.06	1.37	0.30	4.20	2.69	1.43	1.98	6.77	37.74
	B	88.5	5.60	0.84	14.38	1.57	0.34	4.38	2.57	1.72	1.96	6.82	38.18

A—V. L. Bauni-1 B—Contender.

TABLE 2. CHANGES IN AMINO ACIDS (mg/100 g.) DURING POD DEVELOPMENT OF FRENCH BEAN

Days after fruit set	Variety	Lysine	Glycine	Threonine	$\alpha$ -Alanine	Cysteine	Methionine	Valine	Leucine
6	A	1.80	4.45	6.88	4.60	9.09	3.24	5.74	6.62
	B	1.84	4.38	6.74	4.73	8.74	3.27	5.72	6.60
12	A	2.18	3.72	6.74	7.01	4.84	4.73	6.38	5.73
	B	2.24	3.64	5.72	7.01	3.62	4.74	6.28	5.74
18	A	2.22	2.18	5.82	8.08	8.52	6.42	6.07	4.49
	B	2.42	2.22	5.20	8.20	5.93	5.94	6.06	4.52
22	A	2.79	2.48	4.83	23.00	4.34	4.78	5.80	4.30
	B	1.94	2.36	4.73	25.00	4.72	4.82	5.13	4.27
25	A	3.79	2.42	2.91	16.40	2.74	3.63	2.42	3.78
	B	2.87	2.34	2.87	14.80	2.41	3.52	2.38	3.68

A—V. L. Bauni-1; B—Contender.

TABLE 3. CORRELATION BETWEEN DAYS OF PICKING AND DIFFERENT BIOCHEMICAL CONSTITUENTS

Sl. No.	Biochemical constituents	Variety*	Regression equation	R <sup>2</sup>	Sl. No. of variable with which significantly correlated
1.	Moisture	A	$Y = 94.5908 - 0.2388X$	0.8343	2, 3, 5, 9
		B	$Y = 91.7272 - 0.1627X$	0.8268	2, 3, 5, 6, 9
2.	T. S. S.	A	$Y = 3.6408 + 0.0857X$	0.9063	1, 5, 9
		B	$Y = 3.3636 + 0.0854X$	0.9392	1, 3, 5, 9
3.	Total minerals	A	$Y = 0.5392 + 0.0138X$	0.9716	1, 5, 9
		B	$Y = 0.4816 + 0.0149X$	0.9864	1, 5, 9
4.	Ascorbic acid	A	$Y = 14.5444 + 0.0406X$	0.3901	—
		B	$Y = 13.4067 + 0.0625X$	0.4869	7
5.	Crude fibre	A	$Y = 0.4852 + 0.0343X$	0.8776	1, 2, 3, 9
		B	$Y = 0.3425 + 0.0487X$	0.8827	1, 2, 3, 7, 9
6.	Total Sugar	A	$Y = 3.9660 + 0.0055X$	0.1723	—
		B	$Y = 3.6698 + 0.0272X$	0.6446	1, 5
7.	Reducing Sugar	A	$Y = 2.6344 + 0.0051X$	0.1473	—
		B	$Y = 2.7739 + 0.0133X$	0.4846	5
8.	Protein	A	$Y = 1.8020 + 0.0018X$	0.0062	—
		B	$Y = 1.8599 - 0.0027X$	0.0213	—
9.	Crude fat	A	$Y = 0.1055 + 0.0088X$	0.9665	1, 2, 3, 5
		B	$Y = 0.1207 + 0.0085X$	0.9683	1, 2, 3, 5

\*A—V. L. Bauni-1; B—Contender.

fibre, crude fat and total minerals increased with pod development (Table 1). Similar trend was also observed by early workers in other varieties of French bean,<sup>19</sup>

**Protein and amino acids:** It was found that protein content decreases with pod development upto 22 days, after fruit set, but after that, it increases in both the varieties.

Lysine, glycine, threonine,  $\alpha$ -alanine, cysteine, methionine, valine and leucine were identified at all the stages of the pod development (Table 2) in both the varieties. However, the concentration of amino acids varied at different stages of pod development. The concentrations of lysine,  $\alpha$ -alanine and methionine increased with pod development. Methionine and  $\alpha$ -alanine increased upto 18 and 22 days respectively and afterwards decreased.

The increase in the concentration of lysine,  $\alpha$ -alanine and methionine could be due to the reduced demand for their metabolites as growth processes are slowly taken over by ripening process in the final stages of maturity.<sup>20</sup> Selvaraj *et al.*,<sup>21</sup> observed that the concentration of  $\alpha$ -alanine increased several folds during the later developmental stages in grape.

Glycine, threonine, cysteine, valine and leucine showed decrease in concentration at the overmature stage. However, their concentrations at 22 days after fruit set were found higher than at 25 days. The erratic fluctuations in most of the amino acids indicate their involvement in intermediary metabolic processes during development. Out of eight amino acids identified in French beans, six were found to be essential amino acids.

Taking into consideration all the biochemical constituents, the period between 18 and 22 days after fruit set, appears to be the appropriate time for picking the pods of bean for vegetable purpose. At this period, the ascorbic acid and reducing sugars were at their maximum level and most of the essential free amino acids were found to be in adequate amount. The crude fibre content was also within tolerable limits (Table 3).

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# A Method for Measuring the Firmness of Cooked Soyabean (*Glycine max*)

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A simple method for objective determination of cooking firmness of soyabean has been described. The apparatus used can be improvised in the laboratory. The cooking firmness of three soyabean (*Glycine max*) varieties 'DS-24-2', 'Lee' and 'UPSM-19' has been studied by this method.

The firmness or tenderness, besides cooked weight, cooked volume and cooking losses is an important trait which determines the cooking quality of a pulse. The determination of cooked weight, cooked volume and cooking losses is simple. The routine methods for the determination of firmness are to press the cooked grains between two fingers or between two glass slides.<sup>1-5</sup> These methods have inherent disadvantages: (i) these are subjective and (ii) the results cannot be expressed quantitatively. In this communication, we report a method for objective determination of firmness of cooked soyabean; the method is simple and the apparatus can be fabricated in the laboratory. Besides, the proposed method offers the advantage of digital representation of results.

## Materials and Methods

Three varieties of soyabean (*Glycine max*) viz. 'DS-24-2', 'Lee' and 'UPSM-19' were used in the experiment.

**Apparatus:** The main features of the apparatus are shown in Fig. 1. It consists of a metal stand (I) with a horizontal arm (II). On the horizontal arm is placed the test specimen (III) whose firmness is to be determined. A 2cm. long loop of nylon thread or a very fine wire (IV) is passed over the specimen (Fig. 1b). The thread/wire should not touch the arm of the stand (II). A pan (V) with three iron chains (VI) is connected to the lower end of the loop. The total weight of the pan, the chains and the thread/wire is 22.5 g, the diameter of the pan is 6.5 cm. and the vertical length of the chains 5 cm. (Fig. 1a). Care is taken to keep the plan of the pan (V) parallel to the arm of stand (II).

**Theory:** The force required to cut the grain transversely represents the index of firmness. Greater

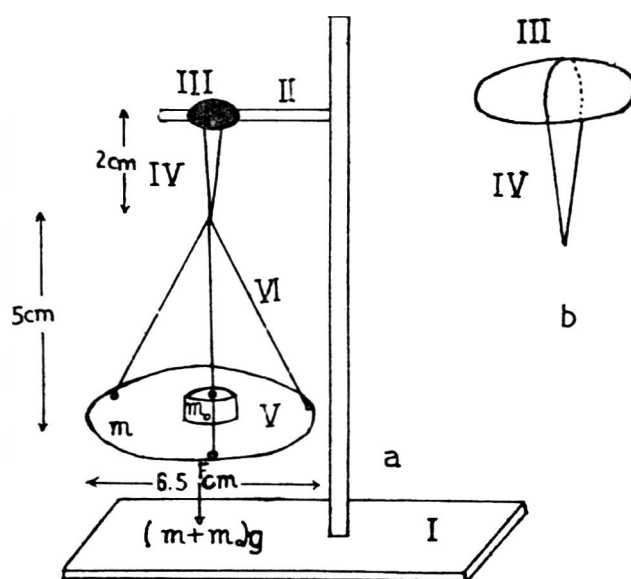


FIG. 1. Description of apparatus: I. Stand. II. Arm of stand III. Test sample. IV. Nylon thread/wire. V. Pan VI. Connecting iron chain.

the firmness, greater the force required to cut it.

The force ( $F$ ) required to cut the sample is represented by:

$$F = (m_0 + m) \times g$$

Where  $m$  is the weight of the pan,  $m_0$  is the weight needed to be put on the pan for cutting the grain into two halves and  $g$  is the acceleration due to gravity. The force required for extending the thread/wire is very small and is neglected.

If  $m_0 = m_a$  for variety 'A' and  $m_b$  for variety 'B', the forces  $F_a$  and  $F_b$  required to cut them respectively are:

$$F_a = (m_a + m) \times g$$

$$F_b = (m_b + m) \times g$$

If  $F_a > F_b$ , the variety 'A' is more firm than variety 'B'. The relative firmness of varieties 'A' and 'B' at a particular time of cooking can be expressed as follows:

$$F_a = F_b \left( \frac{m_a + m}{m_b + m} \right)$$

Or variety 'A' is  $\frac{m_a + m}{m_b + m}$  times firmer than variety 'B' at a particular time of cooking.

An arbitrary unit of firmness is defined as the force produced by a weight of 1 g which cuts the sample into two halves.

*Cooking of soyabean:* Distilled water (100 ml.) was added in a beaker containing soyabean grains. The beaker was kept in an autoclave and cooked for different lengths of time at a pressure of 15 psi (1.05 kg./cm.<sup>2</sup>).

*Measurement of the weight required to cut the sample:* The soyabean grain was placed on the arm and loop passed over it as described earlier. Weight was increased on the pan by 10 g at a time till the grain was cut into two halves. This gave the approximate weight required for cutting the grain. For the next observation, a weight well below recorded in the first observation was put on the pan and additions of 1 g at a time were made slowly till the grain was cut into two halves. This value was recorded and twenty replications were made to get the average value.

## Results and Discussions

Table 1 shows that the effect of cooking the three varieties of soyabean at 15 psi (1.05 kg/cm<sup>2</sup>) in autoclave for three different lengths of time. At all the three time intervals there was a significant

difference in firmness of the varieties tested. Given 5 min. of cooking, the variety 'DS-24-2' is 1.09 and 1.69 times were more firm than 'Lee' and 'UPSM-19' respectively. The comparative firmness at 15 and 20 min. of cooking also included in Table 1, show that the variety 'UPSM-19' is the least firm and therefore, possessed the best cookability compared to varieties 'DS-24-2' and 'Lee'.

The variety 'DS-24-2' when cooked at 15 psi (1.05 kg/cm<sup>2</sup>) pressure for 15 min. gave firmness values (mean  $\pm$  S.D.) of 105 $\pm$ 4 and 105.5 $\pm$ 6 on two different occasions. The differences were not significant, thereby showing the reproducibility of the observation by the method.

While cooking, the outer skin (testa) of some of the grains gets detached. The cooking firmness when measured (mean  $\pm$  S.D.) with skin was 105.5 $\pm$ 4 as compared to 63.5 $\pm$ 5 got with the skin removed. The values are significantly different at  $p > 0.001$ . Therefore, proper care should be taken for the selection of grains. Need for avoiding the frictional forces by ensuring the absence of touch of loop with the horizontal arm has already been stated. Caution is also required in placement of weights on the pan to avoid the involvement of forces due to jerks.

An objective evaluation of different soyabean varieties for their cooking firmness can help the plant breeders in evolving varieties with high cookability in addition to their high yielding quality. Most varieties of soyabean take a long time for cooking and certain chemical pre-treatments can be given to reduce their cooking time. In such studies, the above method can be of paramount utility to assess the relative efficacy of different treatments on cooking quality. Though the method has been developed for soyabean, it may offer scope for the study of other pulses also.

TABLE 1. COMPARISON OF THE COOKING FIRMNESS OF THREE VARIETIES OF SOYABEAN

Time of cooking* (min)	Firmness (units)**			F <sub>1</sub> /F <sub>2</sub>	F <sub>1</sub> /F <sub>3</sub>	F <sub>2</sub> /F <sub>3</sub>
	DS-24-2 (F <sub>1</sub> )	Lee (F <sub>2</sub> )	UPSM-19 (F <sub>3</sub> )			
	Mean $\pm$ S.D.	Mean $\pm$ S.D.	Mean $\pm$ S.D.			
5	159.5 $\pm$ 5 <sup>a</sup>	146.5 $\pm$ 4 <sup>b</sup>	94.5 $\pm$ 4 <sup>c</sup>	1.09	1.69	1.55
15	105.5 $\pm$ 6 <sup>a</sup>	99.5 $\pm$ 6 <sup>b</sup>	72.5 $\pm$ 4 <sup>c</sup>	1.06	1.46	1.37
25	88.5 $\pm$ 4 <sup>a</sup>	84.5 $\pm$ 4 <sup>b</sup>	50.5 $\pm$ 4 <sup>c</sup>	1.05	1.46	1.40

\* Cooked at 15 psi (1.05 kg/cm<sup>2</sup>)

\*\*  $m+m_0$  expressed in g; values represent average of 20 replications.

a, b, c Different superscripts in the same line indicate that the varietal differences are significant at  $P < 0.001$ .

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## Breakage of Rice During Milling

### I. Types of Cracked and Immature Grains

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About 150 randomly-selected hand-dehusked grains are examined in transmitted light in a modified microbiological colony counter. Grains with (a) a single transverse crack (STC), (b) multiple transverse cracks (MTC), and (c) longitudinal (or irregular) ( $\pm$  transverse) cracks (LC) can be distinguished; immature grains can be identified by their size, colour and opacity. During progressive ripening of paddy on stalk, STC appeared and increased first, but MTC and LC increased rapidly at the later stages. Controlled hydration and drying of paddy showed that sorption of water led to regular transverse cracks, while longitudinal (irregular) cracks were formed mostly upon fast drying.

