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## Lipids of Kokum (*Garcinia indica*) and Dhupa (*Veteria indica*). Part II.

H.T. THIPPESWAMY\* AND P.L. RAINA

Department of Nutrition and Food Safety, Central Food  
Technological Research Institute, Mysore - 570 013, India.

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**Total lipids of Kokum and Dhupa seed flour extractable with chloroform : methanol (2:1 v/v) was resolved into neutral, glyco- and phospholipids using silicic acid column chromatography. Each of these fractions were further characterised, quantitated and fatty acid composition determined. The study indicated that the total lipids of kokum and dhupa contained a major proportion of neutral lipids (88.00 and 75.80%) comprising predominantly of triglycerides (84.9 to 68.7%). The glycolipids 4.0% of kokum were digalactosyl diglyceride (30.9%) and monogalactosyl diglyceride (19.3%) whereas, the major glycolipids (23.1%) of Dhupa were sterolglycoside (23.5%) and acylsterolglycoside (15.6%). The phospholipid contents were identical in both kokum and dhupa, having phosphotidyl ethanolamine (75.4 and 48.5%) as a major constituent. All the lipid fractions showed palmitic and stearic acids as major fatty acids.**

Although our country abounds in having a wide variety of oilseeds of plant and tree origin, the latter group have not been studied for their lipid composition. Besides the traditional ones, the oilseeds of tree origin comprise nearly 25 varieties and the potential availability of seeds is of the order of 15 million tonnes<sup>1</sup>. Hardly 7 per cent of the available seeds are collected at present which contribute about 1 lakh tonnes of oil. Even if 50 per cent of this wealth is tapped, India can substantially meet her requirements of edible and non-edible oils. Some of these secondary seed oils can be used for edible purposes by upgrading them by suitable blending and refining. The non-edible grades of these fats are used in soap and candle making, paint and varnish manufacture, pharmaceutical, cosmetic formulations, etc.

Kokum and Dhupa fats have high contents of stearic and oleic acids. They are used as edible fats by some tribal people<sup>2</sup>. Efforts have been made, particularly with respect to kokum fat to upgrade it with suitable blending and refining and the resultant fat is used as a substitute for cocoa butter by the confectionery industry<sup>3,4</sup>. They have also been used as sources of palmitic, stearic and oleic acids<sup>5</sup>.

The importance of these two secondary sources of fats in food and other industries prompted us to study their chemical characteristics in detail.

### Materials and Methods

Sample preparation and analytical procedures were as described earlier<sup>6</sup>. The neutral, glyco- and phospholipid fractions obtained by resolution of the total lipids on silicic acid column were further characterised and quantitated by thin layer chromatography and densitometry.

*Thin layer chromatography:* For qualitative work, glass plates of 20×20 cm were coated with silica gel G (for neutral and phospholipids) and silica gel H (for glycolipids) layer of 0.25 mm using an adjustable hand applicator. For quantitative work, 1 mm thickness of the gel was used. The coated plates were air-dried and then activated in a hot air oven at 110°C for 2 hr. The compounds were identified by running the authentic standards simultaneously on the plate and also making use of their R<sub>f</sub> values.

After the development of neutral, glyco- and phospholipids on TLC plates, the chromatoplates were air-dried and sprayed very lightly with 50 per cent sulphuric acid. The plates were heated in an air oven at 180°C for 20 min in order to char the spots. These plates were then scanned in the direction of the solvent development using an automatic Camag TLC scanner - Model 2, mounted on a Fluorimeter-Model III (Turner Associates, California), attached to a W+W Recorder 1100 (Scientific Instruments, Switzerland). Scanning conditions were: lamp 110-850 (visible 360 nm), primary filter 110-811 and secondary filter 110-823 (10 per cent neutral density), plate speed 2 cm/min; chart speed, 1 cm/min. Relative percentages were calculated by triangulation of the peak areas<sup>7</sup>.

### Results and Discussion

The total lipid contents of kokum and dhupa were 49.42 and 37.82 per cent respectively. Silicic acid fractionation showed that the major portion of this lipid on flour weight basis was neutral lipids (88.0 and 75.8 per cent) followed by glyco (4.0 and 23.1 per cent) and phospholipids (3.0 and 1.0 per cent).

\*Present address: Department of Biochemistry, Government Medical College, Mysore.

*Characterisation of neutral lipids:* The neutral lipid fractions of kokum and dhupa (Table 1) when analysed by TLC and densitometry showed triglyceride (TG) as the major component (84.9 and 68.7) followed by free fatty acids (FFA) (7.9 and 10.7) and diglycerides (1,2- and 1,3-) (2.8 and 8.1 per cent). The sterol spots could not be detected separately, as these had close  $R_f$  values with FFA. Thus, both the spots were together scraped off from the plates, recovered with chloroform : methanol mix (2:1) and rechromatographed in the solvent system containing petroleum ether:diethyl ether:acetic acid (90:10:1). Spots were visualised under UV light after a light spray of Rhodamine 6 G. The  $R_f$  values of the

two spots were distinct. Thus, these were scraped off and recovered from the material with chloroform:methanol (2:1) and quantitated by gravimetry. The quantity so determined for both sterol ester and sterol was 2.6 in kokum and 5.7 per cent in dhupa. Dhupa, however, had an additional spot near the origin which could not be identified.

*Characterisation of glycolipids:* The compositions of the glyco-lipids in kokum and dhupa are given in Table 2 and found to vary considerably. Kokum showed digalactosyl diglyceride (DGDG) as the major glycolipid (30.9 per cent) followed by monogalactosyl diglyceride (MGDG) (19.3 per cent). In addition, 12.5 of acyl sterol glycoside (ASG)

TABLE 1. COMPONENTS OF NEUTRAL LIPIDS AND THEIR FATTY ACIDS

Component	%	Fatty acids (%)					US/S ratio	Cal. $I_2$ value
		12:0	14:0	16:0	18:0	18:1		
<b>Kokum</b>								
Sterol ester	1.8	—	2.1	31.5	33.6	32.8	0.48	28.27
Triglyceride	84.9	—	TR	3.0	47.9	49.1	0.96	42.32
FFA	7.9	—	TR	19.9	42.4	37.6	0.60	32.41
Sterol	0.8	—	—	—	—	—	—	—
1,3-diglyceride	1.5	—	TR	11.4	53.0	35.6	0.55	30.68
1,2-diglyceride	1.3	—	TR	5.4	44.8	49.8	0.99	42.93
Polar lipids + MG (origin)	1.0	—	TR	20.0	43.8	36.2	0.56	31.20
<b>Dhupa</b>								
Sterol ester	3.0	9.5	10.6	35.7	16.3	28.9	0.40	24.91
Triglyceride	68.7	4.3	TR	23.8	12.0	59.9	1.49	51.63
FFA	10.7	3.9	1.5	27.0	27.4	40.1	0.67	34.61
Sterol	2.7	—	—	—	—	—	—	—
1,3-diglyceride	4.3	2.0	TR	15.5	27.2	55.3	1.24	47.67
1,2-diglyceride	3.8	1.6	TR	10.4	24.2	63.8	1.73	54.99
Unidentified spot	1.9	3.0	TR	14.6	25.3	60.1	1.50	51.80
Polar lipids + MG (origin)	2.2	10.2	TR	27.7	22.0	40.4	0.67	34.82

FFA: Free fatty acids; MG: Monoglyceride.

TABLE 2. COMPONENTS OF GLYCOLIPIDS AND THEIR FATTY ACIDS

Components	%	Fatty acids (%)						US/S ratio	Cal. $I_2$ value
		12:0	14:0	16:0	18:0	18:1	18:2		
<b>Kokum</b>									
Pigments+NL components	20.4	6.1	9.6	34.6	17.1	17.8	14.7	0.48	40.80
MGDG	19.3	4.1	9.4	56.9	7.9	9.8	11.8	0.27	28.89
DGDG	30.9	4.9	8.1	37.0	21.6	13.2	15.2	0.40	37.71
ASG	12.5	4.2	5.5	33.7	19.2	24.5	12.9	0.60	43.46
SL + unidentified	16.8	6.1	10.1	47.2	15.8	10.1	10.6	0.26	27.06
<b>Dhupa</b>									
Pigments + NL components	10.3	3.5	4.2	26.8	29.9	35.6	—	0.55	30.69
MGDG	8.4	14.6	22.6	47.4	2.2	8.0	5.2	0.31	15.89
SG	23.5	26.0	20.6	29.2	3.8	7.8	12.6	0.26	28.54
DGDG	12.3	12.8	15.6	39.6	8.2	10.4	13.3	0.31	31.99
ASG	15.6	27.4	16.2	34.8	4.8	9.7	7.1	0.20	20.65
Unidentified	13.4	18.0	25.3	33.3	5.6	9.5	8.2	0.21	22.39
SL + unidentified	16.6	31.1	16.1	28.2	4.9	9.3	10.3	0.24	25.85

MGDG: Monogalactosyl diglyceride; DGDG: Digalactosyl diglyceride; ASG: Acyl sterol glycoside; SL: Sulpholipid.

and a considerable quantity of pigments (20.0 per cent) were also detected. In dhupa, however, sterol glycoside (SG) formed major component comprising 23.5 per cent followed by ASG (15.6 per cent), DGDG and MGDG comprised 12.3 and 8.4 per cent respectively. Sulpholipid (SL) was found merged with an unidentified glycolipid, having a very close  $R_f$  value which could not be separated clearly by repeated chromatography. However, this compound was present in almost equal proportions in both kokum and dhupa.

**Characterisation of phospholipids:** The nature of phospholipids present in both kokum and dhupa was almost identical as shown in Table 3. Phosphatidyl ethanolamine (PE) was the major phospholipid comprising 75.4 in kokum and 48.5 per cent in dhupa. Lysophosphatidyl ethanolamine (LPE) and phosphatidic acid (PA) were 14.0 and 8.6 in the former and 28.9 and 19.0 in the latter. Phosphatidyl glycerol (PG) formed a smaller proportion in both.

**Fatty acid composition:** Palmitic and stearic acids were the major saturated fatty acids in both kokum and dhupa, together comprising more than 50 per cent of the total in almost all the neutral lipid components (Table 1). The diglycerides have a higher proportion of these two fatty acids (90) and least were found in the sterol ester (SE) fractions (65). The other major fatty acid was oleic acid. The TG and the two diglycerides have maximum amount of this fatty acid whereas the SE and FFA fractions have lowest. The oleic acid content of the neutral lipids of dhupa was higher than that of kokum. Due to the higher oleic acid content, these components were more unsaturated as is evident from their unsaturated/saturated fatty acid ratio (US/S ratio) and Calculated Iodine values (Cal  $I_2$ ).

The glycolipids of kokum and dhupa (Table 2) showed similar fatty acid pattern as those of the neutral lipids in having palmitic (26.8-56.9) and stearic acids (2.2-29.9) as the major saturated fatty acids. The MGDG of both kokum and dhupa was however, different from the rest of the glycolipid components in having the highest concentrations of palmitic

acid (56.9 and 47.4) and lowest stearic acid (7.9 and 2.2). The two lower fatty acids viz., lauric and myristic acids were also found in considerable quantities in the glycolipids when compared to those of neutral lipids. Between the two, the dhupa glycolipids had fairly higher percentages of lauric and myristic acids. Among the unsaturated fatty acids, the proportions of oleic and linoleic acids present were in almost equal quantities in the respective components of both kokum and dhupa. Linoleic acid was found in small quantities in bound and firmly bound forms<sup>6</sup>. The US/S ratio was less than 0.6 in all the components indicating that these compounds were more saturated than those of neutral lipids.

In phospholipids, palmitic acid was the major fatty acid uniformly distributed in all the components, kokum having 37.7-42.7 and dhupa 31.8-38.5 (Table 3). Stearic acid was found more in PE of kokum (18.8) and LPE of dhupa. Lauric and myristic acids were also present in all the components. Oleic acid was found in much lower proportions compared to total<sup>6</sup> and neutral lipids. Small quantities of linoleic acid were also observed in kokum (4.2-7.4) and dhupa (9.1-13.7). Similar to neutral and glycolipids, the phospholipids of kokum were also more saturated as shown by their US/S ratio and Calculated  $I_2$  values. These values do not exceed 0.2 and 20 respectively, which are the lowest found among three lipid fractions. However, the phospholipids of dhupa were more saturated than the glycolipids.

Several reports on seed lipids showed that TG forms the major portion of neutral lipids<sup>8,9</sup>. The NL of kokum and dhupa (Table 1) also comprised mainly of TG (84.9 and 68.7) accompanied by FFA (7.9 and 10.7), sterols along with their esters (2.6 and 5.7), 1,3-DG (1.5 and 4.3), 1,2-DG (1.3 and 3.8) and MG with polar lipids (1.0 and 2.2).

It has been reported that in non-photosynthetic tissues, DGDG formed a major glycolipid<sup>10,11</sup>, while in photosynthetic tissues MGDG is in higher proportion. However, varied proportions of these glycolipids have been reported in many photosynthetic and non-photosynthetic tissues<sup>12</sup>.

TABLE 3. COMPONENTS OF PHOSPHOLIPIDS AND THEIR FATTY ACIDS

Components	%	Fatty acids (%)						US/S ratio	Cal. $I_2$ value
		12:0	14:0	16:0	18:0	18:1	18:2		
<b>Kokum</b>									
Phosphatidic acid	8.6	12.1	17.2	40.0	18.0	5.5	7.2	0.14	17.21
Phos. glycerol	2.0	14.1	16.6	42.7	13.1	11.2	4.2	0.18	16.92
Phos. ethanolamine	75.4	9.9	19.9	37.7	18.8	7.1	7.4	0.16	18.94
LPE	14.0	22.7	21.3	38.9	4.9	7.2	5.0	0.14	14.86
<b>Dhupa</b>									
Phosphatidic acid	19.0	5.5	12.0	38.5	17.0	15.9	11.1	0.39	32.93
Phos. glycerol	3.5	1.5	6.8	33.2	19.0	30.3	9.1	0.65	41.88
Phos. ethanolamine	48.5	4.5	8.4	31.8	16.0	25.6	13.7	0.65	45.79
LPE	28.9	2.0	5.3	32.5	20.0	30.6	9.5	0.67	42.83

LPE: Lysophosphatidyl ethanolamine.

Ginkonuts (*Ginkgo biloba*)<sup>13,14</sup>, cowpea (*Vigna catjang*)<sup>15</sup>, Hinoat oats (*Avena sativa* L.)<sup>16</sup> and cucumber (*Cucumis sativus*)<sup>17</sup> containing glycolipids in different proportions are found to have DGDG as the major glycolipid component. However, the cucumber seeds contained DGDG only 10 per cent with ASG and cerebrosides as the major glycolipids amounting to 21.9 and 20.2 respectively of the total glycolipid fraction. Similarly, the rice bran lipid of *tongil* and *japonica* varieties also contained esterified sterol glycoside (ASG) as the major glycolipid component in both. In the present study (Table 2), kokum contained DGDG (31 per cent) as the major glycolipid whereas in dhupa it was sterol glycoside, (23.5 per cent). However, the DGDG content (12.3) in dhupa was more than MGDG (8.4). The contents of other glycolipids in both kokum and dhupa were comparable. Exceptionally high contents of sulpholipids were found in both kokum and dhupa lipids. Examination of this component by repeated TLC, indicated the presence of another lipid containing sugar moiety along with sulpholipid. This glycolipid could not be identified.