A recent review of the factors responsible for breakage of rice grains during milling by us<sup>1</sup> revealed certain gaps in information. This prompted us to undertake a comprehensive study on the relationship of various types of kernel defects, grain shape and size, moisture content, and type of milling equipment—individually and in their interaction—to rice breakage during milling.

There are a few reports<sup>2,3</sup> that different types of cracks develop in rice depending on the circumstances of its exposure to moisture stress. We also made similar observations during our studies on artificial aging of rice by humid-heat treatment.<sup>4</sup> The types of cracks observed in rice grain and on the possible circumstances of their development were therefore, first examined and are presented here. A method of

evaluating grains for kernel defects (cracked and immature grains) is also described. This information was later used in evaluating the data on rice breakage presented in subsequent papers of this series.\*

### Materials and Methods

All paddy samples were procured from the University of Agricultural Sciences Experiment Station at Mandya.

To study the effect of the maturity stage of paddy at harvest on its content and type of defective kernels, five varieties of paddy (one plot each) were harvested at different stages. About 50 plants selected at random from each in duplicate were reaped (at 10 a.m. every time) at each stage as per the general procedure of Mahadevappa *et al.*<sup>7</sup> The plants were threshed immediately and a small portion of the grain was

\*Two of these papers have already been since published<sup>5,6</sup> in a journal, which then returned it as it ceased publication.

The present paper was unfortunately held up for two years

stored in a closed bottle for determination of moisture. Remaining paddy was dried in the shade for 2–3 days, carefully winnowed to remove chaff, but not immature grains, and stored in closed polyethylene bags until examined for defective grains. Unfortunately, the season was exceptionally wet, with overcast sky and intermittent rain, and the actual counts of defective kernels may not be typical. The trend of their change with harvest stage, however, can be taken as fairly representative.

To study the effects of (a) wetting and (b) fast drying on the types of cracks formed in paddy grain, a few samples were (a) soaked in water at room temperature (RT=25–30°C) for different times and dried in shade, and (b) soaked and then dried in a cabinet dryer at different temperatures.

Cracks and immature grains were visualised in transmitted light essentially by the method devised earlier.<sup>8</sup> However, a microbiological colony counter, with its ruled-glass viewing window replaced with a piece of plain glass, was used instead of the stool with a hole and a flashlight from below used earlier. With its dark background and a strong diffused fluorescent light from below, which moreover generated little heat, this instrument was ideal for this purpose. About 150 paddy grains (3 g) were selected with a

sample divider, dehusked by hand, and stored in a closed petri dish. About 20 of these kernels were taken at a time on a petri dish placed on the viewing window of the instrument, and were examined carefully while being gently moved around with a spatula. Grains with cracks of different categories and other defects were collected separately and weighed.

Moisture content (wet basis) was determined by oven drying.<sup>9</sup>

### Results and Discussion

*Types of cracks:* Most samples of paddy, when examined in transmitted light (after manual shelling), showed kernels with different kinds of cracks—viz. kernels having (i) a single transverse crack (STC) (Fig. 1a), (ii) two or more (multiple) transverse cracks (MTC) (Fig. 1b, c), and (iii) longitudinal (or irregular), usually with some transverse cracks (LC) (Fig. 1d, e, f). This classification, important for milling studies showed<sup>5,6</sup> that grains with MTC and LC were the most prone to break. They were also very fragile when tested by pressing between fingers.

*Immature grains:* Immature brown rice kernels also tend to break easily during milling.<sup>8</sup> Their main distinguishing features as observed in transmitted light (Fig. 2c,d,e) are: (i) invariably smaller than the

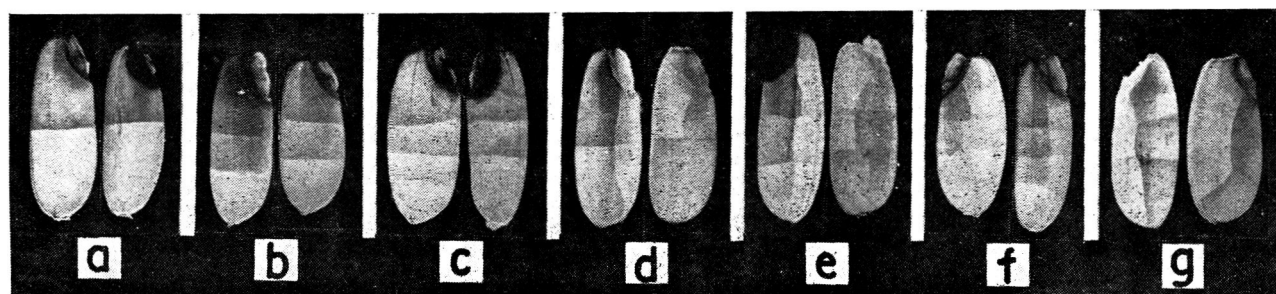


Fig. 1. Types of cracks in brown rice grain. (a) Single transverse crack (STC), (b) two transverse and (c) three transverse cracks (MTC), (d) longitudinal crack (LC) with STC. (e) LC with 2 TC, (f) LC with 3 TC, (g) badly cracked grains.

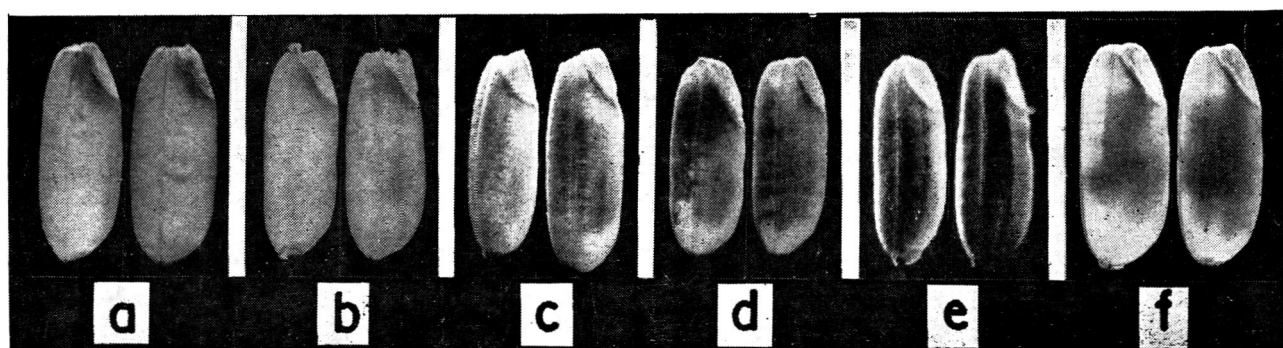


Fig. 2. Photograph of (a) sound and mature, (b) slightly smaller, but otherwise sound and mature, (c), (d), (e) various degrees of immature, and (f) mature, but chalky-centre brown rice grains.

TABLE 1. REPRODUCIBILITY OF THE DEFECTIVE-KERNEL ANALYSIS METHOD\*\*†

No. of replicate sub-samples	No. of replicate analyses	Sound	STC	MTC	LC	Immature	Total defective
1	6	48.8	15.5	17.9	6.4	11.4	51.2
	C.V.	2.4	7.2	5.0	9.7	3.4	2.3
7	1 each	51.4	18.2	16.1	5.9	8.5	48.6
	C.V.	7.0	13.6	11.9	5.8	14.2	7.4

\*A sample of 'IET 1522' variety, harvested fairly late, was analysed in seven replicate subsamples of 3 g each; one of the subsamples was analysed six times, the rest only once.

† Results are expressed in mean contents in % by weight.

C.V. = Coefficient of variation = s.d. expressed as % of mean.

mature grains (Fig. 2a, b), (ii) usually (but not necessarily) green in colour, and (iii) invariably partly or fully opaque. Chalky, but mature kernels (Fig. 2f) can be easily distinguished from immature kernels.

*Reproducibility of defective-kernel analysis:* Some information on the reproducibility of the method for defective-kernel analysis is given in Table 1. Precision of replicate defective-kernel analysis in a single subsample was excellent. Precision of the analysis in replicate subsamples was lower but still good.

*Effect of stage of harvest:* There were appreciable varietal difference in the count of defective kernels, but too much could not be read into this because of the intermittent rains and because different varieties came up for harvest on different days. Two typical sets of results—one of a long-slender (Ls) and the other of a medium-bold (Mb) variety as per our recent classification<sup>10</sup>—are shown in Table 2. The main points that emerge from these results are:

(a) A very small amount of cracked kernels was present even as early as 25–30% grain moisture.

TABLE 2. CONTENT OF DEFECTIVE GRAINS IN PADDY HARVESTED AT VARIOUS STAGES

Variety*	No. of days after flowering	Moisture content at harvest (%)	Cracked kernels (%)				Immature grains (%)
			STC	MTC	LC	Total	
MR 301	24	29.2	2.1	0.9	0.0	3.0	13.3
	29	28.6	6.2	3.1	0.0	9.3	5.8
	33	23.4	4.8	0.9	0.0	5.7	4.4
	39	22.4	17.2	14.2	3.0	34.4	5.1
	45	19.1	12.8	13.6	5.1	31.5	6.0
	51	16.9	14.1	16.6	6.0	36.7	6.0
	64	16.9	21.8	31.9	9.9	63.6	3.3
	72	17.6	17.8	31.2	10.8	59.8	7.8
IET 3305	24	25.3	3.4	0.0	0.0	3.4	21.0
	29	25.9	8.0	2.1	0.0	10.1	16.4
	34	23.5	8.2	2.2	0.0	10.4	13.0
	38	17.5	13.1	5.9	1.3	20.3	8.9
	44	17.9	24.9	11.4	1.3	37.6	9.7
	54	15.1	29.3	26.3	7.3	62.9	5.6
	68	14.8	25.2	22.6	18.6	66.4	8.8

\*The length (mm) and the length/breadth ratio, respectively, of the varieties (brown rice) were: 'MR 301'—6.5, 3.2; 'IET 3305'—5.6, 2.2.



(b) Not only did the total content of cracked kernels increase with increasing ripening of the crop, which is well known, but the proportion of grains with multiple and irregular cracks increased. This is important, for it is these latter rice grains which are the most prone to break during milling.<sup>5,6</sup>

(c) Single transverse cracks (STC) were the first to appear, rapidly increasing with time, but soon reaching a more or less steady value. Multiple transverse (MTC) and longitudinal cracks (LC) appeared later, increased rapidly and finally exceeded the STC. Clearly, the first-formed STC grains were gradually converted into MTC and LC grains as time passed.

(d) Content of LC grains became appreciable only when the moisture content had dropped to quite low levels.

(e) Immature grains were plentiful in early samples, but decreased rapidly, finally reaching a more or less steady value.

(f) It was observed that all the cracks, including the STC, became somewhat deeper and more pronounced as the harvest was delayed. In early samples, the cracks were usually very thin, and some

of them were only fractional (less than full across the kernel).

*Effect of artificial damage:* Examination of the grains after soaking in water (followed by shade drying) and after hot-air drying suggested (Table 3) that sorption of water led mostly to regular transverse cracks, as has been observed in milled and brown rice<sup>2,3,11</sup>. Rapid desorption, however, gave rise to appreciable numbers of longitudinal (or irregular) cracks, particularly at the terminal stages of drying. However, it is to be noted that a good number of transverse cracks were also formed during desorption. With progressive drying, both the number and proportion of STC soon decreased and those of MTC and LC increased. This need not mean, however, that STC are the first to form which are then gradually transformed into MTC and LC. For, as has been shown, cracks form not during drying, but after the drying has ceased<sup>12,13</sup>. It appears that under mild stress, most grains suffer only one crack (i.e., STC); but under more severe conditions, several grains crack more than once (MTC). LC, however, form only under severe drying conditions. These results also explain

TABLE 3. EFFECT OF SOAKING AND OF HOT-AIR DRYING ON NUMBER AND TYPE OF CRACKS IN PADDY

Variety	Time of soaking at RT (hr)	Drying temp.* (°C)	Final moisture (% w.b.)	Cracks (%)			
				STC	MTC	LC	
Bangara Sanna	0.0	Nil	12.5	5.7	2.0	0.0	
	0.5	RT	—	25.5	4.2	0.0	
	3	RT	—	31.2	4.9	0.0	
Taichung 65	0	Nil	12.0	26.6	3.2	2.4	
	0.5	RT	—	53.5	5.4	5.6	
	1	RT	—	42.3	12.8	9.1	
Bangara Sanna	0	Nil	22.3	—	—	—	
	0	60	14.9	6.0	1.8	0.0	
			14.0	15.6	5.2	1.8	
			13.0	21.3	22.0	2.0	
			12.3	26.8	32.5	4.0	
			11.5	27.3	46.8	8.4	
Coimbatore Sanna			9.9	8.9	67.7	14.7	
	0	Nil	24.2	4.1	0.0	0.0	
		60	13.5	24.3	12.6	1.1	
		70	9.2	4.1	36.6	44.1	
Adt 27	0	Nil	12.9	3.4	1.0	0.0	
	4	RT	11.3	35.6	13.2	3.1	
	70	80	15.8	}	15.7	8.3	30.8
		RT	12.5				
		60	13.5				
	6	85	9.6		3.1	2.2	90.3

\*RT (room temp.) indicates dried in shade for 1—3 days.

the appearance of LC in late-harvested grain. STC and MTC in the standing crop can arise both from wetting (rain or dew) and drying (by sun).

As in late-harvested samples, cracks in paddy damaged severely by drying also were deeper and more severe compared to less damaged samples.

Stermer<sup>3</sup>, working with milled rice, had reported that the cracks developed by moisture sorption were transverse, whereas those developed by desorption were irregular in shape. This fact is of course well known and can be verified easily by putting a few kernels of milled rice (a) in water and (b) in the sun. Within a few minutes transverse cracks in (a) and numerous minute irregular cracks in (b), respectively, would be evident even to the naked eye. However, the latter cracks, which are the same as seen in Stermer's figures, are mostly on the surface and tend to disappear again on tempering the rice. The irregular cracks observed in late-harvested and hot-air dried paddy, referred to in the present work (Fig. 1), on the other hand, were much fewer in number but considerably longer in size, as well as deeper and permanent. It would appear that they are formed primarily under severe conditions of drying. As has been shown, grains with such cracks are the most prone to break under the stress of milling.<sup>5</sup>

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## Component Acids of Some Ornamental Seed Oils

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Seed oils of *Muntingia calabura*, *Dracaena reflexa*, *Indigofera wightii*, were found to contain (wt. %) the following acids: capric 2.4, 0.1, nil; lauric 1.4, 0.8, 4.6; myristic 1.9, 1.7, 4.2; palmitic 19.1, 15.0, 29.1; stearic 10.0, 6.1, 12.1; arachidic 1.4, 2.0, 2.2; behenic 1.1, 2.2, 2.5; oleic 18.6, 41.2, 6.6; and linoleic 44.1, 30.8, 38.7 respectively.

*Muntingia calabura*, Linn. (N. O. Tiliaceae) is cultivated all over the tropics for ornament and for monotypic genus and native of South America, its edible fruit. The fruit is a berry, smooth, red

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globose or obovoid, containing many seeds embedded in a juicy pulp. The fruits are sweet and pleasant to taste and can be made into Jams. Infusion of the leaves is used as a tea, and that of flowers for headache and incipient colds.<sup>1</sup>

*Dracaena reflexa*, (N. O. Liliaceae) is ornamental rosette of densely clustering, short and narrow leathery leaves and self branching stem.<sup>2</sup>

*Indigofera wightii*, Grah, (N. O. Leguminosae) is a small erect shrub, with numerous branches. The leaves are 1.5 to 2.5 in. long and petioles 0.25 to 0.5 in. long. It is a rare plant.<sup>3</sup>

There are no reports on the component acids of the oils obtained from the seeds of these species.

### Materials and Methods

The seeds were collected from the plants grown in the Botanical Gardens of Karnatak University, Dharwad. The seeds were extracted with light petroleum ether (40–60°) to yield the oil. A portion of the oil was hydrolysed in cold condition with ethanolic potassium hydroxide and non-saponifiable matter and mixed acids were obtained.

The thin layer chromatography of mixed acids and their methyl esters revealed the absence of oxygenated acids. The mixed acids (a) after hydrogenation, (b) without other treatment and (c) after oxidation<sup>4</sup> were examined by reverse phase partition chromatography.<sup>5</sup>

The iodine values and saponification equivalents calculated from the results agree well with those measured experimentally.

### Results and Discussion

The seeds of *M. calabura*, *D. reflexa* and *I. wightii* contain 5.0%, 2.0% and 7.0% of fatty oil respectively. *M. calabura*, *D. reflexa* and *I. wightii* seed oils have iodine values of 94.0, 93.8 and 73.4 and saponification equivalents of 265.9, 275.5 and 266.3 respectively.

The mixed fatty acids, hydrogenated fatty acids, and oxidised fatty acids were examined by reversed phase partition column chromatography on paraffin columns in each species. The chromatographic results on paraffin columns in terms of mole % were obtained. The wt. % thus obtained are given in Table I.

The seed oil of *M. calabura* contains 62.7% of unsaturated acids. The unsaturated acids are oleic (18.6%) and linoleic (44.1%). The saturated acids are capric (2.4%), lauric (1.4%), myristic (1.9%), palmitic (19.1%), stearic (10.0%), arachidic (1.4%) and behenic (1.1%).

TABLE I. COMPONENT ACIDS OF ORNAMENTAL SEED OILS

Fatty acids	<i>M. calabura</i> (wt. %)	<i>D. reflexa</i> (wt. %)	<i>I. wightii</i> (wt. %)
Capric	2.4	0.1	—
Lauric	1.4	0.8	4.6
Myristic	1.9	1.7	4.2
Palmitic	19.1	15.0	29.1
Stearic	10.0	6.1	12.1
Arachidic	1.4	2.0	2.2
Behenic	1.1	2.2	2.5
Oleic	18.6	41.2	6.6
Linoleic	44.1	30.8	37

*D. reflexa* seed oil contains 71.0% of unsaturated acids. The principal components among the unsaturated acids are oleic (41.2%) and linoleic (30.8%). This seed oil contains 15.0% of palmitic acid as the major component among the saturated acids with smaller amounts of capric (0.1%), lauric (0.8%), myristic (1.7%), stearic (6.1%), arachidic (2.0%) and behenic (2.2%).

*I. wightii* seed oil contains 54.7% of saturated acids and 45.3% of unsaturated acids. The palmitic acid (29.1%) is the major component among the saturated acids with next major component as stearic (12.1%) acid. The other saturated acids are lauric (4.6%), myristic (4.2%), arachidic (2.2%) and behenic (2.5%). The linoleic acid (38.7%) is the principal component amongst the unsaturated acids along with a small amount of oleic (6.6%).

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# Minor Seed Oils I. Component Fatty Acids of Some Seed Oils

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Seed oils of *Fluggea microcarpa*, *Ixora parviflora*, and *Diospyros melanoxylon* were found to contain (wt. %) the following acids: capric nil, 1.3, nil; lauric nil, 3.1, 4.6; myristic 5.4, 4.7, 9.9; palmitic 19.8, 11.4, 37.9; stearic 9.7, 11.9, 16.1; arachidic 1.4, 2.9, 5.3; behenic 1.2, 2.0, 5.3; oleic 6.1, 18.7, 19.7 and linoleic 56.4, 44.0, 1.2 respectively.

*Fluggea microcarpa*, Blume, syn. *F. virosa* (N. O. Euphorbiaceae) is distributed throughout India. The roots are used to cure gonorrhoea. The leaves made into paste with tobacco are used to destroy worms in sores.<sup>1</sup>

*Ixora parviflora*, Vahl, (N. O. Rubiaceae) is a small much branched evergreen tree. The bark is thick reddish brown.<sup>2</sup> The root or bark is given to females when the urine is highly coloured.

*Diospyros melanoxylon* Roxb, Syn. *D. tuperu* Buch-Ham, (N. O. Ebenaceae), is a moderate sized tree. It grows mixed with teak; but is heavier and harder than teak. The lighter part of sapwood is valuable as black heartwood, which is used in place of true ebony. The fruit is edible.<sup>3</sup>

There are no reports on the component acids of oils obtained from seeds of these trees.

## Materials and Methods

The seeds were collected from the plants grown in the Botanical Gardens of Karnatak University, Dharwad. The seeds were extracted with light petroleum ether (40–60°) to yield the oil. A portion of the oil in each case was hydrolysed in cold condition with ethanolic potassium hydroxide and non-saponifiable matter and mixed acids were obtained.

The thin layer chromatography of mixed acids and their methyl esters revealed the absence of oxygenated acids. The mixed acids (a) after hydrogenation, (b) without other treatment and (c) after oxidation<sup>4</sup> were examined by reverse phase partition chromatography.<sup>5</sup>

The iodine values and saponification equivalents calculated from the results agree well with those measured experimentally.

## Results and Discussion

The seeds of *Fluggea microcarpa*, *Ixora parviflora* and *Diospyros melanoxylon* contain 12.5%, 23.5% and 3.5% of fatty oil respectively.

*F. microcarpa*, *I. parviflora* and *D. melanoxylon* oils have iodine values of 105.3, 98.2, and 19.5, and saponification equivalents of 272.5, 270.0 and 264.2 respectively.

The mixed fatty acids, hydrogenated fatty acids, and oxidised fatty acids of oils were examined by reverse phase partition column chromatography on paraffin columns in each species. The chromatographic results on paraffin columns in terms of mole % were obtained. The wt. % thus obtained are given in Table 1.

*F. microcarpa* seed oil contains linoleic acid as the major component (56.4%) among the unsaturated acids, with smaller amount of oleic acid (6.1%). The saturated acids are myristic (5.4%), palmitic (19.8%), stearic (9.7%), arachidic (1.4%) and behenic (1.2%).

*I. parviflora* seed oil contains 33.3% of saturated acids and 62.7% of unsaturated acids. The saturated acids are capric (1.3%), lauric (3.1%), myristic (4.7%), palmitic (11.4%), stearic (11.9%), arachidic (2.9%), and behenic (2.0%). The linoleic acid (44.0%) is the major component amongst the unsaturated acids with small amount of oleic acid (18.7%).

*D. melanoxylon* seed oil contains (79.1%) of saturated acid. The principal component amongst the saturated

TABLE 1. COMPONENT ACIDS OF SEED OILS

Fatty acids	<i>F. microcarpa</i> (wt. %)	<i>I. parviflora</i> (wt. %)	<i>D. melanoxylon</i> (wt. %)
Capric	—	1.3	—
Lauric	—	3.1	4.6
Myristic	5.4	4.7	9.9
Palmitic	19.8	11.4	37.9
Stearic	9.7	11.9	16.1
Arachidic	1.4	2.9	5.3
Behenic	1.2	2.0	5.3
Oleic	6.1	18.7	19.7
Linoleic	56.4	44.0	1.2

acids are palmitic (37.9%), and stearic (16.1%) with appreciable amount of myristic (9.9%). However, this seed oil also contains lauric (4.6%), arachidic (5.3%), and behenic (5.3%) acids. The major component among the unsaturated acids is only oleic (19.7%). However, very small amount of linoleic acid (1.2%) is observed in the present investigation.

#### Acknowledgement

Authors are grateful to Prof. E. S. Jayadevappa for his keen interest and encouragement.

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## A Study on Seliwanoff Colour Reaction for Estimation of Sucrose in Dairy Products

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The suitability of high temperature and short time for colour development in the estimation of sucrose in dairy products by Seliwanoff reaction is investigated. Lactose contributes towards colour after prolonged heating at high temperatures. However, interference by lactose is negligible when heating is restricted to 15 min. in a boiling water bath.

A method was reported<sup>1</sup> for the estimation of sucrose in ice-cream by Seliwanoff reaction.<sup>2</sup> Though the Seliwanoff colour reaction distinguishes a ketose from an aldose and thus makes it possible to estimate sucrose in the presence of lactose, there are reports that degradation products of aldoses could contribute to the colour.<sup>3</sup> Thus, interference due to degradation products has to be taken into account while employing high temperatures for colour development. Applicability of Seliwanoff reaction for estimation of sucrose in dairy products under different experimental conditions is reported in this paper.

#### Materials and Methods

*Test solution:* The test solution was essentially the same as reported earlier.<sup>1</sup> About 5 g of the sample was made to a convenient volume with distilled water. To an aliquot of the solution, 2 or 3 drops of a saturated solution of lead acetate were added, made to volume and filtered through Whatman No. 4 filter paper.

*Colour development:* To 2 ml. of the filtrate containing not more than 0.1 mg of sucrose/ml.

taken in a test tube, 2 ml. of 0.1% resorcinol in water and 6 ml. of concentrated HCl were added and heated at different temperatures for different duration of time. The colour developed was measured in a spectrophotometer at 490 nm. Sucrose concentrations were read from a standard curve using sucrose.

#### Results and Discussion

Sucrose solutions on hydrolysis give a colour reaction when treated with resorcinol in acid medium. This reaction takes place at a faster rate at higher temperatures. Degradation products of carbohydrates might contribute towards colour intensity when higher temperatures are employed.<sup>3</sup> Further, a colour change from pink to yellow is observed during heating at higher temperatures. Absorption spectra of the resorcinol colour complex read at different stages during the colour change from pink to yellow showed absorption maxima around 405 nm at which wavelength absorbance increased with the temperature and duration of heating (Fig. 1). Though the absorbance was comparatively less at 490 nm, it was largely unaffected by small changes in heating time at boiling water

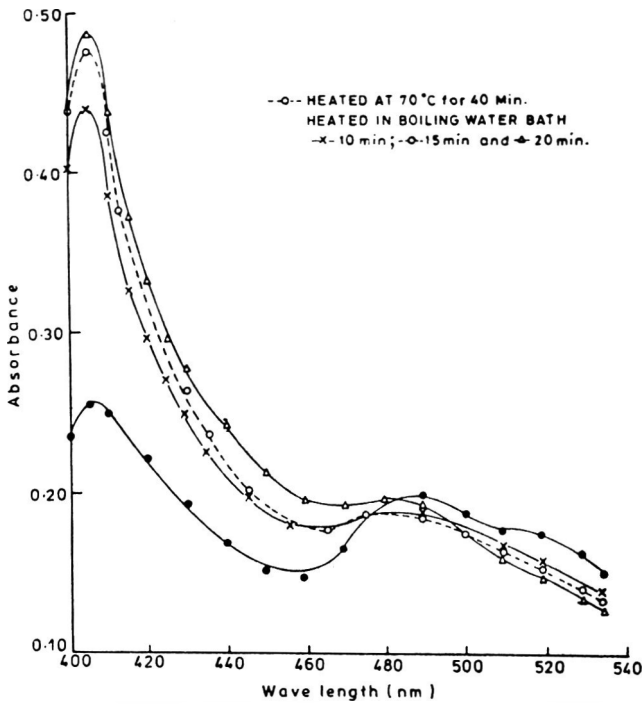


FIG. 1. Absorbance spectra of resorcinol sucrose colour complex.

bath temperature. Therefore, in further studies for the selection of suitable time-temperature combination for colour development, absorbance measurements were made at 490 nm as it would give better reproducibility. Further, interference from aldoses was less at this wave length as revealed by a study on lactose.