Phosphatidyl choline (PC) has been reported to be the major phospholipid identified in many non-photosynthetic tissues. It is the major phospholipid in wheat, bajra, mango kernel and cashew kernel<sup>10,11,18,19</sup>. Unusually, phospholipids of kokum and dhupa do not contain PC. The phospholipids present in kokum and dhupa were PA, PG, PE and LPE (Table 3). Their proportions vary considerably in the two lipids. PA is known to occur in small amounts (1.5) in plants<sup>20</sup>, but it was found to the extent of 8.6 in kokum and 19 in dhupa similar to PA content of mustard seed lipid<sup>21</sup>. The relatively high content of PA may be attributed to phospholipase-D-activity during extraction process<sup>12,20</sup>. PE was major phospholipid in both kokum and dhupa containing 75 and 48.5 respectively. PE content of phospholipid are known to vary widely (11.83)<sup>18,22</sup>. LPE has been reported only in florescence and leaves. However, there are few reports of its presence in seed as well. The contents of this phospholipids in kokum and dhupa were found to be higher than the reported values<sup>16</sup>.

Several reports<sup>8,23,24</sup> on fatty acid composition of the neutral lipids of plant sources showed that these lipids were rich in linoleic and palmitic acids. Kokum and dhupa neutral lipids do not contain linoleic acid, instead these lipids contain high contents of stearic and oleic acids followed by relatively smaller quantities of palmitic acid (Table 1).

Unsaturated fatty acids such as linoleic and linolenic acids dominate in most plant glycolipids. Among the glycolipid components, MGDG was more unsaturated than the DGDG and palmitic acid predominates generally in DGDG<sup>24</sup>. The fatty acid composition of MGDG and DGDG in kokum and dhupa give a different picture. The MGDG's in both the lipids were not only less unsaturated than the other glycolipids but also contained higher amounts of palmitic acid. The glycolipids of dhupa contained higher quantities of lauric and

myristic acids also. In general, the glycolipids of kokum fat were less unsaturated than those of dhupa as indicated by their US/S fatty acid ratio and calculated iodine value. Several workers reported palmitic acid as the predominant fatty acid esterified to ASG in plant sources<sup>24,28</sup>. However, there are reports indicating linoleic acid as the predominant fatty acid in ASG<sup>29,31</sup>. In the present study, the ASG of kokum and dhupa were found to be enriched with palmitic acid. The ASG of kokum was slightly different from that of dhupa in having more oleic and linoleic acids. This dissimilarity in the fatty acids esterified in various glycolipid fractions has been reported earlier<sup>11</sup>. Thus, in the present context, the enzymes catalysing the biosynthesis of ASG seem to preferentially pick up palmitic acid from the common pool.

Comparing the fatty acid composition of the phospholipids of kokum and dhupa with those of the triglycerides, it was found that the former was different from the latter in not agreeing with the Kennedy pathway which postulates common pathway between TG's and PL's<sup>32</sup>. Similar results have been reported with soybean lipid<sup>33</sup>, which contains higher percentages of linoleic acid in PL's than TG's.

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## Production of Partially Defatted Groundnuts Using an Inclined Hydraulic Cage Press

V.D. DEVDHARA, B. VEERANJANEYULU, T.N. MURTHI, J.S. PUNJRATH AND R.P. ANEJA  
National Dairy Development Board, Anand - 388001, Gujarat, India.

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**A process for manufacture of partially defatted groundnuts using an inclined hydraulic cage press specially designed and fabricated indigenously with cage capacity of 100 kg per batch is described. Among different processing conditions studied, predrying of the kernels to 3.5-6.0% moisture from an initial moisture of 5.8-8.0%, rate of application of pressure, use of partition plates for obtaining an optimum oil recovery (52.0%) are described. The pressed nuts were hydrated to an optimum moisture content of 13%, and dried at 110°C for 30 min to regain the original shape of the nuts easily. These nuts were further roasted at 120°C for one hour to have acceptable crispness and flavour. The oil obtained during pressing the nuts at ambient temperatures (30°C) is of superior quality due to its low FFA (0.08%) and light in colour (5 units, Y+5R), while pressing the nuts at 70°C resulted an oil having 0.5% FFA and 8 units colour.**

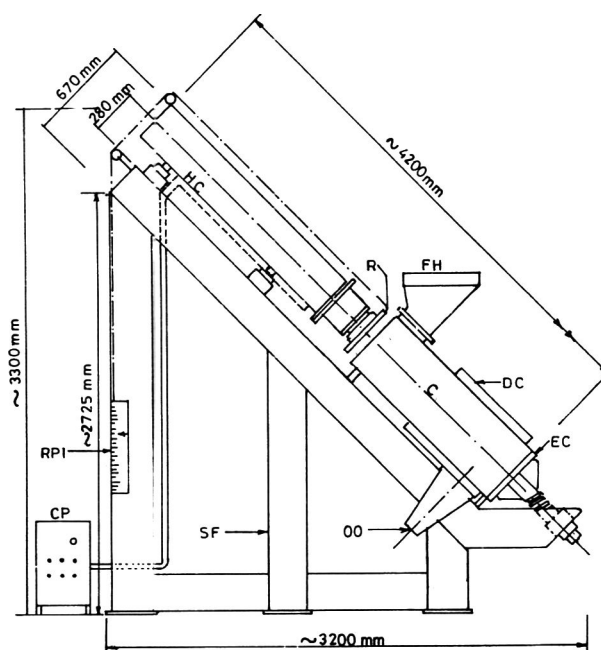
Groundnut is the most important oilseed crop of India: its utilization as a source of protein for human consumption leaves much to be desired. In developed countries, particularly USA, only groundnut kernels unfit for direct human consumption which is about 20 per cent of the crop<sup>1</sup> are crushed for oil while the rest are utilized for edible purpose in many snack products. Considering the high convenience, nutritional value and consumer appeal which are important for developing a commercially viable protein from oilseeds<sup>2</sup>, the ideally designed product would be partially defatted groundnuts<sup>3,5</sup> since, it derives directly from the nut itself. Such a technology if standardized, would not only recover a portion of high quality and light coloured oil for domestic use but can also help in the better utilization of high quality oilseed protein. Therefore, pilot-plant studies were carried out in a newly developed inclined hydraulic cage press of 100 kg capacity per batch for the manufacture of an acceptable partially defatted groundnut and the processing conditions are reported here.

### Materials and Methods

The sound and cleaned groundnut kernels used in these experiments were HPS grade having 5.8 to 8.0 per cent moisture, 48.0 per cent oil and 25.0 per cent protein. A hydraulic cage press of the batch type which has been designed exclusively for partially defatting groundnuts (Fig.1) and fabricated indigenously was used in all these experiments. It consists of an inclined cylindrical perforated (slits) cage made of mild stainless steel to hold 100 kg sound groundnut kernel of 0.60 g/ml bulk density. For maintaining temperature, electrical heaters were also provided on the top of the cage.

Standardization of processing conditions for the optimum recovery of oil without affecting the final shape of the partially

defatted nuts and their acceptability was effected by pre-drying the kernel and by varying the pressing time, temperature and the thickness of the charge at the optimum operational pressure of the press (61.8 kg/cm<sup>2</sup>). The effects of applying the pressure continuously and rapidly were also studied separately.



HC: HYDRAULIC CYLINDER; C: CAGE; R: RAM; FH: FEED HOPPER; DC: DUST COVER; EC: END CAP; OO: OIL OUTLET; SF: SUPPORT FRAME; CP: CONTROL PANEL RPI: RAM POSITION INDICATOR

Fig.1. Inclined hydraulic cage press for the manufacture of partially defatted groundnuts.

Sound HPS grade and cleaned kernels were pre-dried from 5.8-8.0 to 3.5-6.0 per cent moisture in a dryer at 100°C for one hour. These pre-dried kernels were immediately cold pressed (30°C) at 61.8 kg/cm<sup>2</sup> in the cage press after inserting the partition plates and also without these plates. Separately, a series of trials were conducted varying the quantity of charge per batch at 10, 20, 40 and 100 kg pre-dried kernels with and without partition plates. The number of partition plates inserted varied from one to four depending upon the size of the batch, normally keeping one plate for every 20 kg of material during pressing. The effect of temperature on the recovery of oil during pressing 100 kg of pre-dried groundnut kernels was studied separately at ambient (30°C) and at 70°C with and without insertion of the partition plates. In all these experiments, the maximum pressure used was 61.8 kg/Cm<sup>2</sup>. Also, the effect of rate of applying pressure on the nuts in the cage press was studied by increasing the pressure rapidly from 29.8 to 61.8 kg/Cm<sup>2</sup> within 60 min pressing time and holding the charge at that pressure for the remaining 60 min. In other trials, the pressure was applied slowly but continuously from 24.7 to 61.8 kg/Cm<sup>2</sup> in about 120 min. In all these experiments, the recovery of oil was measured periodically during pressing at an interval of 15/30 min.

The chemical characteristics of the kernels namely moisture, oil and protein before and after pressing and the quality of oil (FFA and Lovibond colour in 1.27 cm cell) was determined as per the AOCS methods<sup>6</sup>.

## Results and Discussion

**Effect of pre-drying:** From the Table 1, it can be observed that the oil recoveries for a charge of 25 to 50 kg kernels without pre-drying were 17 to 23 per cent. When the kernels were pre-dried from 5.8 - 8.0 to 3.5-6.0 per cent moisture, the oil recoveries increased to 46 - 54 per cent. Though, the pre-dried kernels with moisture content below 4 per cent gave more oil recovery, the quantity of broken bits (30 per cent) were higher during pressing.

**Effect of size of the batch:** When the cage press was filled with 10 kg nuts (Fig. 2) the recovery of oil was 32.3 per cent of the available oil during first 15 min pressing and increased during next 15 min pressing and reached optimum of 51.5 per cent during remaining 15 min. When the quantity of kernels per batch was increased from 20 to 100 kg, the recovery of oil without partition plates was 15.5, 8.0 and 6.2 per cent respectively for 20, 40 and 100 kg batches during first 15 min and reached a maximum of 45.2 per cent oil recovery for 20 kg batch during 75 min pressing. The oil recovery for 100 kg batch was the lowest (33 per cent) during a pressing time of 150 min.

**Effect of partition plates and temperature:** However, with partition plates, one plate for every 20 kg material, the oil recoveries were 20.6 per cent for 20 kg batch during first 15 min and 51.6 per cent after 75 min pressing time. As the

TABLE 1. EFFECT OF PRE-DRYING THE KERNELS ON THE RECOVERY OF OIL DURING COLD PRESSING

Batch (kg)	Moisture (%)		Oil recovery*	
	Initial	After pre-drying	Without pre-drying and without partition plates <sup>a</sup>	With pre-drying and with partition plates <sup>b</sup>
25	8.0 (7.5 - 8.6)	6.0 (5.8 - 6.2)	17.0 (16.6 - 17.4)	46 (44 - 48)
50	6.6 (6.2 - 7.0)	5.0 (4.9 - 5.1)	24.0 (23.5 - 24.5)	49 (48 - 50)
50	6.0 (5.9 - 6.1)	4.0 (3.8 - 4.2)	25.0 (23.4 - 26.6)	51 (50 - 52)
50	5.8 (5.7 - 5.8)	3.5 <sup>c</sup> (3.4 - 3.6)	25.0 (24.0 - 26.0)	54 (53 - 55)

Figures in parenthesis indicate range

\*Average of ten runs each

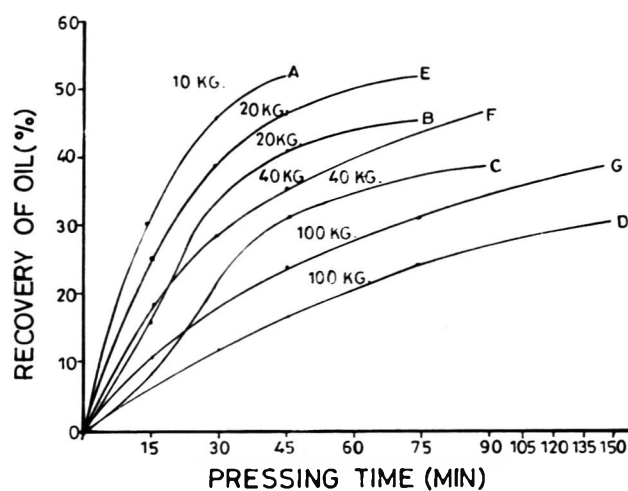
<sup>a</sup> After 45 min of pressing time

<sup>b</sup> After 90 min of pressing time

<sup>c</sup> More (30%) splits were observed

+ pressing time cold

pressing at 61.8 kg/Cm<sup>2</sup>



A, B, C, D : WITHOUT PARTITION PLATES

E, F, G : WITH PARTITION PLATES

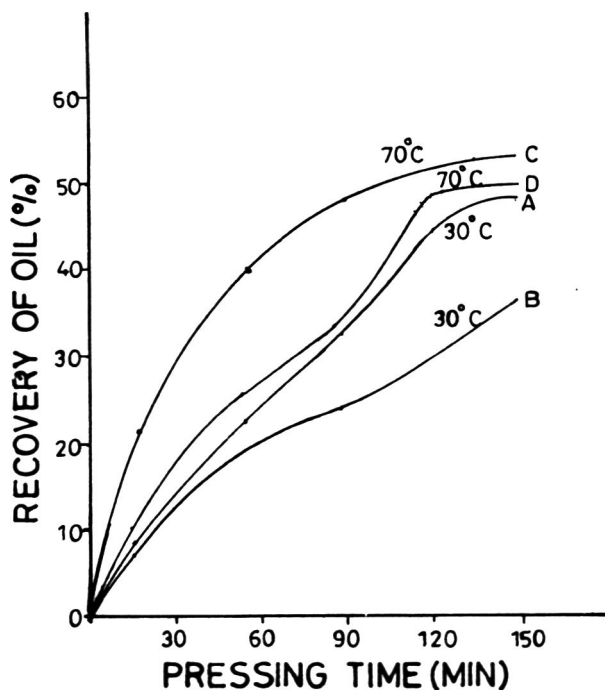
Fig. 2. Effect of batch size on the recovery of oil during cold pressing of groundnut kernels in a hydraulic cage press at 61.8 kg/cm<sup>2</sup>.

quantity of kernel increased from 20 to 100 kg, the maximum oil recovered varied from 49.2 to 51.6 per cent, but the pressing time varied from 75 to 150 min. Thus, to recover 49.2 per cent oil, the time taken for pressing a full capacity charge (100 kg) was greater (150 min). Even for pressing of nuts at 30°C (cold pressing) insertion of partition plates increased the recovery from 36.4 to 48.6 per cent which further improved to 49.8 to 52.0 - 53.2 per cent by raising the temperature of the batch to 70°C (Fig. 3). Though, the oil recovery (53.2 per cent) was improved with increasing the

temperature of the charge (70°C) the pressing time still remained same.