The absorbances at 490 nm of test solutions heated to 60, 70, 80 and 90°C and boiling water indicated

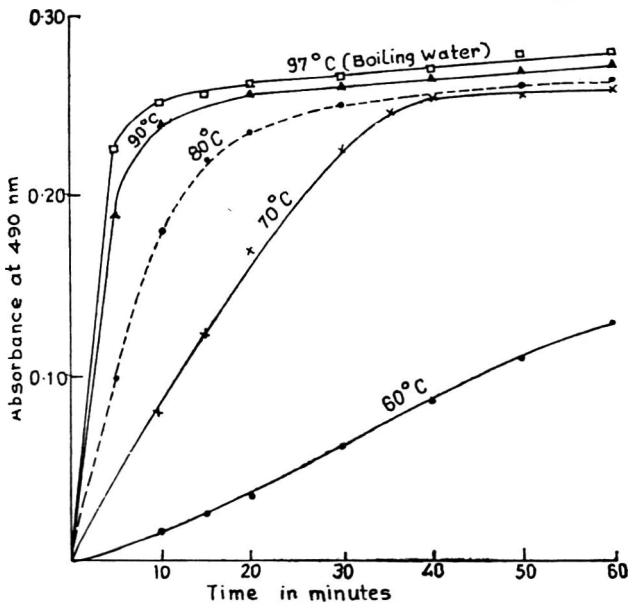


FIG. 2. Effect of heating time and temperature on colour development.

that the colour development was slow at 60°C and the optimum was not reached even after 60 min. of heating (Fig. 2). Optimum absorbance could be achieved by maintaining the temperature at 70°C for 40 min.; higher temperatures however, lowered the heating time. Use of high temperatures however, had to be carried out with caution as it would favour the reaction of aldohexoses and strong acids to form hydroxy methyl furfural.<sup>4</sup> This compound is known to react with resorcinol and other phenolic compounds to form coloured products.<sup>5</sup> The formation of such coloured products from glucose produced by the inversion of sucrose would not be expected to interfere with the estimation when reference curve for sucrose concentrations is prepared under identical conditions. However, the interference due to lactose present in the milk product needed investigation.

Pure sucrose and lactose solutions as well as mixtures of sucrose and lactose in the ratio 2 : 1 were used for colour development at 70°C and at boiling water bath temperature. Absorbance measurements at 490nm showed no influence of lactose when the test solutions were heated at 70°C for upto 60 min. However, at the temperature of boiling water, lactose contributed towards colour, especially during prolonged heating (Fig. 3). Nevertheless, the influence of lactose was negligible when the duration of heating was restricted to 15 min. or less and when the proportion of lactose to sucrose was as high as 1 : 2. As the proportion of lactose would be much lower in dairy products like icecream and condensed milk, heating for 15 min.

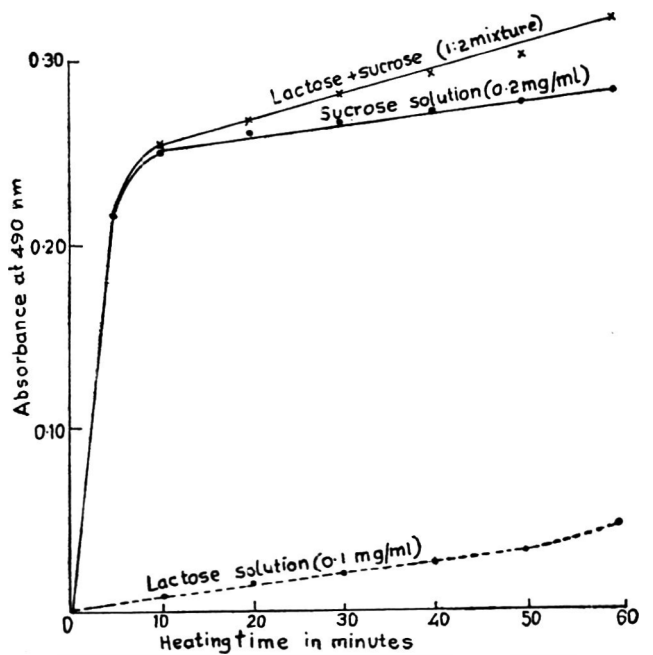


FIG. 3. Effect of lactose on colour development in a boiling water bath.

TABLE 1. SUCROSE CONTENT (PER CENT) OF DAIRY PRODUCTS AS DETERMINED BY LANE-EYNON AND RESORCINOL METHOD

Sample no.	Lane-Eynon	Resorcinol	
		Heating at 70°C	Heating at boiling
Ice cream			
1	15.43	15.33	15.38
2	11.69	11.93	11.88
3	13.32	13.76	13.38
4	15.63	15.43	15.29
5	10.00	9.70	9.50
6	15.11	15.47	15.70
7	15.95	15.64	15.56
8	12.37	13.01	12.73
9	16.69	16.75	17.19
10	16.32	16.53	16.99
11	14.16	14.25	14.63
12	13.00	12.63	12.74
13	13.04	12.84	12.73
14	15.04	15.16	15.39
15	15.18	15.70	15.47
Mean	14.20	14.28	14.30
Condensed milk			
1	41.17	40.05	38.50
2	41.04	41.96	40.06
3	42.50	40.00	43.30
4	45.40	44.40	45.72
5	41.17	40.00	40.75
6	40.81	42.20	41.76
7	41.04	40.05	40.06
8	40.96	39.76	39.50
9	41.07	40.05	42.40
Mean	41.68	40.95	41.34

at boiling waterbath temperature could be employed with advantage. When the proportion of lactose is higher, as in flavoured milk, heating at lower temperature should be preferred.

The two time-temperature combinations, 40 min. at 70°C and 15 min. at boiling water bath were, therefore, selected for the estimation of sucrose in dairy products. Table 1 gives the sucrose contents in 15 samples of icecream and 9 samples of condensed milk as determined by the Lane-Eynon volumetric

TABLE 2. SUCROSE CONTENT (PER CENT) IN THE SAME SAMPLE OF ICE CREAM DETERMINED BY RESORCINOL METHOD AT TWO TEMPERATURES

Estimation no.	Heating at 70°C	Heating at boiling
1	15.70	15.47
2	15.32	15.64
3	15.38	15.33
4	15.56	15.43
5	15.49	15.72
6	15.47	15.39
7	15.29	15.68
8	15.46	15.44
9	15.36	15.61
10	15.63	15.49
Mean	15.47	15.52
Std. dev.	±0.14	±0.13

method<sup>6</sup> and the resorcinol colorimetric method employing these two time-temperature combinations. Statistical analysis showed that both the combinations gave satisfactory results comparable to standard volumetric method.

The reproducibility of the colorimetric method determined by replicate estimations on the same sample of icecream showed that the development of colour at higher temperatures did not affect the precision of the colorimetric method (Table 2).

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# Studies on the Production of *Kulfi* Part—I. The Acceptable Level of Total Milk Solids

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*Kulfi* is an indigenous frozen dessert. Composition of *Kulfi* as sold in market varies widely. A study has been taken up to standardize the composition of *Kulfi*. The acceptable level of total milk solids in *Kulfi* prepared from cow milk was determined by sensory evaluation by a panel of judges. On the basis of trials, the concentration of milk to 26 % T. S. is found to yield *Kulfi* with better body, texture and overall acceptability.

*Kulfi* is an indigenous frozen milk product and like icecream it is palatable and nutritious. The involvement of equipment and machinery in the manufacture of *Kulfi* is minimum. Due to its palatability and comparatively low cost, *Kulfi* is popular in many parts of the country. Very little work has been reported on standard method of preparation and factors that affect the quality of *Kulfi* in terms of chemical composition or on consumer's preference. The method of producing *Kulfi* varies widely resulting in variation in composition, quality and cost of the product. Due to non availability of standardised process for its manufacturers, very few organisations are manufacturing on a commercial scale. Another drawback on large scale production is that PFA regulations do not differentiate between plain icecream and *Kulfi* with regard to its composition. Manufacture of *Kulfi* has been described by various workers.<sup>1-3</sup> However, detailed study such as the effect of total solids on textural qualities and overall acceptability have not been worked out. The present investigation was therefore, undertaken to standardise the method for the production of *kulfi* on commercial scale and evaluate the product by sensory methods.

## Materials and Methods

**Cow milk:** Pooled raw cow milk obtained from NDRI experimental dairy was standardised to a fat content of 3.5%.

**Sugar:** White crystalline sugar was used as sweetening agent and it was presumed to contain 100 per cent total solids.

**Freezing mixture:** Ice and coarse salt in the ratio of 4 : 1 was used for preparation of freezing mixture for freezing *Kulfi*.

***Kulfi* cones:** Aluminium cone, with screw type lid of 100–120 ml. capacity was used in the experiments.

**Pitcher:** Earthen pot of 29.1. capacity was used for freezing *Kulfi* cone with ice-salt mixture

**Heating and concentration unit:** Stainless steel jacketted steam kettle and laddle for stirring were used.

**Standardization and concentration of mix:** Raw cow milk standardized to 3.5% fat and 8.5 per cent SNF was concentrated in an open pan jacketted steam heated vessel with constant stirring until the required solids content was attained. Slow heating was done to avoid burnt particles in final product. *Kulfi* mix was then filled in *Kulfi* cones. The filled cones were immersed in freezing mixture contained in earthen pot and frozen with intermittent shaking according to method of Salooja.<sup>4</sup> In all the trials, 2–5 kg. of *Kulfi* mix of desirable composition was prepared. The details of various steps in *Kulfi* preparation are

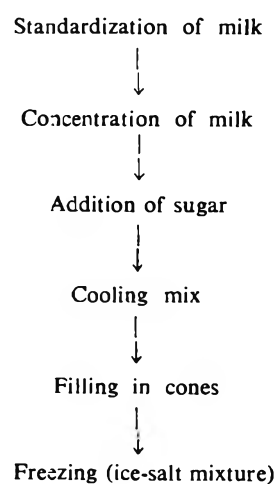


Fig. 1. Flow chart for *Kulfi* preparation.



presented in the flow chart (Fig. 1). The *Kulfi* mix was prepared from standardized cow milk concentrated to following total milk solids levels: 17 per cent (T 1), 20 per cent (T 2), 23 per cent (T 3), 26 per cent (T 4) and 29 per cent (T 5). These levels of concentration were arbitrarily chosen. In all trials, sugar was maintained at 13 per cent level.

**Sensory evaluation:** A panel consisting of six judges evaluated *Kulfi* samples for body, texture and overall quality by a 9-point hedonic scale. The panelists were familiar with the characteristics of the product.

**Statistical analysis:** The data were subjected to analysis of variance according to procedure outlined by Snedecor and Cochran.<sup>5</sup>

### Results and Discussion

Table 1 gives the composition of *Kulfi* prepared with different levels of milk solids. The average fat and solids-non-fat (SNF) contents of *Kulfi* increased with the increase in total milk solids. For instance, when mix was concentrated to 29 per cent total milk solids, the fat content of *Kulfi* increased from the initial value of 3.5 to 8.7 per cent. The increase in fat and SNF per cent in *Kulfi* mixes increased in proportion to the concentration of standardised cow milk used in these trials. Data pertaining to sensory evaluation of *Kulfi* are presented in Table 2.

**Body:** The average scores for body attribute were 5.19, 6.17, 6.90, 7.20 and 6.99 for 17, 20, 23, 26 and 29 per cent total solids respectively. The different treatments were observed to be significantly different. ( $P < 0.05$ ). *Kulfi* with 26 per cent total solids was rated superior by panelists. *Kulfi* with 29, 26 and 23 per cent T. S. scored almost same values indicating that the body of *Kulfi* with these total solids was almost similar in quality.

The reason for comparatively low rating for higher

TABLE 1. FAT, SNF AND TOTAL MILK SOLIDS CONTENT OF *KULFI* PREPARED FROM DIFFERENT LEVELS OF MILK SOLIDS

Milk solids content (%)	Fat (%)	SNF (%)	Total solids (%)
	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
17	5.18 $\pm$ 0.066	11.98 $\pm$ 0.066	17.16 $\pm$ 0.051
20	6.12 $\pm$ 0.049	14.26 $\pm$ 0.194	20.38 $\pm$ 0.225
23	7.02 $\pm$ 0.049	16.22 $\pm$ 0.16	23.24 $\pm$ 0.150
26	7.88 $\pm$ 0.037	18.30 $\pm$ 0.152	26.18 $\pm$ 0.139
29	8.68 $\pm$ 0.020	20.52 $\pm$ 0.065	29.22 $\pm$ 0.120

TABLE 2. SENSORY EVALUATION OF *KULFI*

Milk solids content (%)	Body	Texture	Overall acceptability
17	5.19	4.99	4.76
20	6.16	6.39	5.70
23	6.90	6.73	6.76
26	7.20	7.36	6.86
29	6.99	7.13	6.59

total solids (29 per cent) may be due to heavy and soggy body in *Kulfi*. Ice cream with higher total solids content also gives a heavy and soggy product.<sup>6</sup> Less total solids in *Kulfi* on the other hand give weak and crumbly body. Hence, optimum level of total solids are desirable to have good body characteristics.

**Texture:** The average scores for texture were 4.99, 6.39, 6.73, 7.36 and 7.13 for 17, 20, 23, 26 and 29 per cent total solids respectively. It is also seen that the texture scores for different total solids content were also significant ( $P < 0.05$ ); highest score for texture was obtained for *Kulfi* containing 26% total solids. Sommer<sup>7</sup> reported that an increased total solids content resulted in apparent improvement of texture, which was due to incomplete freezing of product. This may be the reason for insignificant difference in critical difference.

In case of ice cream, higher total solids help to improve the smoothness. That the highest solids level under present study (29 per cent) had scored less in terms of texture, may be due to presence of coarse denatured particles in *Kulfi* on account of heating and concentration of milk.

**Over all quality of the product:** The average score for over all quality for T 1, T 2, T 3, T 4 and T 5 were 4.76, 5.70, 6.76, 6.86 and 6.59 respectively. The overall quality scores for different treatments were not significantly different. Highest average score (6.76) was for *Kulfi* with 26% total solids. *Kulfi* with 23, 26 and 29 per cent total solids gave almost similar average scores.

The present study has shown that if cow milk with 3.5% fat and 8.5% SNF is used in the preparation of *Kulfi*, the concentration of milk to 26 per cent total solids is expected to yield *Kulfi* of better body texture and over all quality. But at this level of total milk solids, the *Kulfi* is expected to have an average of 7.9% fat. But this *Kulfi* sample will fall short of statutory regulation (minimum of 10%) with regard to fat content.

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## A Study on the Bionomics of Major Insect Species Infesting Cashew Kernels

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In a survey conducted at the major cashew processing units, *T. castaneum*, *E. cautella* and *O. surinamensis* were identified as the major insect pests of processed cashew kernels. Laboratory investigation on five species of insects showed that *T. castaneum* had a low population growth rate as measured by 'intrinsic rate of natural increase per week' and 'theoretical rate of self multiplication'. However, *T. castaneum* was the most predominant and was successfully growing under the ecological conditions prevalent in the cashew processing units. Though the temperature and humidity conditions prevailing at the processing units were conducive to the faster multiplication of the moth species, the interspecific competition and probably, ecological adaptability of *T. castaneum* will continue to make it the most important pest infesting processed cashew kernels.

In a commodity of high monetary value like cashew kernels, the tolerance level of insect infestation approaches zero. Though earlier studies report a large number of insects associated with cashew processing units<sup>1-3</sup>, they do not provide an insight into the status of individual species as pests of processed cashew kernels. A survey of major foci of insect development and identification of the species found associated with cashew kernels and raw nuts in processing units, provided the basic foundation for laboratory studies. *Tribolium castaneum* (Herbst) was the most predominant insect in all processing units surveyed. *Oryzaephilus surinamensis* (Linn) and *Ephestia cautella* (Walker) were the other major species infesting cashew kernels. The imported rawnuts in storage showed infestation by *O. surinamensis*, *T. castaneum*, *Sitophilus oryzae* (Linn), *Cryptolestes ferrugineus* (Stephens) and *E. cautella*.

Samples of dust material in gunny bags used for storage of rawnuts contained dead *Tenebroides mauritanicus* (Linn), *Ahasverus advena* (Waltl.) *Necrobia rufipes* (Degeer), *T. castaneum* and *O. surinamensis*. Cracks and crevices of processing floor, store and

grading room frequently harboured *T. castaneum*, *E. cautella* and *C. ferrugineus*. *T. castaneum* and *Alphitobius piceus* (Oliv) were observed in empty baskets and polyethylene covers.

Population dynamics has often been calculated from the laboratory experimental data and the method is based on life and fecundity tables adapted by Leslie and Park<sup>4</sup> and Howe<sup>5,6</sup>.

### Materials and Methods

One hundred, 1 day old eggs of *T. castaneum* were released on to 125 g of freshly processed W-320 grade cashew kernels kept in 77 mm (dia), 93 mm (ht) open top sanitary can. The mouth of the can was covered with a cloth and fixed by rubber band. One set of treatment with four replications was incubated at  $25 \pm 2.5^\circ\text{C}$  and  $65 \pm 5\%$  R.H. (ambient conditions in the laboratory) and another set of four replications at  $32 \pm 1^\circ\text{C}$  and  $80 \pm 1\%$  R.H. in a Callenkamp humidifying oven. The developmental period from egg to adult 'd' in weeks and the percentage of eggs which matured into adults 'p' were recorded according to Howe.<sup>5</sup>

TABLE 1. LIFE TABLE AND FECUNDITY DATA ON FIVE SPECIES OF STORED PRODUCT INSECTS BREEDING IN CASHEW KERNELS

Insect species	Incubation temp. (°c)	R. H. (%)	Developmental period 'd' (wk)	Oviposition period 'l' (wk)	No. of eggs laid / female '2n'	Maturity proportion 'p'
<i>T. castaneum</i>	25 ± 2.5	65 ± 5	6	28	360	0.28
	32 ± 1.0	80 ± 1	5	7	539	0.43
<i>O. surinamensis</i>	25 ± 2.5	65 ± 5	7	30	211	0.48
	32 ± 1.0	80 ± 1	4	21	287	0.72
<i>E. cautella</i>	25 ± 2.5	65 ± 5	4	1	103	0.54
	32 ± 1.0	80 ± 1	3	1	108	0.48
<i>C. cephalonica</i>	25 ± 2.5	65 ± 5	7	1	97	0.32
	32 ± 1.0	80 ± 1	5	1	126	0.41
<i>S. paniceum</i>	25 ± 2.5	65 ± 5	8	2	71	0.58
	32 ± 1.0	80 ± 1	8	1	58	0.37

Simultaneously, 15 pairs of freshly emerged adult *T. castaneum* were released to 10 g finely powdered meal of fresh cashew kernels sieved to pass through 35 mesh standard Tyler's sieve and kept in petri dishes. The meal was sieved in 35 mesh sieve twice weekly to recover the eggs and recorded as eggs per 15 females, from which number of eggs per female '2n' and number of female eggs per female 'n' were computed. The oviposition period 'l' in weeks was arrived at by recording the beginning and cessation of egg laying. The experiments were done in four replications and at the two temperature and humidity levels.

The two sets of experiments were repeated with *O. surinamensis*, *E. cautella*, *C. cephalonica* and *S. paniceum*. The life table and fecundity data from the experiments are presented in Table 1.

### Results and Discussion

Ecological significance is natural to an assumption that the unhindered growth of a population which has settled down to a stable proportion of age groups is geometric, the numbers increasing by a constant factor 'λ' called 'the theoretical rate of self-multiplication per week'. 'The intrinsic rate of natural increase' represented by 'r<sub>m</sub>' is an index of increase of a species under specified set of physical conditions given adequate food supply and space<sup>4</sup>. The 'r<sub>m</sub>' and 'λ' values computed from the life and fecundity data under the two temperature and humidity conditions in respect of the five pest species studied is presented in Table 2.

The intrinsic rate of natural increase per week (r<sub>m</sub>) as well as the 'theoretical rate of self multiply

per week' (λ) was greater at the higher temperature and humidity conditions in all species studied except *S. paniceum*. At 32 ± 1°C and 80 ± 1% R.H., *E. cautella* showed the highest intrinsic rate of natural increase and theoretical rate of self multiplication. At 25 ± 2.5°C and 65 ± 5% R.H. the test species in descending order of their r<sub>m</sub> and λ per week

TABLE 2. STATISTICS OF POPULATION DYNAMICS OF FIVE INSECT SPECIES IN CASHEW KERNELS

Insect Spp.	Intrinsic rate of natural increase/wk. (r <sub>m</sub> )		Theoretical rate of self multiplication/wk. (λ)	
	25°C and 65% RH	32°C and 80% RH	25°C and 65% RH	32°C and 80% RH
	<i>E. cautella</i>	0.32	0.40	2.09
<i>C. cephalonica</i>	0.16	0.26	1.44	1.81
<i>T. castaneum</i>	0.08	0.24	1.22	1.75
<i>O. surinamensis</i>	0.08	0.14	1.19	1.38
<i>S. paniceum</i>	0.14	0.12	1.40	1.32

Note:  $r_m = \frac{\log np}{d + \frac{1}{2}l}$       λ = the natural antilog of r<sub>m</sub>.

Where, n is the number of female eggs\*,  
p is the proportion of female eggs that survive and mature.  
d is the development period in units of weeks.  
l is the oviposition period in units of weeks.

\*Number of eggs that will hatch out into female insects, where sex-ratio is assumed to be 1 : 1, this is half the total number of eggs.

were *E. cautella*, *C. cephalonica*, *S. paniceum*, *T. castaneum* and *O. surinamensis*. The results showed good agreement with the reported<sup>7</sup> estimate of optimal conditions for population increase for the species. Although the innate capacity for increasing of a pest species derived from laboratory experiments are figures of limited application, as opined by Solomon<sup>8</sup>, the parameter gives an approximate measure of actual characteristic of the species which enter fundamentally into its population dynamics, however, greatly their expressions may be modified in nature.

A predominance of *T. castaneum* was observed in the natural ecosystem of cashew processing units over species with higher intrinsic rate of natural increase and theoretical rate of self multiplication under laboratory conditions. This is attributed to the modification of population dynamics due to ecological factors like interspecific competition, availability of food and foci of development. This inference is well borne out by the findings of Adeyemi<sup>9</sup> on competition between *T. castaneum* and three moth species infesting groundnuts in which *T. castaneum* reduced the intrinsic rate of moth increase, while moths did not affect the rate of increase of *T. castaneum* to the same extent under conditions of competition for food and space. Hence as a result of competition for food and or space with *T. castaneum*, the moth species are likely to be exterminated despite their apparently higher intrinsic rate of increase. Under ecological condition existing in the processing units, the extermination of moth species by natural inter-

specific competition is very unlikely, though predominance of *T. castaneum* is widely observed.

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## RESEARCH NOTES

### BIOCHEMICAL EFFECTS OF RAPE SEED MEAL IN ALBINO RATS

A 4-week trial was conducted with male albino rats to ascertain the effect of different protein levels of rape seed meal (RSM) on growth rate and changes in carbohydrate and protein metabolism in various tissues. The levels of muscle aminotransferases increased, whereas liver glycogen decreased significantly in RSM (10%) fed animals as compared to casein fed animals. The other biochemical parameters examined remained unaltered. The animals fed RSM at 5% level showed an increase in liver glycogen content while blood glucose, protein of both liver and muscle as well as aminotransferases of muscles decreased significantly in comparison with group fed 10% RSM. However, the muscle glycogen and liver aminotransferases did not vary significantly in both RSM fed animals. Furthermore, significant decrease was also observed in growth rate of both RSM groups. Thus, it is suggestive that RSM intake disturbs the metabolic homeostasis and cannot be recommended as such for animal consumption.

The maintenance of metabolic homeostasis depends upon the simultaneous interplay of several regulatory mechanisms. The biological values of rape seed meal (RSM) have earlier been carried out by studying the growth<sup>1</sup>, nitrogen metabolism<sup>2</sup> and its nutritive utility.<sup>3</sup> The changes in metabolic and enzyme activity patterns on feeding rape seed protein at different levels to animals have not been delineated. In view of this, the present study was designed to investigate changes in protein and carbohydrate metabolism in various tissues which included protein, aspartate (GOT) and alanine (GPT) aminotransferases as well as glycogen and glucose on feeding different levels of rape seed protein in albino rats.

Male albino rats (Wistar strain maintained at H.A.U. Hissar) weighing 80–120 g, were housed individually in cages in a room under a conventional lighting schedule with a dark night. Food pellets from M/s Hind Lever Ltd. India were supplied in fixed aluminium pots for 4–5 days to adapt the animals to new surroundings and water was made available *ad libitum*. The animals were then reweighed and divided into three groups. Each rat was weighed weekly and a record of daily food intake was maintained.

The rape seed (*Brassica campestris*, var. *toria*) grown in Amritsar district (India) was procured, cleaned, ground and made free from oil using successive extraction. Experimental diets containing

casein 10%(A), RSM (rape seed meal) to provide 10%(B) and 5%(C) protein were prepared<sup>4</sup> and was given *ad lib* for four weeks to respective groups.

At the end of the experimental period, the animals were starved for 12–15 hr and then killed by decapitation. Their blood was collected by using anticoagulant for determining blood glucose.<sup>5</sup> Part of the liver and gastrocnemius muscle of each hind leg were rapidly excised for the determination of glycogen,<sup>6</sup> protein<sup>7</sup>, alanine and aspartate amino transferases.<sup>8</sup> These samples were blotted free of blood, placed in small polythene bags and stored at –20°C. Estimations were carried out either on fresh samples on the day of sacrifice or on samples stored at –20°C. The analysis of all biochemical parameters were performed in duplicate. Means and standard errors were calculated for each group and significance was found out by applying paired 't' test.

The changes in growth, carbohydrate and protein metabolism on feeding different levels of rape seed protein in albino rats are presented in Table I. Groups B and C consumed on an average only 58 and 64% of food, 55 and 31% protein respectively as compared to the casein 10% group (A). Growth rate of group B animals was significantly lower than group A, but it is similar to that of group C. The fasting levels of GOT and GPT in muscles increased, whereas liver glycogen decreased significantly in group B as compared to group A animals. The other biochemical parameters investigated did not show any appreciable changes.

The liver glycogen contents of group C animals increased, while blood glucose, protein of both liver and muscle and GOT and GPT of muscles decreased significantly in comparison to group B. On the other hand, muscle glycogen, liver GOT and GPT did not vary significantly in groups B and C.

The present study reveals the effect of quality (B Vs A) and quantity (B Vs C) of protein intake on the various biochemical parameters in rats. The results indicate a decrease in liver glycogen and simultaneous increase in GOT and GPT activity of muscles of rats fed on RSM 10% in comparison to casein 10%. However, such changes are not associated with a parallel increase in blood glucose. This decreased level of blood glucose might be due to their increased capacity to move glucose from blood into peripheral tissues for utilization which may be accompanied by increased stimulation of  $\beta$ -islets of langerhans of pancreas. Similar findings have earlier been demonstrated in malnourished animals<sup>9,10</sup>.

TABLE 1. BIOCHEMICAL EFFECTS OF RAPE SEED MEAL DIET ON RATS

Diet	Tissues	Casein 10%(A) Mean $\pm$ S. E.	RSM 10%(B) Mean $\pm$ S. E.	RSM 5%(C) Mean $\pm$ S. E.
Food intake (g/rat/28 days)	—	178.92 $\pm$ 24.08	104.37* $\pm$ 21.20	114.24 $\pm$ 19.32
Body wt. (g/rat/28 days)	—	115.60 $\pm$ 6.20	69.00* $\pm$ 1.79	77.80* $\pm$ 3.34
Glucose (mg/100 ml)	Blood	111.18 $\pm$ 13.77	95.83 $\pm$ 6.53	65.83* $\pm$ 4.55
Glycogen (mg/g)	Liver	48.80 $\pm$ 1.20	16.40* $\pm$ 1.30	29.20* $\pm$ 4.00
	Muscle	11.00 $\pm$ 1.30	10.40 $\pm$ 0.30	11.10 $\pm$ 0.07
Protein (mg/g)	Liver	136.49 $\pm$ 2.80	142.75 $\pm$ 3.45	122.52* $\pm$ 0.94
	Muscle	169.70 $\pm$ 1.10	169.90 $\pm$ 0.78	137.98* $\pm$ 1.48
Aspartate aminotransferase (units/100 mg)	Liver	20.70 $\pm$ 0.13	22.60 $\pm$ 0.33	21.30 $\pm$ 0.36
	Muscle	20.20 $\pm$ 0.63	22.70* $\pm$ 0.30	17.60* $\pm$ 0.16
Alanine aminotransferase (units/100 mg)	Liver	33.30 $\pm$ 0.96	36.50 $\pm$ 0.30	35.00 $\pm$ 0.30
	Muscle	26.60 $\pm$ 0.80	29.50* $\pm$ 0.20	20.90* $\pm$ 0.46

Enzyme units =  $\mu$ g of oxaloacetate or pyruvate released/min  
Initial body wt for all groups were 107.00  $\pm$  4.35  
Feeding *ad libitum* for 4 weeks.