**Effect of application of pressure:** Application of pressure rapidly (Fig.4) on the nuts in the cage from 29.8 to 61.8 kg/Cm<sup>2</sup> in about 60 min gave an oil recovery of 32.3 per cent and holding the charge at maximum pressure for the remaining 60 min increased the oil recovery to 45 per cent only. However, application of pressure slowly and continuously from 24.7 to 61.8 kg/Cm<sup>2</sup> in about 120 min increased the recovery of oil to 52.0 per cent for 100 kg batch with partition plates at 70°C. Increasing the pressing time further by 30 min (Fig.3) did not improve the recovery of oil substantially.

**Re-shaping of partially defatted nuts:** The partially defatted nuts were hydrated to an optimum moisture content of 13 per cent by spraying salt solution (10 per cent conc.) at ambient temperature (30°C) in a rotatory coating pan (Cadmach, 91.4 cm dia and 25 r.p.m.). After conditioning the pressed nuts for 15 min at this moisture, the nuts were slowly dried in the same pan at 110°C for 30 min to regain the original shape and size. This method of reshaping the nuts is more superior than the soaking the pressed nuts in salt solution at 60°C as the nuts regained the shape easily in a minimum quantity of water and with a minimum effect on the hydrolytic rancidity of oil present in the nut. The nuts after regaining the shape were further roasted at 120°C for acceptable flavour and crispness.



B, D : WITHOUT PARTITION PLATES  
A, C : WITH PARTITION PLATES

Fig.3. Effect of temperature on the recovery of oil during pressing of groundnut kernels in a hydraulic cage press.

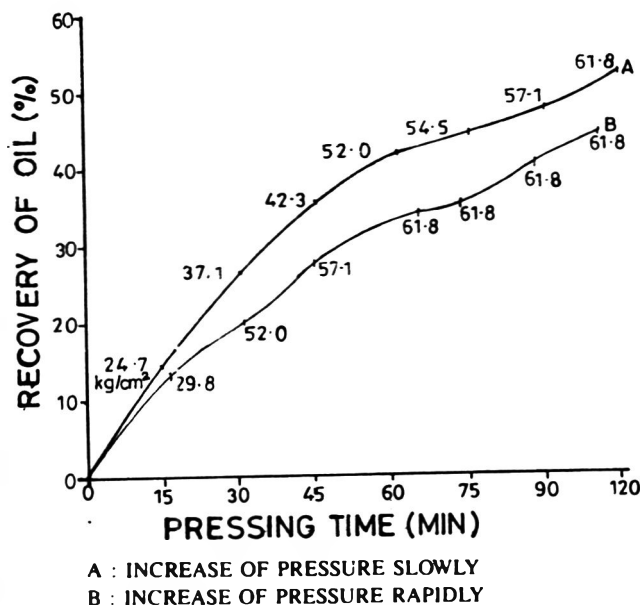


Fig.4. Effect of application of pressure on the recovery of oil during pressing of groundnut kernels in a hydraulic cage press.

**Quality of oil and partially defatted nuts:** The partially defatted nuts, thus, obtained (Table 2) had 4.0 moisture, 33 oil and 35 per cent protein. The cold pressed oil (at 30°C) had a FFA of 0.08 per cent with a colour of 5 units, Y+5R in 1.27 cm cell in a Lovibond Tintometer. However, the oil obtained at 70°C pressing temperature had a FFA of 0.50 per cent and the colour of the oil in same cell showed 8 units, Y+5R. Thus, pressing nuts under cold condition gave light coloured and low FFA oil.

In a specially designed hydraulic cage press, the recovery of oil for 100 kg batch was higher (49.2 per cent) for the nuts when pre-dried to an optimum moisture content of 4.0 per cent than without pre-drying (33.0-36.4 per cent) for the same pressing time (150 min) and pressure. Pressing of nuts at 70°C for 120-150 min with other conditions remaining same, resulted increased oil recovery of 52.0-53.2 per cent. However, the oil colour and FFA were also higher compared to the cold pressed oil.

The pressed nuts regained the shape easily when the hydrated nuts (to 13 per cent moisture) were dried at 110°C

TABLE 2. QUALITY OF RAW GROUNDNUTS, PARTIALLY DEFATTED GROUNDNUTS AND PRESSED OIL

Material	Moisture (%)	Oil (%)	Protein (%)	FFA (%)	Lovibond colour in 1.27 cm cell Y+5R
Groundnut raw	8.0	48.0	25.0	—	—
Partially defatted nuts	4.0	33.0	35.0	—	—
Cold pressed oil	0.2	—	—	0.08	5.00
Oil pressed at 70°C	0.1	—	—	0.50	8.00
Average of ten trials					

for 30 minutes. The crispness and the desired flavour in the defatted nuts were obtained by roasting them at 120°C for one hour.

Thus, pre-dried nuts to an optimum moisture content of 4 per cent should be pressed in the cage by slow but steady increase of the pressure from minimum (24.7 kg/Cm<sup>2</sup>) to the operational maximum pressure (61.8 kg/Cm<sup>2</sup>) with the partition plates. A pressing time of 120 min and the temperature of the charge (70°C) are other important parameters which should also be maintained for obtaining an optimum recovery of 52.0 per cent.

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# Ingredient Selection for Production of a Low-fat Butter Flavoured Spread

P.S. PRAJAPATI\*, S.K. GUPTA, A.A. PATEL AND G.R. PATIL  
Dairy Technology Division, National Dairy Research Institute,  
Karnal - 132001, Haryana, India.

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**A 50:50 blend of hydrogenated fat and soybean oil was better in terms of rheological and sensory characteristics of the spread. Addition of carrageenan (0.1%) and tri-sodium citrate (1.0%) enhanced water binding, whereas glycerol mono-stearate (0.3%) improved the emulsion characteristics. Reconstituted skim-milk powder formed the source of protein.**

The problems associated with traditional butter such as high cost and poor spreadability at temperatures below 15°C have created interest in alternatives devoid of such limitations. Non-dairy fat blends in spread comprise of one or more hydrogenated vegetable oils or both hydrogenated and un-hydrogenated oils. The non-fatty constituents, particularly proteins, play an important role in texture of spreads, and their levels influence the plasticity and spreadability of the product<sup>1</sup>. In addition to contributing flavour, milk solid-not-fat (MSNF) acts as a preservative by sequestering metals that promote oil oxidation<sup>2</sup>. Milk proteins have been found to provide good water binding, spreadability and stability to spreads<sup>3</sup>. However, little information is available in literature regarding the relative merits of the different forms of MSNF as the non-fat component of spreads, particularly of water-in-oil (W/O) type, which are considered to have better shelf life as compared to oil-in-water (O/W) type spreads<sup>4,5</sup>. The present study was, therefore, undertaken to examine the suitability of different dairy by-products as the source of MSNF/protein in a W/O type butter spread based on a selected hydrogenated fat-vegetable oil blend. Effects of additives such as stabilizers, emulsifiers, emulsifying salts and plasticizers on the rheological and sensory characteristics of the product were also evaluated in the course of this investigation.

## Materials and Methods

**Vegetable fat and oils:** Hydrogenated fat ("Dalda" of Lipton India), refined vegetable oils viz., safflower ("Saffola" Bombay Oil Industries), groundnut (Amrit Banaspati), soybean (Surya Agroils) and palm oil (imported by State Trading Corporation), and filtered coconut oil (Bombay Oil Industries) were used as different sources of fat.

**Source of milk protein/MSNF:** Skim milk, sweet cream buttermilk and cheddar cheese whey (used to obtain a non-fat solids level of 5 per cent in the spread) were received from the Experimental Dairy of the Institute. Spray-process skim

milk powder, also obtained from the Experimental Dairy, was reconstituted to 35 per cent TS and used to standardize the spread composition with respect to the MSNF content when different levels (5, 10 and 15 per cent) were studied.

**Additives:** Carrageenan, Type II (Sigma, USA), glycerol-mono-stearate (GMS) ("Gemesol": Fine Organic Industries, Bombay), trisodium citrate (IP/BP, Sarabhai Chemicals) and sorbitol (Sarabhai) were the additives investigated.

**Preparation of spread:** A W/O type spread (40 per cent fat) was prepared by using a Hobart mixer (Model N-50, Hobart Corp., USA). The procedure involved separate preparation and tempering of fat and aqueous or serum phases, before blending and emulsification. The blend of vegetable fat and oil heated to 75°C (and held for 30 min) was cooled to 5±1°C and held overnight at the same temperature without agitation. The cooled fat phase was tempered in an incubator at 30±1°C for 6 hr prior to use. The aqueous phase was pasteurized at 75°C for 30 min, cooled to 5±1°C and held overnight, followed by warming up to 30±1°C. The fat phase transferred to the bowl of Hobart mixer was creamed for 30 sec at a medium speed followed by gradual addition of the serum phase and mixing at a high speed for 30 sec to get fine dispersion of serum droplets. The spread so obtained was transferred to a refrigerator (5±1°C).

**Rheological measurements:** Firmness of the spread was measured in terms of penetration value by employing a Precision Cone Penetrometer (Central Ignition, UK) as outlined by ACCS<sup>6</sup>. Stickiness was measured in terms of extruder friction using FIRA—NIRD extruder (HA-Gaydon, UK). Estimation of oiling off was carried out by the method of deMann and Wood<sup>7</sup> with certain modifications. Sample together with the set of filter papers (Whatman No.1), after holding at 15±1°C for 48 hr, was transferred to a refrigerator (5±1°C) for 30 min. After scraping the solidified fat adhering to the filter-papers, the latter were weighed and then held in a hot air oven (100±1°C) for at least 4 hr. The dried filter

\*Present address: SMC College of Dairy Science, Gujarat Agricultural University, Anand Campus, Anand.

set was cooled and weighed and the values for oiling off and wheying off of the spread were calculated.

**Sensory evaluation:** Sensory evaluation was carried out by a panel of trained judges selected from the staff in the Dairy Technology Division of the Institute. A special laboratory with necessary facilities viz., separate booths, provision for adequate diffused light, and air-conditioned, odour-free environment<sup>8</sup> was employed for sensory evaluation of the spread. Different attributes viz., flavour, spreadability, and body and texture of vegetable fat-oil blends or spread were rated on a 9 point scale ranging from 1 (most undesirable) to 9 (most desirable). Data were subjected to variance and correlation analyses<sup>9</sup>.

### Results and Discussion

During preliminary studies, various vegetable oils (safflower, groundnut, soybean, palm and coconut) were individually blended with hydrogenated fat (melting point, 37.4°C) in different proportions and evaluated for their softening effect and flavour. Soybean and groundnut oils were selected in blends with hydrogenated fat (each at 40:60 and 50:50), to optimise the condition for type of oil and its level in spread making. Moreover, the skim milk based spread was sensorily and rheologically more acceptable than the spread based on whey or buttermilk. Hence, skim milk was used in further trials.

**Optimization of fat-to-oil ratio and MSNF content of the spread:** To obtain a low-fat spread (40 per cent fat) with desired plasticity and spreadability at refrigerator temperature, due consideration must be given to the MSNF content and nature and level of fat blend. Therefore, soybean and hydrogenated fat blends (40:60 and 50:50), and groundnut oil and hydrogenated fat blends (40:60 and 50:50) were studied for

different MSNF levels in the spread (Table 1). It can be seen that soybean oil and hydrogenated fat blend produced a softer product as compared to groundnut oil and hydrogenated fat blend both at 5°C and 15°C and this effect was statistically significant ( $P < 0.01$ ). The lower firmness of soybean oil containing spread was accompanied by better spreadability, body and texture scores. Crumbly and mealy body were some of the defects found in the groundnut oil containing spread. Stickiness of the product measured as extruder friction was significantly lower for soybean oil-hydrogenated fat blend based spread than groundnut oil-hydrogenated fat based one ( $P < 0.05$ ,  $CD=4.6$ ). The flavour score was also significantly higher ( $P < 0.01$ ) for the soybean oil based spread at either level of fat replacement. Thus, soybean oil was more desirable than groundnut oil to get a spread with the desired consistency, spreadability and flavour. Higher level (50 per cent) of soybean oil in the spread (i.e. blend of 50:50 soybean oil and hydrogenated fat) seemed to improve softness which coincided with significantly improved ( $P < 0.01$ ) sensory scores for body and texture and spreadability. Stickiness was also reduced with the use of higher level of oil in the fat-oil blend ( $P < 0.01$ ). Thus, it can be concluded that 50 per cent incorporation of soybean oil in the fat-oil blend was distinctly more desirable than groundnut oil to get optimum sensory and rheological characteristics in the spread.

With the increasing MSNF in the spread (Table 1) the firmness decreased significantly ( $P < 0.01$ ) both at 5° and 15°C which, in turn, resulted in a significant rise in body and texture ( $P < 0.05$ ,  $CD=0.2$ ) and spreadability ( $P < 0.01$ ,  $CD=0.2$ ) scores. The reduction in firmness with increased MSNF may be attributed to increased lactose content which, in solution, presumably acted as a softening or plasticizing agent<sup>10</sup>. Extruder friction of the spread tended to decline

TABLE 1. EFFECT OF THE TYPE AND LEVEL OF VEGETABLE OIL, AND MSNF CONTENT ON RHEOLOGICAL AND SENSORY CHARACTERISTICS OF LOW-FAT (40 PERCENT) SPREAD

Blending ratio			MSNF (%)	Rheological properties				Sensory scores		
Hydrogenated fat	Groundnut oil	Soybean oil		Penetration value (0.1 mm) at		Extruder friction (g/cm) at		Flavour	Spreadability	Body & texture
			5°C	15°C	5°C	15°C				
60	40	—	5	111.6	199.8	77.5	77.5	5.8	6.7	6.0
60	40	—	10	121.3	203.3	50.0	50.0	6.1	7.0	6.3
60	40	—	15	128.3	210.1	40.0	37.5	6.6	7.3	6.7
50	50	—	5	144.5	224.9	42.5	40.0	6.3	7.3	6.7
50	50	—	10	153.4	228.1	38.8	26.3	6.8	7.6	7.2
50	50	—	15	161.3	233.8	28.8	25.0	7.0	8.0	7.4
60	—	40	5	127.5	200.4	75.0	62.5	6.4	6.7	6.6
60	—	40	10	135.0	204.3	45.0	40.0	6.9	7.3	6.8
60	—	40	15	154.6	212.1	40.0	40.0	6.9	7.3	7.0
50	—	50	5	154.3	228.6	37.5	30.0	7.0	7.4	6.9
50	—	50	10	172.4	236.5	33.8	25.0	7.4	7.9	7.4
50	—	50	15	175.0	246.2	25.0	22.5	7.6	8.1	7.7

Mean values from duplicate experiments

( $P < 0.01$ ,  $CD = 5.6$ ) with increasing MSNF in the spread. The flavour score increased significantly as the MNFS content increased. Thus, from the flavour, body and texture, and spreadability points of view, the 50:50 soybean oil-hydrogenated fat blend with 15 per cent MSNF would form a highly desirable base for a low-fat butter like spread.

*Effect of additives on the spread quality:* The above spread was sought to be improved further with respect to its rheological and sensory characteristics. It can be seen from Table 2 that significant ( $P < 0.01$ ) decrease in the penetration value and extruder friction of the spread were observed as a result of carrageenan addition, the higher level showing a greater effect at both the temperatures of measurement. Increase in firmness was accompanied by small but significant increase in scores for body and texture ( $P < 0.01$ ) as well as flavour ( $P < 0.05$ ). This could be possibly due to better water binding and mouthfeel characteristic imparted by carrageenan<sup>11,12</sup>. However, increased firmness tended to cause slight impairment of spreadability of the product. An increase in oiling off and decrease in wheying off of the spread were observed with the increased level of hydrocolloid. Presumably, the increased firmness due to addition of carrageenan tended to facilitate expulsion of the liquid oil whereas effective water binding properties of the carrageenan reduced wheying off<sup>3</sup>.

Addition of GMS to a spread (40 per cent fat comprising of a 50:50 soybean oil-hydrogenated blend) resulted in significant ( $P < 0.01$ ) reduction of firmness as well as body and texture score, though spreadability remained unchanged (Table 2). The flavour score, on the other hand, was slightly improved as the GMS content increased from 0.0 to 0.3 per cent. This may be attributed to the ability of GMS to impart a better mouthfeel to the spread<sup>1</sup>. A significant ( $P < 0.01$ ) reduction in oiling off and wheying off of the spread without any perceivable influence on the adhesiveness (determined as extruder friction) of the product was noticed as a result of GMS addition. The combination of 0.1 per cent

carrageenan and 0.3 per cent GMS yielded a product with the highest score for body and texture, spreadability and flavour in addition to minimum wheying off and relatively low oiling off.

A significant ( $P < 0.01$ ) increase in firmness of the spread was noticed with an increase in the sodium citrate content both at 5° and 15°C (Table 3). The increased firmness may presumably be attributed to increased water binding capacity of protein due to the use of the peptizing salt. This was also reflected in a significant ( $P < 0.01$ ) reduction in wheying off and oiling off of the spread. Significantly decreased oiling off with increasing citrate level could be due to better emulsification of the liquid oil component of the fat phase. Improvement in fat emulsification and firming of soybased low-fat spread was also reported by Patel and Gupta<sup>1</sup>. Solubilization of protein by peptizing salt is believed to play an important role in the improvement of emulsion stability of the product<sup>14,15</sup>. Decreased wheying off could, on the other hand, be attributed to enhanced water binding and improved dispersion of free water. The improved firmness, and emulsion stability of the spread were perceived as slightly increased flavour, spreadability and body and texture acceptability. Thus, 1 per cent trisodium citrate yielded a product with most desirable sensory characteristics and minimum oiling off and wheying off.

Addition of sorbitol (0.5 per cent) as a plasticizer did not show any recognizable impact on overall quality of the spread containing trisodium citrate (1.0 per cent) and had only a slight favourable effect on the sensory properties in absence of the emulsifying salt (Table 3). Thus, it can be concluded that addition of sorbitol was considered unnecessary in the spread.

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TABLE 2. EFFECT OF CARRAGEENAN AND GLYCEROL MONOSTEARATE (GMS) ON RHEOLOGICAL AND SENSORY PROPERTIES OF THE SPREAD (40 PERCENT FAT, COMPRISING OF 50:50 SOYBEAN OIL AND HYDROGENATED FAT)

GMS (%)	Carrageenan (%)	Penetration value		Extruder friction (g/cm)		Wheying off at 15°C (%)	Oiling off at 15°C (%)	Sensory score		
		5°C	15°C	5°C	15°C			Flavour	Spreadability	Body & texture
0.00	0.00	174.1	251.8	26.7	16.7	2.6	5.1	7.3	7.9	7.6
0.00	0.05	142.5	242.5	20.0	15.0	1.7	5.2	7.3	7.7	7.7
0.00	0.10	137.8	240.3	13.3	20.0	1.4	5.8	7.4	7.3	7.2
0.15	0.00	178.3	266.7	23.3	15.0	2.2	5.0	7.3	7.9	7.2
0.15	0.05	163.4	262.3	28.3	11.7	1.9	5.4	7.5	7.6	7.3
0.15	0.10	160.5	256.2	23.3	20.0	1.9	5.2	7.7	8.1	8.0
0.30	0.00	186.8	278.2	28.3	11.7	1.9	3.5	7.4	7.9	7.4
0.30	0.05	176.4	261.5	21.7	18.3	1.2	3.4	7.5	7.9	7.4
0.30	0.10	152.3	250.1	13.3	20.0	0.9	4.6	7.7	8.2	8.2

Mean values from triplicate experiments



TABLE 3. EFFECT OF SODIUM CITRATE AND SORBITOL ON RHEOLOGICAL AND SENSORY PROPERTIES OF THE SPREAD\*

Sorbitol (%)	Sod. citrate (%)	Penetration value (0.1 mm)		Extruder friction (g/cm)		Oiling off at 15°C (%)	Wheyng off at 15°C (%)	Sensory score		
		5°C	15°C	5°C	15°C			Flavour	Spread-ability	Body & texture
0.0	0.0	150.9	262.3	38.3	21.7	4.7	0.9	6.9	7.8	7.4
0.0	0.5	138.2	239.3	33.3	21.7	4.1	0.4	7.1	8.1	7.7
0.0	1.0	134.7	237.7	38.3	18.3	4.0	0.3	7.4	8.3	7.9
0.5	0.0	139.0	264.5	23.3	21.7	4.8	0.8	7.1	8.1	7.6
0.5	0.5	134.2	240.9	23.3	21.7	4.8	0.4	7.1	8.1	7.5
0.5	1.0	136.4	239.3	36.7	26.7	3.9	0.3	7.0	8.0	7.8

(40 per cent fat, comprising of 50:50 soybean oil and hydrogenated fat: 0.1 per cent carrageenan and 0.3 per cent GMS)

Mean values from triplicate experiments

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## Processing of Low-fat Butter Flavoured Spread

P.S. PRAJAPATI\*+, S.K. GUPTA, A.A. PATEL AND G.R. PATIL  
Dairy Technology Division, National Dairy Research Institute,  
Karnal - 132 001, Haryana, India.

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**In the process of standardizing the method of manufacture of a low-fat spread similar to table butter, cooling of fat phase, vital to successful emulsification, was investigated. Rapid cooling to 30°C followed by slow cooling to 5°C prior to warming to the emulsification temperature yielded a product with the most desirable texture. Addition of 15 p.p.m. diacetyl and 1.5% salt to the spread acidulated to pH 5.8 was made it flavour-wise most acceptable as a butter-like product. While the spread was akin to butter with respect to flavour, appearance, body and texture, it had a distinctly superior spreadability as judged sensorily and in rheological properties.**

Emulsification and cooling are critical to successful spread-making<sup>1</sup>. The type of emulsion obtained is decided by the ratio of oil phase to aqueous phase and the mode of admixing the two phases<sup>2,3</sup>. The emulsification temperature and the pH of the serum phase also determine the type of emulsion produced in the spread<sup>4,5</sup>. In the present study, temperature to which the fat phase has to be cooled before emulsification and the optimum pH of the serum phase before emulsification were investigated in developing a low-fat water-in-oil (W/O) type spread. Further, various flavourings were also evaluated for their effectiveness in simulating butter flavour in the finished product.

### Materials and Methods

**Spread preparation:** A W/O type spread (40 per cent fat and 15 per cent milk solids-not-fat- (MSNF) was prepared in 1.5 kg lots by using a Hobart mixer (Model N-50, Hobart Corp., U.S.A.). The procedure involved separate preparation and tempering of fat and aqueous phases before blending and emulsification. A 50:50 blend of hydrogenated fat (melting point, 29.9°C) and soybean oil was heated to 50-60°C, followed by addition of glycerol monostearate ("Gemesol": Fine Organic Industries, Bombay) and annatto butter (Colour Sonarome Chemicals, Bangalore) at the rate of 0.3 and 0.25 per cent, respectively. After filtration, the blend was further heated to 75°C for 30 min, cooled in water bath containing chilled water (5°C) to 40,35 or 30°C within 130, 150 or 210 sec, respectively with gentle agitation. The cooled fat phase was held overnight at 5°C and then tempered at 30°C for 6 hr prior to blending with the aqueous phase.

The aqueous phase preparation involved mixing of 35 per cent reconstituted skim milk powder, 0.1 per cent (in the finished product) carrageenan, Type II (Sigma, USA)

dissolved in cold water, 1.0 per cent tri-sodium citrate (IP/BP, Sarabhai Chemicals) and 1.0 per cent common salt. The mixture was pasteurized (75°C/30 min), cooled to 5°C and held overnight at the same temperature without agitation. It was warmed upto 30°C before blending.

The fat phase transferred to the bowl of Hobart mixer was creamed for 30 sec at a medium speed, Lactic acid (Extra pure, E. Merck, India) was added to fat phase during creaming to adjust the pH of the final product. This was followed by gradual addition of the serum phase and mixing at a high speed for 30 sec to get fine dispersion of serum droplets. The spread was packaged in polystyrene cups (100 g capacity) and stored at 5°C. Various flavourings were used to simulate butter flavour in the product at different levels as given below. The levels were selected based on preliminary trials.

(i) Starter distillate: 1.0 per cent (v/w), (ii) diacetyl (Sigma, U.S.A.): 15 p.p.m., (iii) butterbuds 8x (Beatrice Foods, U.S.A.): 1.5 per cent (w/w), (iv) butardol (Naarden, India): 0.23 per cent (v/w). Each flavouring was introduced into the warmed serum phase just before emulsification.

**Analysis of the spread:** Firmness of the spread was measured in terms of penetration value by employing a Precision Cone Renetrometer (Central Ignition, UK) as outlined by AOCS<sup>6</sup> and extruder thrust by using FIRA-NIRD Extruder (HA-Gaydon UK). Stickiness/adhesiveness was measured in terms of extruder friction using FIRA NIRD extruder data, Hocppler consistometer (veb prutgeraete Werk, Medingen, E. Germany) fitted with a normal ball bar (k=100) was used to determine the viscosity of the spread.

The spread was analysed for total solids (TS) fat, total protein and total ash<sup>7,9</sup>. For computing the caloric value of the spread, energy values of fat, protein and carbohydrates

\*Present address: SMC College of Dairy Science, Gujarat Agricultural University, Anand Campus, Anand.

+To whom all correspondence should be addressed.

were taken at 9, 4 and 4 kcal/g, respectively. A digital pH meter was used for pH determination and Rotronic Hygroskop for the determination of water activity ( $a_w$ ).

Sensory evaluation was carried out by a panel of judges selected from the staff and students in the Dairy Technology Division of the Institute. Different attributes viz. flavour spreadability, body and texture and colour of spread at 15°C were rated on a 9-point scale ranging from 1 representing most undesirable to 9 indicating most desirable. Data were subjected to statistical analysis<sup>10</sup>.

## Results and Discussion

**Effect of cooling of fat phase on the quality of spread:** Slow cooling of the fat phase by holding it at 5°C undisturbed for several hours and warming to the blending temperature prior to emulsification was found to result in a grainy texture as noted by the panel of judges. Hence, quick cooling in the presence of agitation was studied. It was observed that at 5°C, the firmness of the spread made using the fat blend cooled to 30°C was higher (indicated by the lower penetration depth) as compared to the higher cooling temperatures (Table 1). However, all spreads were equally soft at 15°C irrespective of the cooling temperature. Spreadability score was better when the fat phase was cooled to 30°C than when cooled to 35 or 40°C. The reduction in stickiness (measured in terms of extruder friction) due to lower cooling temperature was observed more at 15°C than at 5°C. The overall body and texture score was higher (7.7) for 30°C than 35°C or 40°C (7.4) as the cooling temperature. Flavour score of the spread made using the fat blend cooled to 30°C was slightly better than that of those made from fat blends cooled to 35 and 40°C. Improvement in firmness, body and texture and spreadability characteristics of the spread could be ascribed to rapid cooling presumably resulting in formation of smaller crystals<sup>4,11</sup>. Rapid cooling of the liquid fat phase is also helpful in achieving fine dispersion of the serum droplets and thus optimum taste and spreadability, and ensuring minimum microbial deterioration in the final product.

TABLE 1. EFFECT OF COOLING TEMPERATURE OF THE FAT PHASE ON THE RHEOLOGICAL AND SENSORY CHARACTERISTICS OF THE SPREAD

Property/Attribute		Temp of cooled fat phase		
		40°C	35°C	30°C
Penetration value, 0.1 mm	5°C	165.7	162.1	148.4
	15°C	252.1	250.5	253.9
Extruder friction, (g/cm)	5°C	35.0	30.0	30.0
	15°C	35.0	20.0	15.0
Flavour score		7.5	7.5	7.6
Spreadability score		7.6	7.7	7.9
Body & texture score		7.4	7.4	7.7
Mean values from duplicate experiments				

**Simulation of butter flavour in the spread:** The use of flavourings resulted in a significant ( $p < 0.01$ ) increase in flavour score except for butter oil (Table 2). Diacetyl had the most pronounced enhancing effect on the flavour score of the product followed by butterbuds and starter distillate, the differences between all the flavours, being significant. Conventional table butter was flavour-wise superior to the diacetyl (score 7.9) flavoured spread, the difference being non-significant. Thus, it could be concluded that the diacetyl flavoured spread was very close to butter in flavour.

**Effect of salt and pH:** The higher salt level (1.5 per cent) did not have any perceptible influence on firmness and stickiness of the spread (Table 3). Flavour, spreadability and colour scores of the spread slightly improved, but body and texture score slightly decreased with the increased salt content at pH 6.7. Similarly, improvement in sensory score except colour score was observed at pH 5.8 with increased salt level. The pH of the product did not have any appreciable effect on the firmness and stickiness of the spread at 15°C. The spread at 5°C was less sticky at lower pH and 1 per cent salt level.

A maximum flavour score without any loss of other sensory attributes of the acidified spread containing 1.5 per cent salt suggested that the acidulation was highly desirable. Moreover, a low pH is generally required for acceptable keeping quality of spread. A pH in the range of 5.7 - 5.9 has been recommended for most of the spreads<sup>12,14</sup>. A distinct acid taste was noticed in the spreads having a lower pH (5.8) and 1.0 per cent salt.