\*p < 0.05

Munro *et al.*<sup>11</sup> reported that the composition of the diet, specifically the administration of different proteins, decreases the liver glycogen and blood glucose content, which is also observed in the present study. However, the protein content of both liver and muscle remained unaltered (Table 1). The decreased level of blood glucose and increased liver glycogen content in group C animals as compared to B shows a positive correlation.

The low muscle and liver protein concentration of group C rats may be due to less protein content in the diet. Both GPT and GOT activity in muscles decreases in group C as compared to group B animals. These observations are in agreement with those of Heard *et al.*<sup>12</sup> The liver GPT and GOT activity as well as muscle glycogen content appeared to be unaffected by any of these dietary treatments.

It is also necessary to note the greatly reduced rate of growth and food intake. In the present study, both RSM group rats were significantly stunted and equally short of food (energy) as judged by normal requirements (casein 10%). The above results can be explained because of less palatability and digestibility of RSM protein than control protein<sup>2,13</sup>.

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## STUDIES ON THE NUTRITIONAL QUALITY OF SOME VARIETIES OF MUNG BEAN

(*Vigna radiata*)

A study of nutritional composition of nine mung varieties was carried out. The proximate principles except fat, energy value and phosphorus contents did not exhibit any significant differences. Thousand kernel weight, crushing hardness, calcium, iron, methionine and tryptophan ranged from 24.07 to 53.18 g, 1.95 to 3.78 kg/seed, 88 to 198 mg, 3.70 to 8.80 mg, 160 to 360 mg and 219 to 578 mg/100 g dry weight respectively. Values varying from 9.29 to 16.57%, 0.35 to 1.98%, 7.96 to 14.59% and from 52.30 to 66.62% were noticed for total, reducing and non-reducing sugars, and biological value respectively. Varieties 'K 851' and 'PS 16' appear to be quite promising from the nutritional angle.

Pulses are essential components in the human dietary in providing the amino acid balance needed for normal growth and maintenance of health of a majority of Indian population. They contain twice as much protein as cereals and a complementary pattern of amino acids, but are low in oil and sulphur containing amino acids. Yohe and Poehlman<sup>1</sup> have observed wide genetic variations for protein, lysine and methionine contents in large population of mung bean strains. The biological value of pulse proteins ranged from 32.0 to 78.0 per cent<sup>2,3</sup>.

In this communication, we have examined the variability of nutritional quality parameters in nine mung varieties for exploring their possible use for development of nutritionally superior varieties.

Grain samples of 'K 851', 'H 70-16', 'PS 16', 'PS 7', 'T 44', 'T 51', 'Cot 71', 'S-8' and 'S R' were received from the Department of Plant Breeding, Haryana Agricultural University, Hissar during 1977. The unsplit whole grain was used to judge crushing hardness with the help of hardness tester (Ogawa Seiki, Ltd., Tokyo, Japan). Moisture, ether extract, mineral matter, calcium and iron contents were estimated by AOAC methods.<sup>4</sup> Protein content was determined by the method of McKenzie and Wallace<sup>5</sup> and the food energy value was computed from the data of proximate principles assuming that protein and carbohydrates each yield 4.0 Kcal/g and fats 9 Kcal/g. Methionine and tryptophan were estimated by colorimetric methods of Horn *et al.*<sup>6</sup>, Smith and Agiza<sup>7</sup> and their chemical scores were worked out in comparison with hen's egg ratio.<sup>8</sup> Protein quality was assessed from the biological value (B. V.) calculated by chemical score method of Block and Mitchell<sup>9</sup> using the equation,  $Y=102-0.634 \times$  where Y is B. V. and  $\times$  is percentage methionine deficiency in mung with reference to whole egg protein. Phosphorus was determined by the method of Dickman and Bray<sup>10</sup> and reducing sugars by Hulme and Narain<sup>11</sup> and the extract for total sugars was prepared by the

TABLE 1. QUALITY CONSTITUENTS OF MUNG VARIETIES

Constituent	K 851	H 70-16	PS 16	P 57	T 44	T 51	Cot 71	S-8	S × R	Mean
1000 kernel wt (g)	34.57	53.18	27.97	40.55	31.63	29.45	24.03	25.85	35.89	32.57
Seed hardness (kg/seed)	1.93	3.63	2.26	2.12	2.14	2.40	1.96	3.78	2.94	2.57
Moisture (%)	8.78	8.90	9.30	8.90	9.14	8.46	8.82	8.64	8.40	8.82
Crude protein (%)	27.71	27.77	26.19	25.64	26.46	27.11	23.92	25.95	24.66	26.16
Minerals (%)	4.03	3.73	3.96	3.87	3.76	4.03	3.96	3.55	3.88	3.90
Total carbohydrate (%)	58.38	57.34	58.29	60.15	60.09	58.88	60.36	59.89	61.38	59.41
Crude fat (%)	1.10	2.21	2.27	1.44	1.50	1.52	1.50	1.97	1.68	1.69
Energy (Kcal)	354.3	360.3	358.3	356.1	359.7	357.6	350.6	361.3	359.3	357.5
Calcium (mg/100 g)	198	132	154	165	88	152	110	142	98	138
Phosphorus (mg/100g)	461	467	453	461	467	453	461	463	457	461
Iron (mg/100 g)	8.80	4.73	5.94	4.73	5.28	7.85	5.28	3.70	4.36	5.63
Methionine (mg/100g)	360	310	360	250	220	250	160	240	250	270
Chemical score (methionine)	41.9	39.4	44.2	31.5	30.0	33.0	21.6	29.8	32.6	33.2
Biological value (%)	65.17	63.70	66.62	58.60	57.62	59.52	52.30	57.50	57.30	59.80
Tryptophan (mg/100g)	579	542	520	472	571	458	571	446	219	480
Chemical score (Tryptophan)	130.6	121.8	118.7	115.6	135.0	105.6	148.7	107.5	77.53	114.7
Total sugars (%)	11.15	13.31	9.29	14.87	11.15	12.89	13.01	12.89	16.57	12.74
Reducing sugar (%)	1.48	1.11	1.35	1.67	0.36	1.24	1.01	1.42	1.98	1.29
Non-reducing sugars(%)	9.67	11.30	7.94	13.20	10.79	11.65	12.00	11.47	14.59	11.45

method followed by Srinivasan and Bhatia<sup>12</sup> and non-reducing sugars were obtained by difference.

The thousand kernel weight varies from 24.03 to 53.18 g and crushing hardness from 1.93 to 3.78 kg/seed, whereas the mean observed was 32.57 g and 2.57kg/seed respectively (Table 1). Rathnaswamy *et al.*<sup>13</sup> and Manimekalai *et al.*<sup>14</sup> observed varietal differences for seed index, bulk density and specific gravity in red gram. Seed size is an objective index of milling value<sup>15</sup>, whereas the grain hardness is governed by the adhesion between starch and storage proteins.<sup>16</sup>

The moisture content varied from 8.46 in 'T 51' to 9.30 in 'PS 16' with an average of 8.82 per cent. The maximum value for protein content (27.77%) was noticed in 'H 70-16' and minimum (23.92%) in 'Cot 71', whereas the average observed was 26.16 per cent. The total mineral matter (as per cent) ranged from 3.55 in 'S-8' to 4.03 in 'K 851' and 'T 51' and energy value from 350.62 to 360.33 Kcal/100 g. Variety 'K 851' had the lowest fat content (1.10%) and 'PS 16' the highest (2.27%). These results are in agreement with those of Khan and Baker<sup>17</sup>, and Gupta and Wagle.<sup>18</sup>

The calcium content varied from 88 to 198 mg/100 g and the iron content from 3.70 to 8.80 mg/100 g. The variability for phosphorus content among varieties was almost negligible. The lowest calcium to phosphorus ratio of '0.189' was observed in 'T 44' and the highest 0.428 in 'K 851'. Desirable ratio for calcium to phosphorus has been recognised to lie between 2 : 1 and 1 : 1 for adequate nutrition<sup>19</sup>. The higher values 219 to 759 mg/100 g obtained for tryptophan in the present investigation are not in agreement with those reported by Gopalan *et al.*<sup>20</sup> The possible reasons may be the variations in variety, location, environmental conditions, fertilizer dose and analytical methods. Variety 'PS 16' had the highest (360 mg/100 g) content of methionine with a chemical score of 44.20 and biological value of 66.62%, whereas the var 'Cot 71' showed the lowest value for all the above mentioned three constituents. The reported results are not close to the findings of Gupta and Wagle.<sup>18</sup> Values ranging between 32 and 78 per cent for biological value reported by Jaffee<sup>2</sup> and Patwardhan<sup>3</sup> for pulses, lend support to the present findings.

Most of the varieties analysed contained more than ten per cent water soluble carbohydrates and the average value was 12.74 per cent. The variety 'T 44' showed the lowest content of 0.36% and the variety 'S × R', the highest (1.98%) reducing sugars, whereas the non-reducing sugars varied from 7.94 to 14.59 per cent. During four days of germination, soluble sugars varied from 9.00 to 0.33%, whereas reducing

and non-reducing sugars, varied from 2.40 to 0.13 and 7.16 to 0.32% respectively in pulses.<sup>21</sup>

It may, therefore, be concluded that mung bean varieties 'K 851', 'H 70-16' and 'PS 16' appear to be nutritionally superior to other varieties studied.

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## BREAKAGE OF RICE DURING MILLING

### 4. EFFECT OF KERNEL CHALKINESS

In 10 rice varieties tested, chalkiness score and cracks in mature kernels were mutually correlated: more the chalkiness index, the more were the cracks, and *vice versa*. Kernel chalkiness thus predisposed the rice grain to easier cracking under field conditions, and hence indirectly to greater milling breakage. Chalkiness score of mature kernels also increased in field paddy as its harvest was delayed, which may be one reason why rice breakage increases with delayed harvest.

Numerous researchers have repeatedly mentioned in the literature that kernel chalkiness (in otherwise mature grains) is an important contributor to breakage of rice during milling. Yet no experimental evidence in support of this statement is available. On the contrary, Bhattacharya<sup>1</sup> and Matthews *et al.*<sup>2</sup> could obtain no evidence in careful studies that chalky, but mature and uncracked grains in a sample broke more readily during milling than corresponding vitreous grains. Bhattacharya<sup>3</sup> in a recent review hypothesized one possibility. It had been noticed in this laboratory earlier that milled parboiled rice with an ungelatinized opaque core<sup>4</sup> as well as milled raw (mature) rice with chalky portions<sup>5</sup> cracked more readily when put in water than fully vitreous kernels. On this basis, it was hypothesized that varieties with chalky kernels could undergo greater cracking in the field and during drying than those with vitreous grains, and thus indirectly be subject to more breakage during milling.

To test this hypothesis, several varieties available in laboratory stock were analysed for cracks and chalkiness in their kernels. The samples were first

shelled with a Satake laboratory rubber-roller sheller. Then, about 200 randomly selected, mature brown rice grains in duplicate were analysed for cracks with a modified microbiological colony counter as described earlier<sup>6</sup> as well as for chalkiness score as per the following score card:

% Kernel area chalky	0	1-20	>20
Score	0	3	9

The results (Table 1) confirmed that cracks and chalkiness score in mature rice kernels were mutually correlated: more the cracks, the more was chalkiness index, and *vice versa*. This was true in varieties of all sizes and shapes.

Meanwhile, it was noticed that chalkiness in mature rice kernels seemed to increase in a variety as its harvest was delayed. To confirm this, seven paddy varieties in the 1980 summer crop were harvested at several stages and shade dried as described earlier.<sup>6</sup> The samples were then shelled and analysed for kernel chalkiness. The results (Table 2) confirmed that as the day after flowering increased, the chalkiness index also increased. These results are in apparent contradiction to those of Kester *et al.*<sup>7</sup> who found that kernel chalkiness decreased with progressive maturity of rice grains in the field. However, these workers classed only grains which were "at least half chalky" as chalky kernels, which means essentially immature grains; and immature grains naturally decreased with progressive maturity. The present work, on the other hand, concerns fully vitreous *vs.* even minutely chalky grains in the mature crop. In such crop, it is clearly shown that kernel chalkiness increases in rice with delay in harvest after attainment of maturity in the field.

TABLE 1. CHALKINESS SCORE OF CRACKED AND UNCRACKED RICE GRAINS

Variety	Length (mm)	L/B ratio*	Chalkiness score of grains having cracks numbering		
			0	1	>1
Basmati370	6.1	3.4	2.0	5.3	8.0
S705	6.1	3.0	0.3	2.3	4.7
Intan	6.5	3.1	1.6	7.0	7.0
Jenugudu	5.7	3.2	2.1	2.4	-
S749	5.7	2.6	1.4	3.1	1.0
Mahsuri	5.2	2.6	2.5	2.8	2.5
Pankaj	6.1	2.4	2.2	3.6	4.0
Ch2	6.0	2.3	3.0	4.0	4.0
Bala	4.8	1.9	2.3	2.9	3.6
C435	4.0	1.9	1.6	3.8	3.8

\*L, mean length and B, mean breadth of a milled grain.

TABLE 2. INCREASE IN CHALKINESS SCORE IN DIFFERENT VARIETIES OF RICE GRAINS WITH DAYS AFTER FLOWERING\*

Variety	Days after flowering	Chalkiness score	Variety	Days after flowering	Chalkiness score	
ES18	24	0.4	MR301	24	2.5	
	28	0.7		29	4.6	
	33	1.4		32	5.0	
	38	2.0		36	5.4	
	41	2.1		40	5.5	
	45	2.7		27	2.4	
Madhu	20	0.7	Bharani	32	3.3	
	24	1.2		35	3.6	
	29	1.5		39	3.8	
	34	3.2		43	4.2	
	37	3.5		MR365	32	0.4
	41	4.2			37	1.3
Mangala	27	4.2		40	2.2	
	31	4.9		44	2.2	
	36	5.4		48	2.4	
	41	6.3		IET4107	19	6.8
	44	6.6		23	7.4	
	48	7.1		33	7.6	
			40	8.0		

\*The relative increase appears to be more in varieties with low initial score than in those with high initial score. This is because of the score card chosen, wherein kernels having >20% of area chalky were given the maximum score of 9. Therefore, even though the chalky area went on increasing beyond 20%, as it perceptively did in varieties with high initial score, the score could not increase proportionately.

Two conclusions that can be drawn from these data are: (1) While chalky rice kernels *per se* may not break more readily during milling than vitreous kernels, the former invariably undergo more cracking under field conditions than the latter. Varieties with more chalky grains are therefore, more prone to break during milling, for kernel chalkiness indirectly contributes to rice breakage through easier cracking. This finding provides a rational explanation to the persistent popular belief that chalky varieties break more during milling than non-chalky ones—although the assumption, that therefore, chalky grains *per se* are prone to break, is apparently unfounded. (2) Since delayed harvest increases kernel chalkiness, which renders the grain more prone to crack, this fact may be one reason why, as is well known<sup>3</sup>, rice breakage increases with delayed harvest.

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## EFFECT OF DEGUMMING ON KEEPING QUALITY AND REFINABILITY OF INDIGENOUS SOYBEAN OIL

Crude and degummed soybean oils obtained from *Kalitur* (indigenous black soybean) could be stored well for 210 and 120 days respectively. Storage showed no adverse effect on the refinability of these oils.

Indigenous cultivar of soybean with black seedcoat called *Kalitur* (Black soybean) is grown to the extent of 50,000 tonnes in Madhya Pradesh. Data on pilot plant processing by expelling of this variety were presented in an earlier paper<sup>1</sup>. Data on storage studies of expeller crude and degummed oils and their refinability are presented in this note.

Parameters studied are free fatty acids, peroxide value, iodine value, refining and bleaching performance according to AOCS methods<sup>2</sup> and Kreis colour test as described by Mehlenbacher<sup>3</sup>.

Crude oil was degummed for 30–45 min with 5 per cent water at 75°C. The crude and the degummed oils, having phosphatide content of 2.99 and 0.55 per cent respectively, were stored in closed tin containers and the samples were drawn from the same containers at intervals of 30 days for 360 days and analysed. At the end of 560 days, final samples were drawn and analysed. The oils were also refined initially and at the end of 560 days.

The variations observed during storage in free fatty acid (in the range of 1.5), iodine value and Lovibond colour (in the range of 50, Y+10R units in 0.635 cm cell) are not significant being within the limits of experimental errors. But according to peroxide

value, the storability of degummed oil would be 120 days, since by that time, there is an increase of 3 units (over initial 9). For crude oil, the keeping time limit is 210 days, the peroxide value having risen slightly to 7-8 from the initial 6.5. Consideration on the basis of Kreis colour (which is rather erratic) also points out towards the same time, limits for the shelf life of the degummed and crude soybean oils. While storage has no adverse effect on the refining performance, the colours of crude and degummed oils as well as refined and bleached oils from them are slightly darker at the end of 560 days of storage.

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**QUANTIFICATION OF CHOLINESTERASE INHIBITION ON TLC PLATE FOR ESTIMATION OF FENITROTHION**

A simple and sensitive TLC-enzymatic method is developed for determination of fenitrothion (O, O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate) as fenitrooxon (O, O-dimethyl O-(3-methyl-4-nitrophenyl phosphate) employing pig liver acetone powder as enzyme source. By this method, fenitrothion can be detected in amounts ranging from 5 to 50 ng.

TLC-enzymatic methods based on cholinesterase (ChE) inhibition technique were reported as simple and sensitive for detection of organophosphorus pesticides<sup>1,2</sup>. Various raw live sources of enzyme like livers of bovine, pig and sheep<sup>3,4</sup> human serum,<sup>5</sup> bee heads and drosphila heads<sup>6</sup> and peacock plasma<sup>7</sup> were employed for detection of organophosphorus pesticides. Nandakumar *et al.*<sup>8</sup>, reported a TLC-enzymatic method for determination of parathion employing raw rat liver as enzyme source. In these methods, the procurement and preparation of enzyme

suspension are cumbersome and thus delay estimation. Recently, the authors reported<sup>9-11</sup> colorimetric estimation of methyl parathion and dimethoate using liver acetone, powder because of its easy availability and instant use. Thus, it is more advantageous than live enzyme sources. In this investigation, attempts are made to develop an enzymatic method on TLC plate for rapid detection and determination of fenitrothion by using pig liver acetone powder as ChE source and p-nitrobenzene diazonium fluoroborate as chromogenic salt. By this, fenitrothion can be estimated as fenitrooxon within 1 hr and it can be used in residue analysis.

All chemicals used were of analytical grade. Fenitrothion (99%) obtained from Rallis India Ltd., Bombay, was used for preparing acetone solutions of various concentrations. Pig liver acetone powder (1%) was homogenised in ice cold distilled water and was used immediately as enzyme source. The solutions of 0.5% 1-naphthyl acetate and 0.4% p-nitrobenzene diazonium fluoroborate were prepared in acetone.

TLC plates (20 × 10 cm) coated with 450 μm layer of silica gel G were prepared as reported earlier<sup>8</sup> and desiccated before use.

*Thin-layer chromatography:* Different concentrations of pesticide standard solutions (1 to 10 μl) in acetone were spotted on dried TLC plate. The plate was exposed to evenly distributed bromine vapour<sup>8</sup> for complete oxidation of fenitrothion as fenitrooxon. The plate was removed and exposed to air for 5 min to evaporate the bromine vapour. Acetone: hexane (1 : 9) solvent was employed. After chromatographic run, the plate was dried. Pig liver acetone powder suspension was uniformly sprayed over the plate thoroughly wetting the gel. About 5 to 10 ml suspension is required for 20 × 10 cm TLC plate. The plate was kept in moist atmosphere at 37° c for 10 min and was sprayed with 1-naphthyl acetate

TABLE 1. RELATIONSHIP OF ChE INHIBITION WITH FENITROTHION CONCENTRATION AS DETERMINED BY AREA WEIGHT METHOD AND AREA MEASUREMENT METHOD

Fenitrothion (ng)	Area weight method		Area measurement method	
	Inhibition zone (mg)	ChE inhibition (%) Mean ± S. D.	Inhibition zone (sq. mm)	ChE inhibition (%) Mean ± S. D.
5	4	7.60 ± 0.26	18	8.00 ± 0.14
10	7	13.46 ± 1.16	31	13.80 ± 0.62
20	15	28.86 ± 0.95	78	32.11 ± 0.93
30	25	48.07 ± 2.15	108	48.00 ± 1.23
40	33	63.46 ± 3.83	148	66.73 ± 2.65
50	40	76.92 ± 3.50	184	81.75 ± 2.25

Values are the mean of 6 observations.

TABLE 2. ESTIMATION OF FENITROTHION RESIDUES AS FENITROOXON IN FORTIFIED PADDY SAMPLES BY TLC-ENZYMATIC METHOD AND GAS LIQUID CHROMATOGRAPHY

Paddy sample (g)	Fenitrothion fortified ( $\mu$ g)	Fenitrothion recovered by TLC-enzymatic method*		Fenitrothion recovered by GLC** ( $\mu$ g)
		Area wt. method ( $\mu$ g)	Area measurement method ( $\mu$ g)	
		Mean $\pm$ S. D.	Mean $\pm$ S. D.	
50	5	4.80 $\pm$ 1.00	4.65 $\pm$ 0.85	4.85
50	10	8.90 $\pm$ 0.95	8.75 $\pm$ 0.90	9.55
50	20	18.50 $\pm$ 1.85	18.10 $\pm$ 1.25	18.75
50	30	27.65 $\pm$ 2.25	26.75 $\pm$ 2.05	27.50
50	50	46.35 $\pm$ 1.65	45.95 $\pm$ 3.25	46.35

\*Values are the mean of 6 observations.

\*\*Values are average of duplicate observations.

in acetone and was again placed in moist atmosphere for 2 min. The plate was sprayed uniformly with p-nitrobenzene diazonium fluoroborate in acetone. The white spots or orange background were marked.

**Quantification of fenitrothion as fenitrooxon:** Fenitrothion was estimated as fenitrooxon by both area weight method and area measurement methods as reported early.<sup>8</sup>

**Residue estimation in fortified paddy samples:** Paddy samples were fortified with fenitrothion and the residues were estimated as fenitrooxon following clean-up technique.<sup>4</sup>

Fenitrothion was estimated in the fortified samples after cleanup<sup>12</sup> by GLC employing Varian Aerograph series 1400 equipped with 6'  $\times$  1/8" i. d. Pyrex glass column packed with 5% OV-17 on 60-80 mesh Chromosorb W. nitrogen (25 ml/min) was used as carrier gas. Alkali flame ionization detector was used with hydrogen flow rate of 45 ml/min and air flow rate of 260 ml/min maintaining column, injector and detector temperatures at 130°C, 140°C and 170°C respectively at 32  $\times$  10<sup>-12</sup> range. The retention time for fenitrothion is 1.8 min.

Plots of amounts of fenitrothion as fenitrooxon vs ChE inhibition by both area weight and area measurement methods showed a straight line relationship (Table 1). From these standard curves, 5 to 50 ng fenitrothion can be estimated. Pig liver acetone as enzyme source is more advantageous in view of its ready availability, instant use and indefinite storage time at 0°C, unlike the rat liver which has to be procured from a live animal followed by isolation and the raw tissues cannot be stored for longer duration. Hence, this method is more advantageous than the method reported earlier.<sup>8</sup>

Fenitrothion residues as fenitrooxon in fortified paddy samples were estimated by the present method and values are compared with the values obtained by GLC (Table 2) and these are comparable and the method was found to be suitable in residue analysis.

Authors are thankful to Rallis India Ltd., Bombay, for generous supply of fenitrothion sample. One of the authors (SUB) is grateful to CSIR, New Delhi for financial assistance.

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## BOOK REVIEWS

*Nutrition and Food Processing:* by H. G. Muller and G. Tobin, The Avi Publishing Co. Inc., Westport, Connecticut, 1980; pp. 302; Price: \$ 33.00.

The title of the book, Nutrition and Food Processing is some what misleading. It is more like a hand book on nutrition than a book on food processing and its nutrition implications. The book is divided into 14 chapters and deals with chemistry and function of nutrients and their evaluation, nutrient requirements, chemistry and nutritional aspects of various food groups, food additives and some food processing technologies like refining, refrigeration, heat treatment and dehydration. The authors have attempted to deal with the above aspects of food concisely and have attempted to present the latest information on the subjects discussed. However, discussion on nutritional diseases appear to be rather sketchy. On the other hand, detailed description of methodology given in chapter IV may, perhaps be of out of place in a book of this type. There are also several instances of misinformation and errors which the authors could have checked and corrected. Some of the glaring examples are:

Under Table 2.2, histidine is mentioned as essential for children while we know that it may be essential for only infants. On page 36, the word 'carotenoids' is used instead of carotenes, to describe pigments with provitamin A activity. Temperature correction for energy requirements discussed on page 56 was suggested by FAO in 1957, while in the 1973 recommendation, no temperature correction has been suggested.

NPR is described as Net Protein Retention, instead of Net Protein Ratio (page 86). The authors also have failed to discuss the recently suggested method of Relative Protein Value (RPV) for protein evaluation.

Chickpea is described as *L. sativus*, the legume responsible for lathyrism (page 148). Chickpea is *Cicer arietinum*, while *L. sativus* is kesari dal and the toxin present in *L. sativus* is BOAA and not BAPN. BAPN is present in *L. odoratus*.

Poor absorption of raw egg white is attributed to avidin which is reported to be present at 5 per cent level. The factor responsible for poor absorption is 'ovamucoid', the trypsin inhibitor and not avidin, the latter is responsible for causing biotin deficiency.

Except for the above omissions and errors, the book on the whole is well written containing a lot of useful and latest information on food and nutrition.

The book, thus will be quite valuable to the students and teachers of nutrition. All those who are interested in the science of nutrition will find this a very useful book.

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*The Analysis of Dietary Fibre in Food:* by W. P. T. James and Olof Theander, Marcel Dekker Inc. New York, Basel, 1981; pp. 288; Price: \$ 35.00.

As a result of a meeting held to discuss the possible relation between different kinds and nutritional patterns of different communities, with specific emphasis on intake of dietary fibre (DF), a collaborative study was undertaken where common samples from different food groups were analysed for dietary fibre content using various methods and a meeting held in 1980 to discuss these results. The data obtained by the different groups of workers, form the subject matter of this book.

Research on dietary fibre is one of the important fields of study in recent years and this collaborative study was undertaken by pioneers and highly experienced persons in the field. Presentation by the different groups gives details of methodology employed, any modifications effected to original methods and difficulties encountered in analysis. At the end of each paper, a short summary of discussion on the work has also been added.

The final review of the results of the various groups brings out the differences in the data and also the possible reasons for these differences. When the data were compared, values obtained by Sarthgate procedure showed large variations. This could be due to the small aliquots taken for analysis and also due to varying success with which the starch portion was removed. Differences in the values obtained by enzymatic procedures could be due to differences in the effect of degradation of the various enzymes used.

Gravimetric methods are rapid, inspite of the loss of soluble polysaccharides components. Good agreement was observed in the results reported by the groups who have employed the detergent procedures of Van Soest.

At the end, need for further work and cooperation from different groups has been stressed.

This book is an useful guide for all those who are

working in the fields of nutrition, food science, food technology, medicine etc. It serves as a source of reference for the methodology for the measurement of dietary fibre.