**Physico-chemical and sensory quality of the spread:** The product developed by the method including all optimized processing parameters (Fig. 1) was evaluated for its compositional, rheological and sensory characteristics, (Table 4). The fat and protein contents were in the normal range for most of the low-fat spreads which have been reported in literature. The carbohydrate content was appreciably higher than that of traditional butter (less than 1 per cent)<sup>15</sup> but the ash content was fairly comparable. The water activity of the spread was in the range of intermediate moisture foods (0.65-0.90)<sup>16</sup>. The calorific value of the spread (421.5 kcal/100 g) as computed from the composition came out to be 50

TABLE 2. EFFECT OF VARIOUS FLAVOURINGS ON THE FLAVOUR SCORE OF THE SPREAD

Flavouring agent	Flavour score	Remark
Control	7.1	—
Starter distillate	7.4	Akin to diacetyl flavour
Diacetyl	7.9	Diacetyl flavour
Butterbuds	7.5	Atypical taste
Butardol	7.2	Flavour incompatible
Table butter (NDRI)	8.1	—
Critical diff.	0.2	
Means from triplicate experiments		

TABLE 3. EFFECT OF SALT AND pH ON THE QUALITY OF THE SPREAD

pH	Salt (%)	Penetration value (0.1 mm) at		Extruder friction (g/cm) at		Quality score			
		5°C	15°C	5°C	15°C	Flavour	Spreadability	Body & texture	Colour
6.7	1.0	146.0	236.3	30.0	16.3	7.3	8.2	8.0	8.3
6.7	1.5	143.7	240.0	22.5	16.3	7.4	8.3	7.9	8.4
5.8	1.0	144.2	235.1	21.5	20.0	7.1	8.2	7.8	8.4
5.8	1.5	145.8	238.1	25.0	17.5	7.6	8.3	8.0	8.4

Means from duplicate experiments

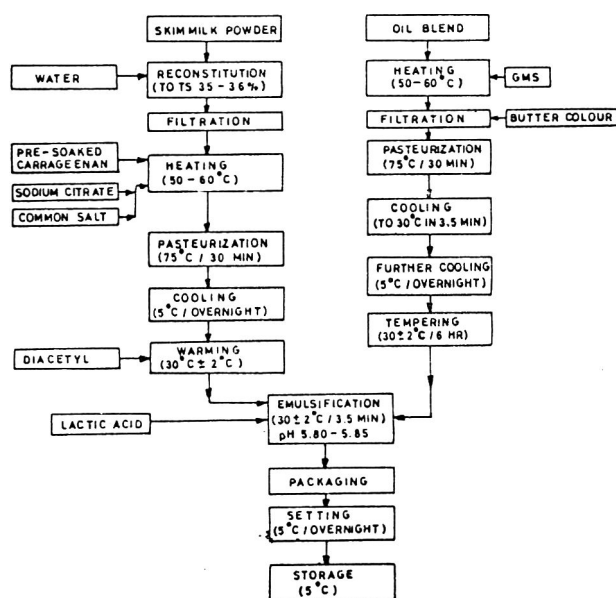


Fig. 1. Schematic diagram of manufacture of butter-flavoured low-fat spread

per cent of that of table butter (729.3 kcal/100 g; fat 80.5, protein 0.5 and lactose 0.7 percent).

It is evident from Table 4 that the newly developed spread was significantly softer than traditional butter as indicated by higher penetration values and lower extruder thrust at both the temperatures of measurement. The superior consistency or plasticity of the spread was confirmed by viscosity measurement, which was considerably lower for the spread at both the temperatures. Table butter was noticed to be crumbling at 5°C unlike the spread. Moreover, butter was considerably more sticky than the spread as indicated by higher extruder friction values at both the temperatures.

The scores for sensory attributes (Table 4) revealed that from the view point of flavour and body and texture characteristics, the spread was fairly close to table butter. Spreadability-wise developed spread was much superior to table butter.

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TABLE 4. PHYSICO-CHEMICAL AND SENSORY QUALITY OF THE SPREAD

Constituent/Attribute	Spread	Table butter
Total solids (%)	57.4	—
Fat (%)	40.6	—
Protein (%)	5.1	—
Carbohydrates (%)	8.8	—
Ash (%)	2.8	—
Water activity ( $a_w$ ) at 15°C	0.9	—
Calorific value. (kcal/100 g)	421.5	729.3
Penetration value. (0.1 mm)	5°C 143.6 15°C 239.9	35.5 90.5
Viscosity. ( $\times 10^7$ ), CP	5°C 42.2 15°C 6.9	440.6 20.6
Extruder thrust. (g)	5°C 617.5 15°C 287.5	3935.0 1286.3
Extruder friction. (g/cm)	5°C 37.5 15°C 20.0	372.5 202.5
Flavour score	7.8 (7.4–7.9)	8.1 (7.9–8.2)
Spreadability score	8.2 (8.1–8.4)	6.1 (5.5–6.6)
Body and texture score	7.8 (7.5–8.2)	7.9 (7.7–8.1)
Colour score	8.2 (8.0–8.5)	8.6 (8.4–8.9)

Means from triplicate experiments

Figures in parentheses indicate range.

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## Studies on Processing and Preservation of *Goshtaba*

A.H. SAMOON AND N. SHARMA  
 Division of Livestock Products Technology  
 Indian Veterinary Research Institute,  
 Izatnagar - 243122, India.

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**Effect of mutton-fat level, hot vs cold-boned meat and manual vs machine mincing was studied on the processing of *Goshtaba* (Kashmiri meat product). Incorporation of mutton-fat at 20% level in the emulsion was found optimum for *Goshtaba*; it could be prepared using chilled mutton. Sodium tripolyphosphate at 0.5% level improved product quality and the effect was more pronounced in samples made from cold boned meat with the use of machine than that of the hand minced. It was possible to store cooked *Goshtaba* samples for seven days in low density polyethylene bags (.25  $\mu$ m thickness) at  $4 \pm 1^\circ\text{C}$ .**

*Goshtaba* is a traditional emulsion type ground meat product of Kashmiri Wazawan. Wazawan represents the traditional Kashmiri cuisine consisting of several speciality meat products processed on large scale for feasts in Kashmir Valley. *Goshtaba* emulsion is obtained by constant pounding of pre-rigor (hot boned) mutton along with mutton fat using a wooden hammer over a smooth flat stone. Emulsions thus obtained are manually shaped into spherical meat balls and cooked in a gravy called "Yakhni" consisting of curd, water, spices and condiments. The product is served hot with rice. The indigenous method of processing *Goshtaba* is laborious, time consuming, limited to use of hot meat and less relevant to suit large hygienic production practices. Hence, studies on processing and shelf life of *Goshtaba* were undertaken.

### Materials and Methods

Lambs of almost same age group (6-9 months) were slaughtered by conventional method. Dressed carcasses were split longitudinally into 2 equal sides. One side was deboned within 2 hr post-mortem to obtain hot boned lean and fat, and other side of the carcass was chilled at  $4 \pm 1^\circ\text{C}$  for 24 hr to obtain cold boned samples. Cow milk curd of pH 3.70-4.00 was prepared in laboratory.

Hot boned chunks of mutton were pounded manually with a wooden hammer on a flat and smooth stone. Mutton fat was pounded separately to obtain a fine paste. Minced fat at 4 different levels (15, 20, 25 and 30 per cent) was added in the pounded lean. Sodium chloride 2.5 per cent, sodium tripolyphosphate 0.5 per cent and cardamom seeds were mixed in the minced lean. Pounding was continued with sprinkling of chilled water or ice laces (10 per cent) to mix the fat until the *Goshtaba* emulsion of desired consistency was obtained. It was moulded manually into spherical balls weighing approximately 50 g each and was allowed to set at  $4 \pm 1^\circ\text{C}$  for 30 min. After several trials, it was found that 20

per cent fat level was optimum for desirable type of product. Hence, only 20 per cent mutton fat was incorporated for further experimental work. Cold boned lean was utilised in the same way but divided into two batches. One batch was treated with 2.5 per cent sodium chloride and the second batch with 0.5 per cent sodium tripolyphosphate in addition to sodium chloride. To compare the effect of hand pounding vs machine mincing, hot and cold mutton chunks were minced separately through 8 mm plate followed by 4 mm plate of a meat grinder. Sodium chloride and sodium tripolyphosphate treatments were given to hot and cold boned mince in the same concentration and manner as described above for cold boned lean. Minced lean was transferred to food cutter (Hobart Model 841820) and chopping was done for 7 min.

Standard recipe for *Goshtaba* is shown in Table 1. *Goshtaba* gravy was prepared by rapid heating of curd and water. Constant stirring was done to avoid formation of lumps of coagulated protein. Hydrogenated vegetable oil was added to

TABLE 1. STANDARDISED RECIPE FOR *GOSHTABA*

Ingredients	Quantity (%)
Raw meat balls (10 × 50 g each)	31.33
Curd	31.33
Water	31.33
Hydrogenated vegetable oil	3.13
Large cardamom	0.08
Small cardamom	0.03
Cinnamon	0.10
Cloves	0.01
Dried ginger powder	0.19
Aniseed powder	0.25
Garlic paste	0.31
Fried onion paste	1.57
Dried mint powder	0.03
Common salt	0.31
Total	100.00

gravy and boiling continued further for 10 min. Afterwards dry spices, garlic paste and onion paste were added. Heating continued until desirable consistency was obtained. Chilled meat balls were reshaped after setting and cooked in a gravy at boiling point in an open vessel for about 3 min. Common salt was added and dried mint powder was sprinkled over cooked product before serving to panelists for sensory evaluation on the first day of its preparation.

From each of the eight treatments, 100 g raw emulsion was collected in low density polyethylene bags for analysis. Appropriate portions of the cooked meat balls soaked in their respective gravies were sealed in LDPE bags and stored in a refrigerator at  $4 \pm 1^\circ\text{C}$ . Samples were drawn at appropriate intervals during storage, for checking of pH<sup>1</sup>, emulsion stability<sup>2</sup>, proximate analysis<sup>3</sup>, TBA value<sup>4</sup> and aerobic plate count<sup>5</sup>. Cooking yields of meat balls and gravy were recorded by calculating the difference in weight of raw and cooked samples and expressed as per cent yield. An 8-point Hedonic scale (8 = extremely desirable, 1 = extremely undesirable) was used for evaluation of *Goshtaba* samples in respect of various organoleptic attributes viz. appearance, flavour, juiciness, texture and overall acceptability. The data were subjected to analysis of variance<sup>6</sup> and all significant main effects and interactions were tested using least significant difference<sup>7</sup>.

## Results and Discussion

The mean values of pH in raw emulsion samples are shown in Table 2. Hot boned samples had a higher mean pH as compared to cold boned samples of emulsion. Higher pH of hot boned emulsions as observed in this study is well documented<sup>8</sup>. Furthermore, higher pH values observed in machine minced as compared to traditionally minced emulsion samples may be due to prolonged processing schedules involved in traditional pounding, which causes a drop in pH. *Goshtaba* emulsion treated with sodium tripolyphosphate had a higher mean pH as compared to salt treated controls. It is in agreement with the findings reported in other meat products<sup>9,10</sup>.

Hot boned emulsion samples were more stable with lower ( $P < 0.01$ ) cooking loss compared to cold processed samples. Superior emulsion stability of pre-rigor meat has been reported earlier<sup>8,11</sup>. Traditional minced emulsion samples were more stable with a lower ( $P < 0.01$ ) cooking loss possibly due to excessive physical disruption of muscle fibres, better myofibrillar protein extraction and more efficient fat dispersion. Similar findings have been reported in meat emulsions subjected to mechanical treatments like beating, massaging and tumbling<sup>12</sup>. Emulsion samples treated with both sodium chloride and sodium tripolyphosphate were more stable with a lower ( $P < 0.01$ ) cooking loss as compared to samples treated with sodium chloride. Synergistic effect of salt and phosphate in improving the stability of treated batters has been reported in various meat emulsions<sup>13,14</sup>. This effect was more pronounced in cold boned machine minced samples. Improvement in stability due to addition of salts and phosphate in hot boned meat by traditional pounding is marginal. Cooking yields ranged from 87.67 to 110.67 and 69.11-70.18 per cent for *Goshtaba* balls and *Yakhni* samples, respectively. Higher cooking yields of hot processed *Goshtaba* balls in comparison to cold processed balls may be due to excellent water and fat binding properties of pre-rigor meat. It is in agreement with the findings reported by various workers in ground beef patties<sup>15,16</sup>. Cooking yield was significantly ( $P < 0.01$ ) higher in traditionally minced as compared to machine minced samples of *Goshtaba* balls. Traditional pounding causes more efficient myofibrillar protein extraction, better fat dispersion into protein matrix and greater emulsification of added fat. This, in turn, causes better binding and emulsion stability. Thus, on subsequent cooking, less fat is lost into gravy in traditionally minced as compared to machine minced emulsion. Higher cooking yield in phosphate treated as compared to salt treated *Goshtaba* balls is due to the effect of phosphate in significantly increasing the water holding capacity, binding and cooking yield of the product. Similar findings have been reported in other ground meat products<sup>13,17,18</sup>. Cooking yield of *Yakhni* obtained for machine minced samples was higher as compared

TABLE 2. pH OF RAW EMULSION, EMULSION STABILITY VALUES AND COOKING YIELDS OF GOSHTABA

Treatments*	Emulsion pH	Cooking loss (%)	Cooking <i>Goshtaba</i> balls	Yield (%) <i>Yakhni</i>
HBTMST	5.97 ± 0.06	7.85 ± 0.52	103.13 ± 1.09	61.35 ± 1.06
HBTMPT	6.10 ± 0.07	6.39 ± 0.36	110.67 ± 1.51	61.51 ± 1.26
HBMMST	6.07 ± 0.08	10.63 ± 0.80	93.27 ± 0.63	63.72 ± 2.91
HBMMPT	6.18 ± 0.07	7.93 ± 0.57	102.53 ± 1.69	62.26 ± 0.58
CBTMST	5.74 ± 0.06	13.11 ± 0.74	94.33 ± 0.46	58.70 ± 1.78
CMTMPT	5.92 ± 0.03	10.32 ± 0.45	99.13 ± 0.72	59.11 ± 0.88
CBMMST	5.87 ± 0.05	20.48 ± 0.38	87.67 ± 0.96	70.18 ± 1.90
CBMMPT	6.08 ± 0.03	16.93 ± 0.80	92.47 ± 0.70	65.26 ± 1.37

HB: Hot boned; CB: Cold boned; TM: Traditionally minced; MM: Machine minced; ST: Salt treated and PT: Phosphate treated.

ES: Emulsion stability  
(Mean ± SE)

to traditionally minced samples. It may be due to loss of a greater amount of fat from machine minced *Goshtaba* balls into *Yakhni* on cooking. However, such differences in cooking yield of *Yakhni* are not practically significant.

**Proximate composition:** Mean values of per cent moisture, protein, fat and total ash are reported in Table 3. On cooking, better fat retention was observed in hot boned traditionally minced and phosphate treated *Goshtaba* balls, with a corresponding decrease in their protein and moisture levels as compared to cold boned, machine minced and salt treated counterparts, respectively. It is in agreement with the findings reported in other meat products<sup>17,18</sup>. It may be postulated that hot boning caused better fat retention due to better emulsifying efficiency of myofibrillar proteins. Similarly, better protein extraction resulting due to traditional pounding of lean caused better fat retention in traditionally minced *Goshtaba* balls. Furthermore, improvement in the emulsifying capacity and fat holding capacity of the meat by phosphate is well documented. In general, the protein levels of the products were above the minimum prescribed standards for meat products.

Lower TBA values in phosphate treated samples as compared to salt treated ones showed that phosphate inhibited oxidative rancidity. (Table 4). Taste panelists also experienced

that the intensity of oxidised flavour was lower in phosphate treated samples of *Goshtaba*. Polyphosphate was used here primarily to improve the functional properties of *Goshtaba* emulsion. However, the antioxidant effect of sodium tripolyphosphate was also observed in this study which is in agreement with the findings reported in other products<sup>19,20</sup>. In general, the overall TBA values of *Goshtaba* samples remained low even at detectable levels of rancidity, in comparison to other meat products. It may be due to interfering factors like low pH of *Yakhni* and higher concentrations of various spices incorporated in this product. Furthermore, a more rapid increase in TBA values was observed in salt treated as compared to phosphate treated *Goshtaba* samples during storage. It may be due to peroxidant effect of sodium chloride treated samples. On the other hand, sodium tripolyphosphate retarded the development of oxidative rancidity in treated sample.