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*Fundamentals of Food Chemistry:* by W. Heimann, AVI Technical Books Inc. U.S.A., 1980, pp. 344, Price: \$ 33.00.

This book is claimed to be an advanced survey of the principles of modern food chemistry aiming to demonstrate the interactions of food chemistry with analytical, organic and biological chemistry in relation to dietetic constituents, health, sickness and medicine.

Having the above goals in view, one wonders if justice can be done in the short spaces devoted to some of the topics. Thus Part 1. Nutrition, not only consists of one chapter but the chapter has only one page. Other examples are to be found in Part 3 of the book where four chapters have five or fewer pages.

Part 2 dealing with the major constituents of foods has been given sufficient space for a meaningful presentation. The presentation in these chapters is detailed and attempt has been made to integrate the organic chemistry of food constituents with their function in foods and biochemistry. The book in this respect is a worthwhile addition to the already available books on food chemistry in English. However, some omissions, errors and possibly poor translation from the original German text mar these chapters.

To give only a few examples, the write ups on optical activity (p. 31) and asparagine and glutamine are confusing. A few of the sentences with errors or bad constructions are—"Small concentrations of salts often increase the solubility (salting-out effect)", page 55. "Lysine is an indispensable essential amino acid" page 42. "Dry heating of hexoses alone or with a small amount of sodium or ammonium carbonate, yields results in caramelization. . . . ." page 149. "The chemical composition of cellulose has been mainly clarified by X-ray investigations." In Table 1, page 132, the isoelectric points for valine, threonine, asparagine, glutamine and ornithine are missing. Some printing errors are, L-glycerinaldehyde (p. 132), D-galactose (p. 132), NaSO<sub>4</sub> (p. 142), cellulose (p. 166) cystine (p. 43) and oxydation (p. 43).

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*Nutrition and Food Science-II:* by Walter Santos Nabuco Lopes, J. J. Barbosa, Dagoberto Chares and Jose Carlos Valente, Plenum Publishing Corporation, New York; 1980; pp. 950; Price: \$ 79.50.

The Proceedings of the Eleventh International Congress on Nutrition, organized by Brazilian Nutrition Society, held in Rio de Janeiro, Brazil, from August 27 to September 1, 1978 have been published by Plenum Press, New York, in three volumes. The three volumes have been captioned as I Food and Nutrition Policies and Programmes, II Nutrition Education and Food Science & Technology and III Biochemical and Pathological Nutrition, respectively. Papers related to topics such as (a) animal and vegetable resources for human feeding, (b) food science and technology, (c) research in food and nutrition, and (d) nutrition education have been clubbed together into the second volume. The inclusion of nutrition education along with the subject of food science and technology appears to be somewhat odd, as the latter could have been put along with similar topics in Volume I.

While the printing, binding and cover designing of the book have been done well, the editing and proof reading part has been poorly done. There are numerous typographical errors throughout the book and occasionally new words have been introduced which are not seen normally in English usage. As the editors are all Brazilian scientists, their difficulty with English language is understandable.

This publication represents the latest research findings and thinking in the area of food and nutrition research education, policies and planning, etc. Hence it is a very useful publication and deserves a place in libraries concerned with these disciplines.

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*Food Control in Action:* by Ed. P. O. Dannis, J. R. Blanchfield and A. G. Ward, Allied Science Publishers Ltd., London; 1980; pp. 290; Price: £ 33.00.

Food control is a wide subject of interest to all those involved in food production, distribution, consumption and also to government agencies, enforcement authorities and standards organisations. It encompasses, besides scientific and technological factors, many procedural and institutional arrangements to ensure effective observance of food regulations by producers and distributors. In order to carry out meaningful food control, it is necessary to have extensive knowledge of the physical, chemical, biochemical, microbiological, nutritional and other

characteristics of behaviour of foods. Besides, a thorough understanding of the principles and practices involved in the technology of manufacture, storage and distribution will be of invaluable help in evolving meaningful and practical specifications of raw materials, packaging materials and finished products. It is praise worthy that Institute of Food Science and Technology of the U. K. recognised the implications of the above concepts and pioneered the evolution of a specialised need oriented course on Food Control in 1978 in collaboration with the Royal Institute of Chemistry and the Institute of Biology. This publication based on a symposium held in July 1979 covers the principles and practice of total food control as applied to food manufacture and distribution.

In the first paper on 'Philosophy of Food Control', J. R. Blanchfield, analyses the reasons and framework for overall control in food manufacturing and distribution operations from the point of view of a food technologist. Food control is concerned with the technical measures taken before, during and after production to ensure that the products comply, not only with all the legal requirements, but also with many other requirements which the law does not and often cannot specify. The scope of food control is limited by four major constraints which include the quality requirements of the market, cost, legislation and organisation.

A series of interlinked papers in specified subjects assigned to various authors with a strict editorial briefing are bunched into four sections under the broad heading components, constraints, interfaces and practical applications.

In the first section on 'Components', three papers on the basis of food control have been included; the first on scientific basis, the next on technological basis and the last on methodological basis. Any attempt to measure the relationship between value and quality of food is the scientific basis of food control. A technological bias is a necessity to study these processes in detail, monitor the relationship between actual and specified performances and provide corrective action whenever there is a deviation. An effective food control operation will depend on a practical administrative system for communicating and defining product manufacturing and distribution standards. There are many methodologies available to aid this objective which have been reviewed.

Many 'Constraints' have been cited as factors affecting food control system in the second section.

These include quality requirements of the market, operating costs, constraints of legislation and organisational constraints. The impingement of the control system on other company functions such as purchasing, R & D, production and productivity and the distribution chain are dealt with in the third section on 'Interfaces'. There are four papers dealing with interfaces with product and process development, material purchasing, quality assurance, production and productivity and distribution and consumer. Though there is some overlapping of the areas covered by each paper on the whole, they bring out the practical aspects of the relationship between food control system and industrial management.

In the last section, the 'Control function' is described as is organised and operated on a day-to-day basis in companies representing four major branches of the food manufacturing industry. Experienced authors describe the practical aspects of integrated food control systems in such diverse sectors as canning, quick freezing of meat, fish products, break-fast cereals, biscuits and cake mixes and chocolate and sugar confectionery.

In his concluding remarks, presented separately as a paper, A. G. Ward has highlighted some few points which merit consideration by the fraternity of food scientists and technologists, national government and industry. The size, mode of operation, technical competence and skill in applying food control are not uniform and many firms get away without any meaningful food control system for which legislative pressure may be the only means of corrective action. The managements of food processing units also must be persuaded to reorient their personnel policy to ensure deployment of control personnel with proper qualifications in the subject.

The code of professional conduct being followed by Institute of Food Science and Technology of the United Kingdom is appended followed by subject index. The publication is highly informative and it puts together in one place the various aspects of quality control and quality assurance. The subject of food quality and its control in developing countries where food industry is in its infancy is all the more relevant and the experience of U. K. as brought out by this publication can be a valuable guide. I strongly recommend its reading by all those involved in the important function of food control.

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

## NEWS:

### INTERNATIONAL CONFERENCE ON LEAF PROTEIN RESEARCH

The above Conference organised by the Society of Green Vegetation Research and co-sponsored by the Indian Universities and Institutes involved in leaf protein research will be held on 5-8 October 1982 at Marathwada University, Aurangabad, India. Five scientific sessions covering screening of vegetation for choice of raw materials, cultivation and agronomic studies for assessment of yields of leaf protein/technical and technological aspects of processing and production, nutritional and biochemical investigations for quality assessment and improvement, and the results of bulk

production and utilisation studies have been planned.

Intending participants are requested to write to Dr. Narendra Singh, Organising Secretary, CFTRI, Mysore 570 013, India for further information and the paper-abstract may be sent to the above address before July end.

British Commonwealth Foundation will sympathetically consider applications for travel grants from the citizens within the Commonwealth. Participants from developing countries may make requests for financial aid directly through their national authorities for use of UNDP funds.

### SIXTH WORLD CONGRESS OF FOOD SCIENCE AND TECHNOLOGY

The above Congress is organised by the Institute of Food Science and Technology of Ireland under the sponsorship of the International Union of Food Science and Technology in Dublin, Ireland from 18-23 September, 1983.

The theme of the Congress will be—Food Science and Technology for Development-Welfare-Peace. The scientific programme will deal with the main food com-

modities under the general headings—Production, Processing and Nutrition. The theme of the Congress will be developed in plenary papers, parallel sessions of invited papers, posters and seminars.

*For details, please contact—*

Secretariat, Sixth World Congress of Food Science and Technology, 44, Northumberland Road, DUBLIN 4, Ireland.

### VIIIth INTERNATIONAL SPECIALISED SYMPOSIUM ON YEASTS

The National Organising Committee of the VIIIth ISSY and the Association of Microbiologists of India are organising this symposium in 'Hotel Oberoi Towers', Bombay, India during January 24-28, 1983. The scientific programme includes—basic biology and generics; basic biochemistry; basic technology; special yeast systems; yeast culture collections, data banks & information transfer systems; ethanolic fermentations; brew-

ing; wines; cider & serry; traditional fermentations; other alcoholic beverages; baking & baked products; food & feed yeasts; chemical feed stocks; and product spoilage & yeasts.

For more information, contact Dr. T. V. Subbiah, Convener, VIIIth ISSY, Alembic Chemical Works Co. Ltd., Baroda-390 003, India.

## ERRATA

- I. In the article "Kinetics of water vapour sorption by wheat flour from saturated atmosphere" by B. P. N. Singh, Maharaj Narain and Harpal Singh, this journal 1981, Vol. 18, No. 5, Page 203:

$$\text{Equation 7, should read: } W_e = \frac{W_n \cdot W_n + 2\epsilon - (W_n + \epsilon)^2}{W_n + W_n + 2\epsilon - 2(W_n + \epsilon)}$$

- II. In the article "Kinetics of moisture absorption by soy flour" by U. S. Shivhare, Maharaj Narain and B. P. N. Singh, this journal 1982, Vol. 19, No. 1, page 27:

$$\text{Equation 4 should read: } W_e = \frac{W_n \cdot W_n + 2i - (W_n + i)^2}{W_n + W_n + 2i - 2(W_n + i)}$$

- III. In the article "Insecticidal residue in vegetables obtained from soil treated with hexachlorocyclohexane" by N. G. K. Karanth, M. Jayaram and S. K. Majumder, this journal 1982, Vol. 19, No. 1, Page 15:

Column 1, last line and column 2, line 12—'R<sub>f</sub> values' should read 'R<sub>t</sub> values'.





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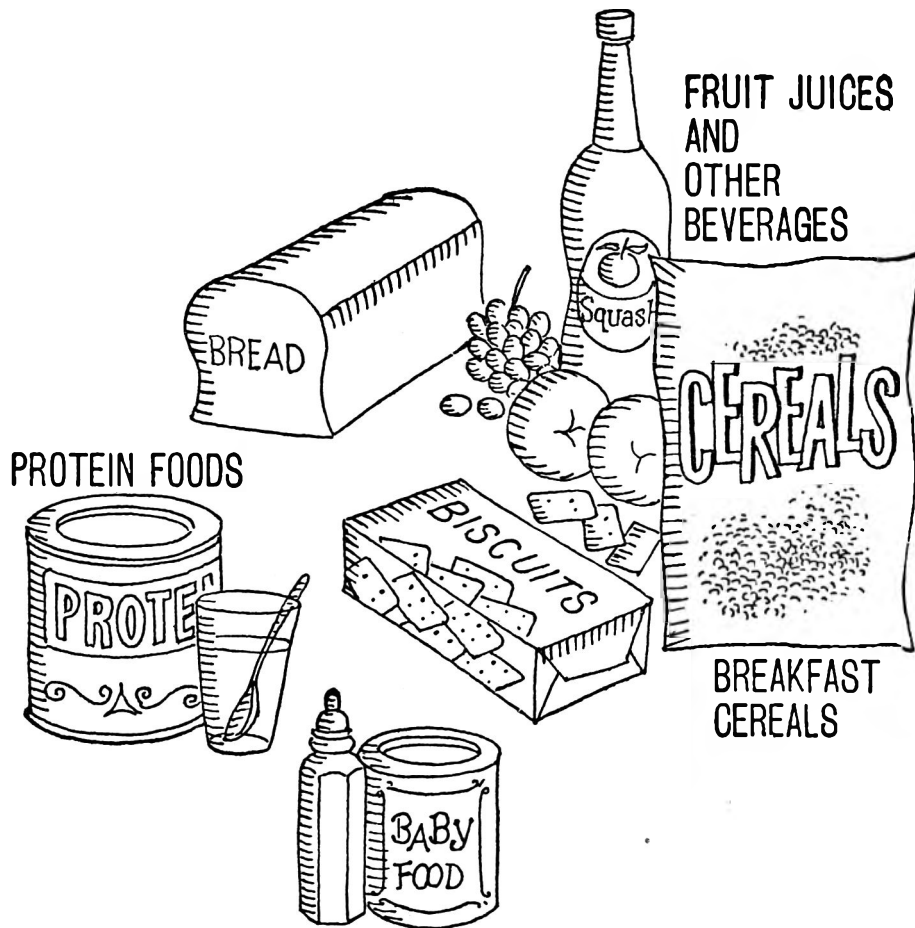
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2. Short communications in the nature of Research Notes should clearly indicate the scope of the investigation and the salient features of the results.
3. Names of chemical compounds and not their formulae should be used in the text. Superscript and 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.
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) *Book:* Venkataraman, K., *The Chemistry of Synthetic Dyes*, Academic Press, Inc., New York, 1952, Vol. II, 966.
  - (c) *References to article in a book:* Joshi, S. V., in the *Chemistry of Synthetic Dyes*, by Venkataraman, K., Academic Press, Inc., New York, 1952, Vol. II, 966.
  - (d) *Proceedings, Conferences and Symposia Papers:* Nambudiri, E. S. and Lewis, Y. S., *Cocoa in confectionery, Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India*, Mysore, May 1979, 27.
  - (e) *Thesis:* Sathyanarayan, Y., *Phytosociological Studies on the Caliculous 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.

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