A maximum aerobic plate count (4.9 log/g) was recorded on the 7th day of refrigerated storage (Table 4). It is below the spoiling limits (6-7 log/g) prescribed for other meat products<sup>21</sup>. Effect of type of boning on aerobic plate count was non-significant. It is similar to findings reported in other ground meat products<sup>8,15,21,22</sup>. Lower microbial counts in salt treated as compared to phosphate treated *Goshtaba* samples

TABLE 3. PROXIMATE COMPOSITION OF *GOSHTABA* (MEAN  $\pm$  SE)

Treatments*	Moisture (%)	Protein (%)	Fat (%)	Total ash (%)
HBTMST	66.98 $\pm$ 0.75	12.01 $\pm$ 0.33	17.30 $\pm$ 0.59	2.51 $\pm$ 0.07
HBTMPT	63.68 $\pm$ 0.62	13.49 $\pm$ 0.22	19.22 $\pm$ 0.92	2.36 $\pm$ 0.25
HBMMST	66.39 $\pm$ 1.35	13.89 $\pm$ 0.20	15.90 $\pm$ 1.12	2.57 $\pm$ 0.15
HBMMPT	63.15 $\pm$ 0.46	15.15 $\pm$ 0.41	18.01 $\pm$ 0.97	2.55 $\pm$ 0.06
CBTMST	66.45 $\pm$ 0.37	13.69 $\pm$ 0.74	14.64 $\pm$ 0.68	2.51 $\pm$ 0.07
CMTMPT	66.79 $\pm$ 0.53	12.96 $\pm$ 0.26	16.69 $\pm$ 0.49	2.49 $\pm$ 0.11
CBMMST	69.74 $\pm$ 0.63	15.22 $\pm$ 2.61	11.46 $\pm$ 0.68	2.75 $\pm$ 0.11
CBMMPT	67.51 $\pm$ 1.12	13.72 $\pm$ 0.44	14.51 $\pm$ 0.65	2.60 $\pm$ 0.04

\*Legend as in Table 2

TABLE 4. TBA AND APC VALUES OF *GOSHTABA* DURING STORAGE (MEAN  $\pm$  SE)

Treatments	Parameters						
	TBA value			Aerobic plate count (log/g)			
	Day 1	Day 4	Day 7	Day 0	Day 2	Day 4	Day 7
HBTMST	0.104	0.273	0.546	3.60	3.90	4.75	4.91
HBTMPT	0.052	0.221	0.390	3.51	3.50	4.30	4.91
HBMMST	0.043	0.260	0.598	3.58	3.77	4.60	4.85
HBMMPT	0.078	0.195	0.598	3.35	3.60	4.27	4.81
CBTMST	0.117	0.299	0.572	3.55	3.82	4.61	4.80
CMTMPT	0.065	0.169	0.403	3.33	3.65	4.40	4.80
CBMMST	0.143	0.234	0.754	3.54	3.80	4.59	4.71
CBMMPT	0.065	0.156	0.494	3.32	3.61	4.39	4.74

TBA : Thiobarbituric acid value (in mg melonaldehyde/1000 g)

\*Legend as in Table 2



TABLE 5. SENSORY SCORES FOR *GOSHTABA* DURING STORAGE

Treatments	Appearance		Flavour		Juiciness		Texture		Overall acceptability	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
HBTMST	7.19	6.71	6.57	5.38	6.86	6.71	7.05	6.62	6.67	6.00
HBTMPT	7.24	6.81	6.86	5.48	7.09	6.81	7.14	6.76	7.19	6.14
HBMMST	6.67	6.14	6.71	5.38	6.43	5.90	5.95	6.00	6.38	5.01
HBMMPT	6.81	6.38	6.93	6.24	6.90	6.24	6.33	6.14	6.95	5.81
CBTMST	7.24	6.67	6.67	5.10	6.57	6.43	6.71	6.52	6.48	5.33
CMTMPT	7.24	6.76	7.00	5.86	6.86	6.76	7.00	6.62	6.86	6.48
CBMMST	6.24	6.24	6.67	5.29	6.05	6.95	5.90	5.86	6.29	5.24
CBMMPT	6.71	6.33	7.00	5.70	6.57	6.19	6.52	6.14	6.76	5.29

\*Legend as in Table 2  
(Mean  $\pm$  SE)

are in agreement with findings reported for beef patties<sup>20</sup> and pork sausages<sup>23</sup>.

Taste panel findings suggest that hot boned, traditionally minced and phosphate treated *Goshtaba* samples got higher scores for various sensory attributes as compared to cold boned, machine minced and salt treated samples respectively (Table 5). However, organoleptic scores for the latter group of samples were also well within moderately to highly acceptable range. In general, the acceptability of *Goshtaba* samples decreased, with detectable levels of rancid flavour on 7th day of storage.

Both pre-rigor and post-rigor mutton can be utilised for processing of *Goshtaba*. However, hot processing offers several advantages like better appearance, texture and palatability, in comparison to processing of these products from conventionally chilled (cold boned) mutton. A comparison of comminution methods revealed that the quality of traditionally processed *Goshtaba* was superior to that of machine minced product. However, use of 0.5 per cent sodium tripolyphosphate significantly improved the eating quality of *Goshtaba*. Furthermore, addition of phosphate appears essential to obtain *Goshtaba* of desired quality from cold boned mutton by employing machine mincing. Cooked *Goshtaba* can be stored in edible condition for seven days at  $4 \pm 1^\circ\text{C}$ , if properly packed in low density polyethylene bags.

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## Moisture Adsorption Isotherm, Fractionation of Bound Water and Storage Stability of Coriander Seed Powder

A. SELOT, M.B. BERA\*, S. MUKHERJEE\*\*, G.P. KESHERVANI AND Y.K. SHARMA  
Department of Food Science and Technology,  
J.N. Agricultural University, Jabalpur - 482 004, India.

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Water activity and equilibrium moisture content data of coriander seed powder were obtained at three temperatures (10, 25, and  $50 \pm 1^\circ\text{C}$ ). The data were analysed for critical moisture content, water activity for maximum storage stability using two and three parameter isotherm equations. Various fractions of bound water i.e., primary, secondary and tertiary bound water were also determined. Coriander seed powder was found to be stable for storage in the water activity range of 0.2 to 0.88, which corresponds to equilibrium moisture content 0.0053 to 0.12 kg water/kg dry solid depending on temperature range (10-50°C). The primary, secondary and tertiary bound water contents were 2.5, 10 and 54.5% (d.b), respectively.

The relationship between total moisture content and corresponding water activity ( $a_w$ ) of a food over a range of value at a constant temperature yields a moisture sorption isotherm (MSI) when graphically expressed. Packaging and storage of any food material can be predicted or manipulated based on their moisture adsorption isotherm<sup>1,3</sup>. Many investigators have reviewed isotherm equations in the literature<sup>4</sup>. In all these studies, the researchers reported equation which gave the best fit to food isotherm. However, the reports on the value of the parameters for each of the equation are rather fragmentary.

Binding energy is defined as the difference between the heat of absorption of water by the solid and the heat of condensation of water vapour at the same temperature. It can be considered as measure of the affinity of food to water<sup>5</sup>. Sockarto and Steinberg<sup>6</sup> postulated that water is bound to food in three different ways i.e., primary, secondary and tertiary and reported characteristics binding energies for each fractions of bound water, using an energy-balance approach.

India is a leading producer of spices in the world and during 1981-82 and 1982-83, the exports were 68,300 and 75,031 tonnes valued at Rs. 93.25 and 92.5 crores respectively. Major spices exported are pepper, cardamom, turmeric, ginger, chillies, cumin and coriander. Loss of volatile oils, caking, microbial spoilage and insect infestation are main deteriorative changes during storage and distribution. Thus, retention of sensory quality takes prime importance during storage<sup>7</sup>.

The present investigations were undertaken to evaluate the moisture adsorption isotherm data of coriander seed (*Coriandrum sativum* L.) powder using commonly used two

and three parameter equations at various temperature levels, to determine various fractions of bound water based on binding energies and to predict storage stability at various temperatures and water activities.

### Materials and Methods

Dried coriander seeds (*Coriandrum sativum* L.) of 11.25 per cent (d.b) moisture content were collected from the local market and ground in an electrical grinder and sieved to pass through 40 mesh sieve (B.S). The proximate composition was determined by standard AOAC procedure<sup>8</sup>.

The equilibrium moisture content (EMC) of the sample was determined using static desiccator method. About 5 g coriander seed powder was weighed directly into glass dish, covered with a lid and placed in the desiccator containing sulphuric acid solution to maintain five selected water activities (0.0142, 0.231, 0.651, 0.878 and 0.980) at three levels of temperature (10, 25 and  $50 \pm 1^\circ\text{C}$ ). The sulphuric acid solutions to maintain desired water activity at particular temperature were prepared by the method of Ruegg<sup>9</sup>. The desiccators were placed in the B.O.D. incubator, where predetermined temperature was adjusted. Sample was weighed along with lid after 48 hr until there was no appreciable change in the weight.

Bruin and Luyben<sup>10</sup> reported that there are about 77 different isotherm equations with varying degrees of fundamental validity available for the purpose of modelling the sorption of water on food materials. Described below are the few commonly used models. These are selected for the present work owing to their simplicity and validity over a wide range of water activity ( $a_w$ ).

\* To whom all correspondence should be addressed.

\*\*Department of Post-Harvest Process and Food Engineering.

Henderson equation<sup>11</sup>, applicable in  $a_w$  range of 0.5 and 0.98, is:

$$1 - a_w = e^{-KTM} \dots\dots\dots (1)$$

The Brunauer - Emmett - Teller (BET) equation<sup>12</sup>, most widely used for variety of food over the range  $0.05 < a_w < 0.45$ , is generally expressed as:

$$\frac{a_w}{(1-a_w)M} = \frac{1}{M_n C_h} + \frac{C_b-1}{M_b C_b} \cdot a_w \dots\dots\dots (2)$$

Caurie's equation<sup>13</sup>, an improvement upon BET equation, is expressed as:

$$\frac{1}{M} = \frac{1}{C_c M} \cdot \frac{(1 - a_w)^{(2C_c/M_c)}}{a_w} \dots\dots\dots (3)$$

The Guggenheim - Anderson de Boer (GAB) model<sup>14</sup>, is a three parameter equation and can be expressed as:

$$a_w/M = a_1 + b_1 a_w + c_1 a_w^2 \dots\dots\dots (4)$$

Where  $a_1 = \frac{1}{M_g C_g K_g} \dots\dots\dots (5)$

$$b_1 = \frac{1}{M_g} \cdot \frac{C_g-2}{C_g} \dots\dots\dots (6)$$

$$c_1 = \frac{K_g}{M_g} \cdot \frac{1 - C_g}{C_g} \dots\dots\dots (7)$$

Rockland<sup>15</sup> developed the concept of stability isotherm where first derivative of the moisture sorption isotherm curve ( $\Delta M/\Delta a_w$ ) when plotted against water activity gave which are related to the point where food products hold minimum moisture and important for storage. The values of  $\Delta M/\Delta a_w$  is obtained after differentiating the Eqn (4) with respect to  $a_w$ . The Eqn (4) is re-expressed as:

$$\Delta M/\Delta a_w = \frac{(a_1 + c_1 + a_w)^2}{(a_1 + b_1 a_w + c_1 a_w^2)} \dots\dots\dots (8)$$

Now by substituting the values of  $a_w$  in the Eqn (8),  $\Delta M/\Delta a_w$  could be obtained.

Rockland and Nishi<sup>16</sup> described the concept of localized isotherm using change in Gibb's free energy data. The change in Gibb's free energy is defined as:

$$\Delta \bar{G} = RT I_n a_w \dots\dots\dots (9)$$

Brunauer *et al.*<sup>12</sup> derived the following equation that related to binding energies for primary bound water (RRI).

$$C_h = K \exp (H_1 - H_L) / RT \dots\dots\dots (10)$$

If the binding energy  $H_B$  is defined as  $\Delta H_1 - \Delta H_L$  then the Eqn (10), becomes,

$$C_h = K_{exp} ( H_B / RT) \dots\dots\dots (11)$$

Thus, an Arrhenious plot of  $I_{ncb}$  against  $1/T$  gives a straight line with slope =  $\Delta H_B/R$ . The slope was multiplied by 'R' (1.987 cal/mol.K) to obtain binding energy for primary bound water.

Similarly, binding energy related to secondary (SEC) and tertiary (TER) bound water is obtained using Gibb's free energy and Clausias Clapeyron equation<sup>17</sup>, expressed as:

$$I_n a_w = \frac{\Delta \bar{H}_B}{R} = \left( \frac{1}{T} \right) + \text{Constant} \dots\dots\dots (12)$$

**Results and Discussion**

The moisture adsorption isotherm for coriander powder is shown in Fig.1; the isotherms were sigmoid in nature (Type-II). The rate of water adsorption was low below the  $a_w$  of about 0.5, but thereafter it increased rapidly showing a steep rise in moisture content. The rate of moisture adsorption increased with increase in temperature of storage. At water activity of 0.98, the coriander powder adsorbed 0.23, 0.29 and 0.38 kg water/kg dry solid at storage temperature of 10, 25 and 50°C, respectively (Fig. 1).

*Characteristics of moisture sorption isotherm (MSI):* Henderson's equation could not fit the data into a single straight line but two straight lines intersecting at values between 0.4 and 0.5 was observed at all temperatures (Fig. 2) This point of discontinuity indicated the change in

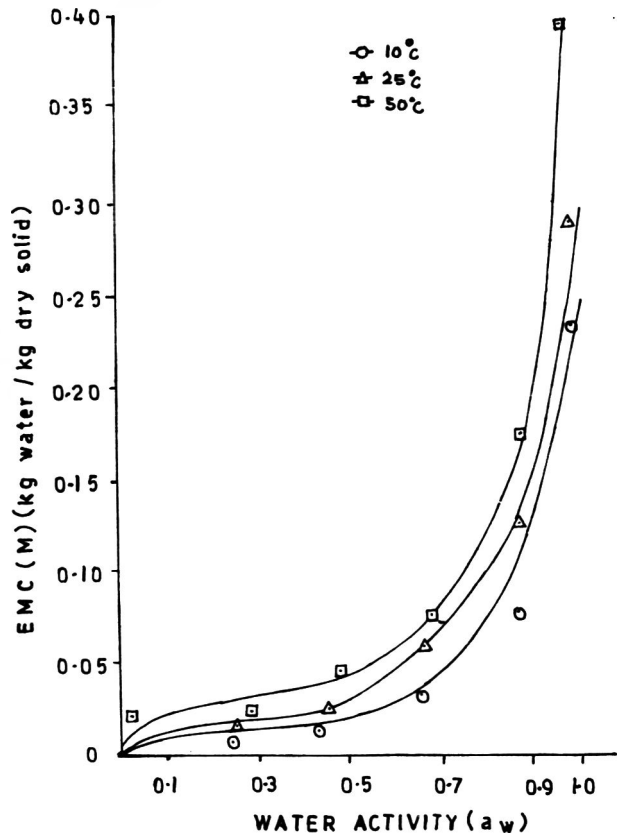


Fig.1. Moisture adsorption isotherm of coriander powder at different temperatures.

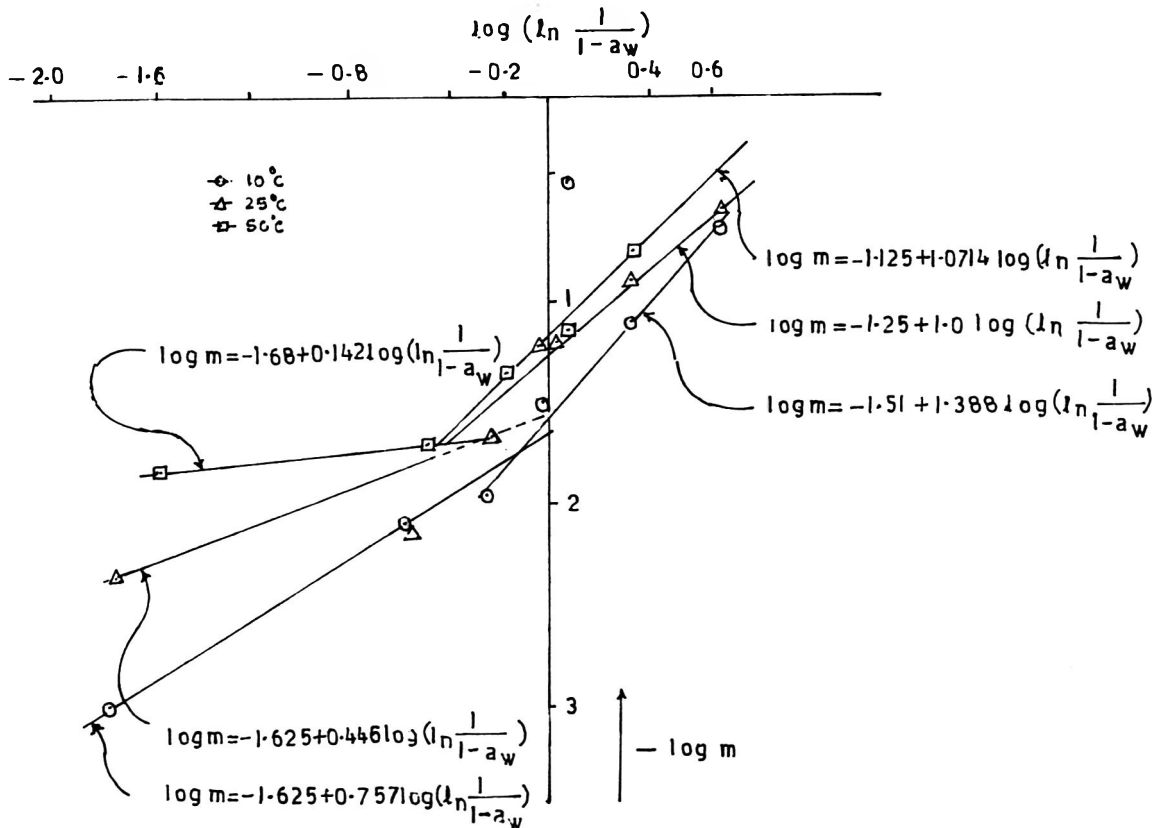


Fig.2. Graphical representation of Henderson's equation of coriander powder at different temperatures.

sorption characteristics or a change in water binding characteristics. Similar observations were reported for fababean flour and dhal<sup>18</sup>. However, Caurie's equation could fit the isotherm data into straight line at all temperatures (Fig.3). The isotherm data were used to fit the BET equation and values of different constants were calculated and presented

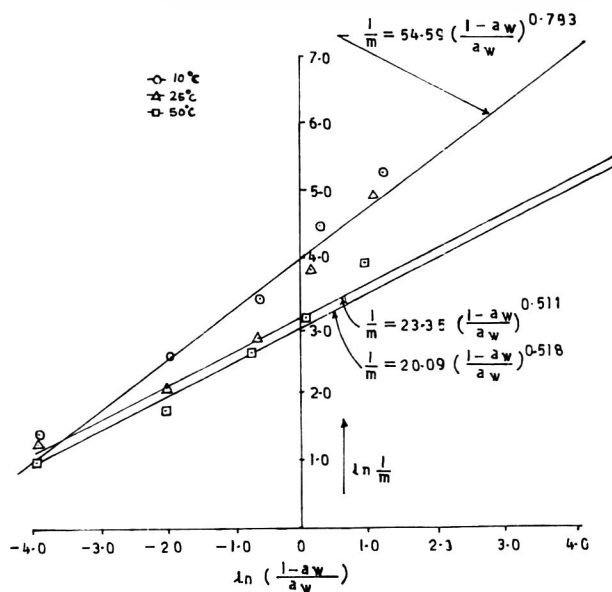


Fig.3. Graphical representation of Caurie's equation of Coriander powder at different temperatures.

in Table 1. The GAB equation has been proved for its accuracy to fit the isotherm data by several researchers<sup>19,20</sup>. Therefore, isotherm data were also analysed by GAB equation and the constants are also presented in Table 1. GAB equation gave best fit ( $r = 0.94$ ) of the isotherm data. Stability isotherms (plot of  $\Delta M / \Delta a_w$  vs.  $a_w$ ) at different temperatures are shown in Fig 4. Discrete minima observed in the curve at 0.20, 0.25 and 0.30 correspond to the moisture content ( $M_s$ ) value of 0.005, 0.018 and 0.020 kg water/kg dry solid, respectively at 10, 25 and 50°C. The minima at different water activities indicate a stage of least inclination to absorb moisture, which may be of importance with regard to the favourable moisture content for storage and keeping quality<sup>16</sup>. The occurrence of the minima may be due to the inability of absorbing further moisture by electrostatic charge interaction<sup>21</sup>. Variation of Gibb's free energy ( $\Delta \bar{G}$ ) with moisture content is shown in Fig 5. These curves showed combination of three intersecting straight line with two point of discontinuity. The water activity and corresponding equilibrium moisture content related to the point of discontinuity are presented in Table 1. The break point in the localized isotherm signified a change in the moisture binding characteristics. The slope of the curve revealed the strength of water binding force; the higher the value of the slope, the greater is the binding strength<sup>16</sup>. The break point in the localized isotherm of coriander powder showed that first point of discontinuity was observed at  $a_w$  of 0.44, 0.46 and 0.27

TABLE 1. COMPARISON OF CONSTANT OBTAINED BY BET, CAURIE AND GAB ISOTHERM

Temp' (°C)	BET Isotherm				Caurie Isotherm				GAB Isotherm			
	$M_h$	$a_w$	$C_h$	E	$M_c$	$a_w$	$C_c$	N	$M_g$	$a_w$	$C_g$	$K_g$
10	0.008	0.014	8.4	1197.2	0.021	0.48	0.852	2.51	0.017	0.43	3.59	14.99
25	0.017	0.300	31.0	2033.4	0.041	0.40	1.047	3.89	0.002	0.02	2.37	20.55
50	0.020	0.280	57.0	2523.4	0.044	0.51	1.129	3.90	0.024	0.28	14.01	29.90

correspond to the moisture content value ( $ML_1$ ) of 0.014, 0.022 and 0.020 kg water/kg dry solid, and second point of discontinuity was observed at water activity of 0.88, 0.88 and 0.69 corresponding to the moisture content ( $ML_2$ ) of 0.08,

0.12 and 0.07 kg water/kg dry solid, respectively at 10, 25 and 50°C.

*Evaluation of constants obtained by BET, Caurie, GAB, Local and Stability Isotherm:* The  $M_h$  value obtained from BET equation was less than the  $M_c$  value obtained from Caurie's equation (Table 1). However, the  $M_h$  and  $M_c$  values (Table 1 & 2) were nearly the same. It is obvious because the GAB equation has been constructed based on BET equation. In general, values of  $M_h$ ,  $M_c$  and  $M_g$  increased with increase in temperature. This may be due to increased rate of moisture adsorption by each sample with increase in temperature. The binding energy (E) based on BET constant also increased with increase in temperature (Table 1). The present studies showed that the number of monolayer ( $N = M_c/C_c$ ) from Caurie's equation at monolayer moisture content also increased with increase in temperature because of the value of  $M_c$  and  $C_c$  also increased with temperature (Table 1). It was interesting to note that monomolecular moisture content ( $M_h$ ), obtained from BET, was also nearly the same as that of moisture content ( $M_h$ ) obtained from stability isotherm. Coriander seed powder at 50°C had  $M_h = 0.02 \approx a_w = 0.28$  correlated well with the minima of the stability isotherm  $M_h = 0.02 \approx a_w = 0.25$ . Similarly, the monomolecular moisture content  $M_h = 0.02 \approx a_w = 0.28$  of coriander seed powder at 50°C were the same where minima of stability isotherm and first break point of localized isotherm  $ML_1 = 0.02 \approx a_w = 0.27$  were observed.

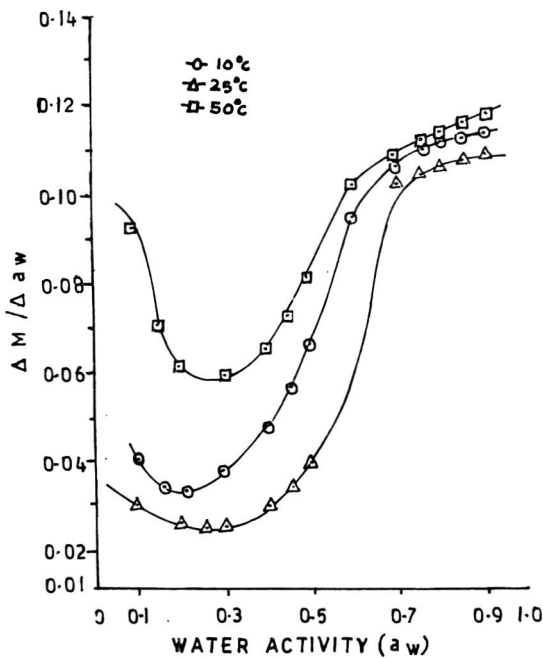


Fig.4. Stability isotherm of coriander powder at different temperatures.

From the analysis of above isotherm models, the coriander powder was most stable at water activity range 0.20 to 0.88, 0.30 to 0.88, and 0.25 to 0.69 corresponding to equilibrium moisture content range of 0.005 to 0.080, 0.018 to 0.120 and 0.020 to 0.080 kg water/kg dry solids, respectively at storage temperature of 10, 25 and 50°C.

*Quantitative analysis of various fractions of bound water based on binding energy:* Binding energy related to primary

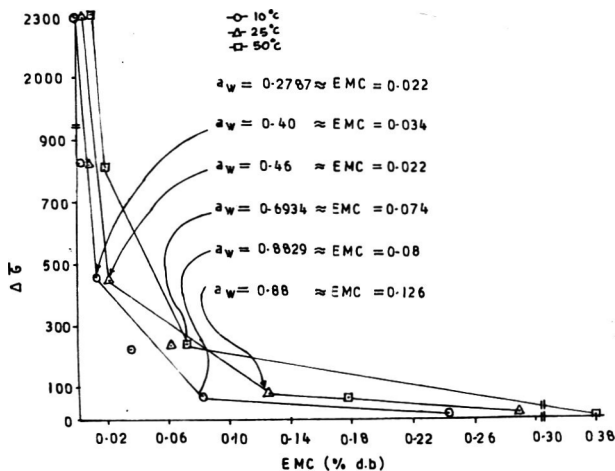


Fig.5. Localised isotherm of coriander powder at different temperatures.

TABLE 2. COMPARISON OF CONSTANT OBTAINED BY STABILITY AND LOCAL ISOTHERM

Temp (°C)	Local Isotherm			Stability Isotherm		
	$ML_1$	$a_w$	$ML_2$	$a_w$	$M$	$a_w$
10	0.014	0.44	0.08	0.88	0.0053	0.20
25	0.022	0.46	0.12	0.88	0.0175	0.30
50	0.020	0.27	0.07	0.69	0.200	0.25

bound water (PRI) of coriander seed powder was determined by following Eqn (12). The value of binding energy is  $-3784$  cal/mol  $H_2O$  and indicated that this binding is exothermic.

The average energy of water binding of coriander powder was determined using Eqn (13). From  $\text{Log}(1-a_w)$  vs  $M$  data, at a given moisture content ( $M$ ),  $a_w$  was determined for each temperature. In  $a_w$  vs  $1/T$  was then plotted, which resulted in a straight line with slope  $\Delta \bar{H}_B/R$ . The graphical representation of  $\Delta \bar{H}_B$  and its corresponding moisture content is shown in Fig.6. It was observed  $\Delta \bar{H}_B$  and corresponding moisture content showed a curvature in the graph and a break point. The break point was observed at 10.0 per cent moisture level. This is the point where secondary bound water ends, then the graph follows a slower, linear decrease in  $\Delta \bar{H}_B$ . The latter line was extrapolated to the abscissa to obtain the moisture content at  $\Delta \bar{H}_B = 0$ , which gave the transition point between bound water and free water, or this is the point where tertiary bound water ends, beyond the tertiary bound water related to the zone of free water which can be removed easily or may be utilized for any biochemical or microbial degradation process. The primary bound water was obtained by plotting the primary binding energy ( $-3784$  cal/mol  $H_2O$ ) as a horizontal line (Fig.6). The intersection of this line with the energy line for SEC gave upper limit of primary bound water.

It appears, now that by combining the BET and Clausius - Clapeyron equation concept, one can determine energy of water binding at each moisture content and quantitatively demarcate the three fractions of bound water. In the present

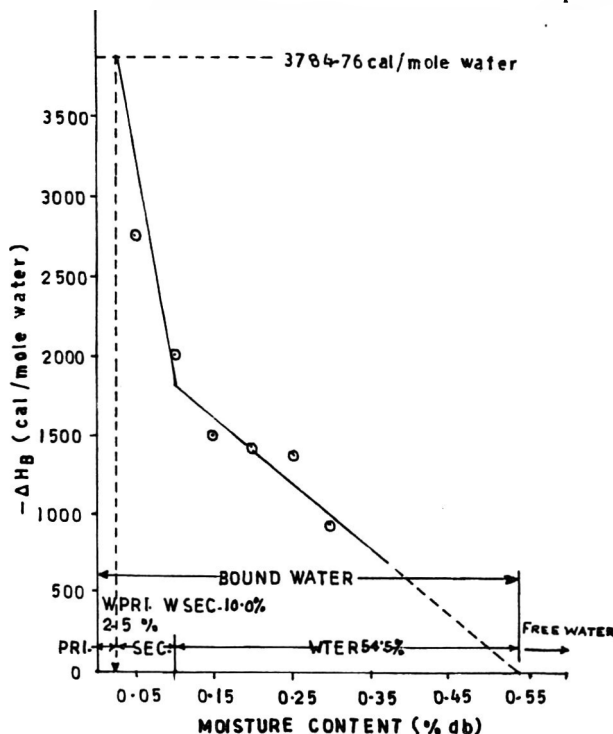


Fig.6. Average energy of moisture binding by Coriander powder at each moisture content.

study, the coriander seed powder contained primary bound water (2.5 per cent), secondary bound water (10 per cent and tertiary bound water (54.5 per cent). However, Sockarto and Steinberg<sup>13</sup> fractionated the bound water of dextrinized tapioca based on binding energy using Lamgmuir and Clausius Clapeyron concept.

The equilibrium moisture content obtained from the GAB, BET and Stability isotherm, fixed the lower limit while Curie's isotherm fixed the upper limit of equilibrium moisture content safe for the storage of this spice product. Various fractions of bound water determined would provide further information regarding selection of suitable packaging materials.

#### Nomenclature

- $a_w$  : Water activity (fraction)  
 K & N : Constant related to Henderson equation  
 T : Absolute temperature (K)  
 M : Equilibrium moisture content (% d.b)  
 $M_b$  : BET's monomolecular moisture content (% d.b)  
 $C_b$  : BET constant related to binding energy  
 E : Heat of absorption ( $RT \ln C_b$ ) (cal/g mole)  
 $C_c$  : Specific gravity of adsorbed water (g/cc)  
 $M_c$  : Curie's monomolecular moisture content (% d.b)  
 $N_c$  : Number of monolayer ( $M_c/C_c$ )  
 $M_g$  : GAB's monomolecular moisture content (% d.b)  
 $C_g$  : GAB's sorption constant related to monolayer properties  
 $K_g$  : GAB's sorption constant related to multilayer properties  
 $\Delta M / \Delta a_w$  : First derivative of the moisture adsorption isotherm curve  
 $\Delta \bar{G}$  : Gibb's free energy change (cal/g mole)  
 R : Universal gas constant (1.987 cal/mole K)  
 K : Constant  
 $H_1$  : Heat of absorption of monolayer (cal/mole)  
 $H_2$  : Heat of condensation of water vapour (cal/mole)  
 $\Delta H_R$  : Binding energy (cal/mole  $H_2O$ )  
 $M_s$  : Moisture content related to minima of stability isotherm (% d.b)  
 $ML_1$  : Moisture content related to first break point of Local isotherm (% d.b)  
 $ML_2$  : Moisture content related to second break point of Local isotherm (% d.b)

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## Comparative Responses of Coffee Species to Phosphine Fumigation

J.R. RANGASWAMY AND V.B. SASIKALA  
Infestation Control and Protectants Discipline,  
Central Food Technological Research Institute,  
Mysore - 570 013, India.

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**Robusta coffee bean on fumigation has a higher phosphine (PH<sub>3</sub>) holding capacity and holds a higher free PH<sub>3</sub> residue than Arabica. Robusta with a higher residue desorbs larger amounts of PH<sub>3</sub> than Arabica. Preliminary work with the wax of Robusta coffee suggests the differences in modes of PH<sub>3</sub> retention by coffee bean and its wax. Oxidation of phosphine in these coffee species is predominant.**

Several measures are in practice to control the coffee bean weevil, *Araecerus fasciculatus* Degeer during storage of coffee seeds. It is generally believed that Arabica coffee (*Coffea arabica*) having less than 13.5 per cent and Robusta, (*Coffea robusta*) with less than 12 per cent moisture content are not likely to be attacked by the weevil. Fumigation of coffee using aluminium phosphide (AIP, Phostoxin) at 3-4 tablets/ton is permitted in Brazil. The PH<sub>3</sub> gas evolved from these tablets was effective against all stages of *A. fasciculatus* within 24 hr under plastic covers<sup>1</sup>. Countinho *et al.*<sup>2</sup> found that in Brazil, complete control of all stages of *A. fasciculatus* was achieved by fumigating coffee with a dose of 15 Phostoxin tablets/ton for 24 hr under plastic sheetings. Puzzi *et al.*<sup>3</sup> found that fumigation of coffee beans with 0.6 g Phostoxin tablet for 4 bags afforded complete eradication of immature stages and adults of *A. fasciculatus*. Dieterich *et al.*<sup>4</sup> reported residue varying from 0.003 to 0.036 p.p.m. in green coffee beans fumigated in paper bags with 0.2 to 2 AIP tablets/ton. Muthu *et al.*<sup>5</sup> have reported a residue of  $0.99 \pm 0.12$  p.p.m. of PH<sub>3</sub> in coffee beans dosed at 6 tablets/ton on exposure of 5 days. Dosage of aluminium phosphide tablets for fumigation, levels of free PH<sub>3</sub> residue during storage and formation of bound residues of PH<sub>3</sub> in coffee beans and in the peaberry were critically examined by Rangaswamy and Sasikala<sup>6</sup>. Brazilian workers have not reported the levels of PH<sub>3</sub> residues in coffee and our experience is that coffee seeds of different species show different amounts of residue for a given dose of fumigation under identical conditions of handling and fumigation. In order to understand the reasons for differential responses by different coffee species, a study was undertaken to examine the implications of fumigating Robusta and Arabica with a dose of 3 AIP tablets/ton and the results are discussed in this paper.

### Materials and Methods

**Fumigation and aeration:** Two kilograms of Robusta and Arabica beans in six replicates were fumigated at a dose of

3 AIP tablets/ton by placing 18 mg Celphos in a paper pack underneath the coffee beans in a 3 l flask. Nearly 6 mg PH<sub>3</sub> should have been produced by 18 mg AIP formulation. The flasks were closed with gas-tight silicon greased glass stoppers. At the end of one week exposure at room temperature (28 - 31°C), the initial PH<sub>3</sub> residue<sup>7</sup>, the trend in desorption and the fall in the residue during storage from pre-aired samples<sup>6</sup> were determined. The remaining samples were aired by spreading them as a thin layer in the open for 24 hr and the PH<sub>3</sub> residue was again determined. Inorganic phosphorus in coffee seeds was determined as reported earlier<sup>6</sup>.

Wax in these coffee species was determined by soaking 20 g beans in 50 ml diethyl ether overnight with intermittent shaking. Solvent ether was removed at room temperature after filtration. The residue of wax left behind was dried to a constant weight. The residue was redissolved in CH<sub>3</sub>OH for UV spectral analysis.

### Results and Discussion

Trends in phosphine desorption from pre-aired Robusta and Arabica coffee beans during first 8 hr immediately after opening the stopper are shown in Fig. 1. At the first hr Arabica desorbs 0.0214 p.p.m. PH<sub>3</sub>, while that from Robusta it is only 0.0115 p.p.m. The desorption of PH<sub>3</sub> from Arabica during first 4 hr shows a slight variation, while that from Robusta is uniform. Over the last 3 hr in the desorption experiments, curves from both run almost parallel to each other. At any given instant of time, Robusta is desorbing a larger amount of PH<sub>3</sub> than Arabica. Absorption spectra of the chromophores from pre- and one-day aired Robusta and Arabica present very interesting features (Fig. 2.) Chromophores from all the four samples exhibit an intense band at 400 nm due to free PH<sub>3</sub> residue. The bands due to chromophore of free PH<sub>3</sub> residue from pre- and one-day aired Robusta, appear at 400 - 410 nm and those from the corresponding samples of Arabica appear at 400-420 nm. As



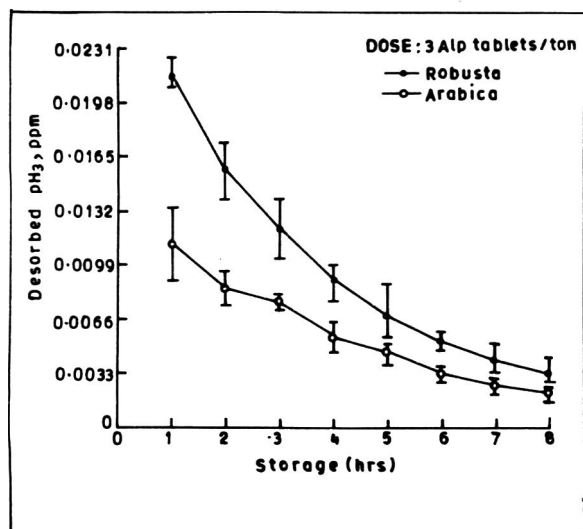


Fig. 1. Trends in desorption of  $\text{PH}_3$  from pre-aired coffee bean samples.

indicated by the intensities of these bands at 400 nm, the loss of free  $\text{PH}_3$  from Robusta is very much less during one-day airing compared to that from Arabica. During one-day airing, loss of 14.7 per cent of  $\text{PH}_3$  was observed from Robusta, while the same from Arabica is 33.8 per cent (Fig. 2), although larger amounts of free  $\text{PH}_3$  are desorbed from Robusta than that from Arabica during the initial 8 hr period. This discrepancy in amounts of  $\text{PH}_3$  desorbed from Robusta as observed by two methods suggests the presence of another source of  $\text{PH}_3$  in Robusta which is responsible for the observations presented in Fig. 1 (later discussion). Additional band at 560 nm due to  $\text{PH}_3$  residue -  $\text{AgNO}_3$  chromophore is observed only from Robusta samples (Fig. 2).

Although absorption spectra of non-fumigated Robusta and Arabica samples show two bands at 230 and 270 nm due to crop extractives, on fumigation and airing, these bands are observed only in the spectra of one-day aired Robusta samples. The reasons for the absence of these two bands in the spectra of other three samples are obscure.

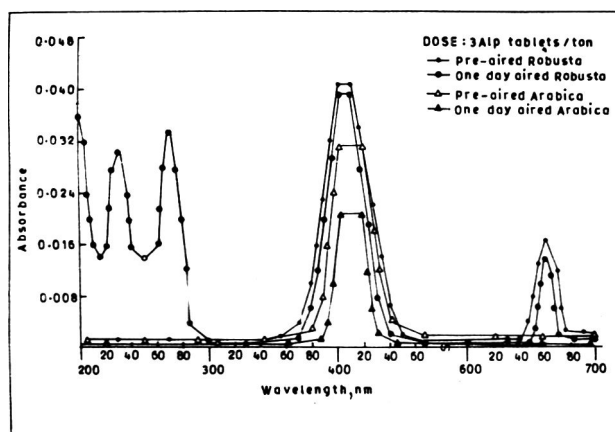


Fig. 2. Absorption spectra of  $\text{PH}_3$  residue- $\text{AgNO}_3$  chromophore from Arabica and Robusta coffee beans.

As phosphine is also retained in the lipid portion of the cashew kernel<sup>89</sup>, an attempt is made here to find out the role of the waxy layer in these two coffee species in retaining  $\text{PH}_3$ . On estimation of wax from several batches of Robusta and Arabica, it was found that wax in the former is  $0.14 \pm 0.003$  per cent while that in the latter varied from  $0.29 \pm 0.02$  to  $0.40 \pm 0.05$  per cent. No change in the weight of wax was noticed after fumigation. But the absorption spectra of the wax from non-fumigated and fumigated coffee seeds of both species showed interesting variations in their character (Table 1). Absorption spectra of 5 per cent solution in methanol, wax from control, pre-aired and one-day aired Arabica show two bands one each at 220 and 270 nm. The bands are more intense in the spectra of wax from one-day aired Arabica samples. These bands in the spectra of pre-aired samples are less intense than those in that of control, suggesting that 24 hr are needed for full development of  $\text{PH}_3$  - wax chromophore.

Although two bands are noticed in the spectra of control samples of Robusta, there is a variation in the position of the bands. The band at 270 nm shows continuous increase in its intensity from control to one-day aired. The band at 219 nm in the spectra of control slightly changes its position on fumigation and appears at 219.5 nm with an increased intensity from pre-aired samples. A new band with highest intensity appears in the spectra of one-day aired samples. It is not known whether the band at 219 nm in the spectra of control sample has changed its position and intensity on fumigation with  $\text{PH}_3$  (under investigation). The differences in the pattern and intensity of bands in the spectra of coffee beans (Fig. 2) and of wax (Table 1) from these samples suggest the differences in the modes of  $\text{PH}_3$  retention by these two portions.

Determined free and computed  $\text{PH}_3$  residues in pre- and one-day aired samples during storage are shown in Table 2. Robusta holds a higher initial free  $\text{PH}_3$  residue (0.095 p.p.m.) than Arabica (0.077 p.p.m.) and at the end of one-day airing, the free  $\text{PH}_3$  residue in these species decreased

TABLE 1. INTENSITY OF ABSORPTION BANDS FORMED DUE TO  $\text{PH}_3$ -WAX INTERACTION CHROMOPHORE IN COFFEE SEEDS

Samples	No. of bands	Absorption bands at nm				
		215.5	219.0	219.5	220.0	270.0
<b>Arabica</b>						
Control	2	—	—	—	0.221	0.146
Pre-aired	2	—	—	—	0.170	0.107
One-day aired	2	—	—	—	0.337	0.284
<b>Robusta</b>						
Control	2	—	0.378	—	—	0.410
Pre-aired	2	—	—	0.490	—	0.435
One-day aired	2	0.599	—	—	—	0.484

TABLE 2. PHOSPHINE AND PHOSPHORUS RESIDUES IN ARABICA AND ROBUSTA COFFEE BEANS

Period of airing	PH <sub>3</sub> (p.p.m.)		Inorganic P (mg/100 g)	Inorganic P inside tube, (µg)
	Determined	Computed		
<b>Arabica</b>				
<b>Pre-aired</b>				
Immediately	0.077 ± 0.027	0.121 ± 0.015	0.94 ± 0.13	—
After storage	B (29)	0 (29)	1.40 ± 0.19	2.60 ± 0.28
<b>One-day aired</b>				
Immediately	0.041 ± 0.021	0.057 ± 0.008	0.95 ± 0.12	—
After storage	B (20)	0 (20)	1.22 ± 0.10	2.12 ± 0.22
<b>Robusta</b>				
<b>Pre-aired</b>				
Immediately	0.095 ± 0.043	0.186 ± 0.017	1.22 ± 0.42	—
After storage	B (28)	*0 (28)	1.52 ± 0.13	4.81 ± 0.47
<b>One-day aired</b>				
Immediately	0.81 ± 0.046	0.084 ± 0.018	1.28 ± 0.35	—
After storage	B (31)	0 (31)	1.38 ± 0.47	5.29 ± 0.72

B = Below detectable limit of the method; Figures in parentheses indicate number of days.  
Values given as mean ± SD of 12 replicates from 3 fumigations

by 14.7 and 33.8 per cent, respectively. This is in agreement with the data presented in Fig. 2. So even one-day aired Robusta shows a higher residue (0.081 p.p.m.) than the corresponding Arabica samples (0.041 p.p.m.). Due to variation in desorption rates during storage, pre-aired samples of Robusta and Arabica show residue below detectable limit of the method at 29 days. Although free PH<sub>3</sub> residues in one-day aired samples of Robusta and Arabica reach a level of 0.002 p.p.m. in about 17 days in storage, the residue is zero in about 20 days in Arabica, while small residue (0.0007 p.p.m.) lingered on upto 30 days in Robusta due to stratification of desorbed PH<sub>3</sub>; Robusta shows zero level in about 31 days. Sullivan and Murphy<sup>10</sup> have reported green coffee beans packed in paper bags fumigated with 45 tablets and 450 tablets/11000 cft showed PH<sub>3</sub> residue of less than 0.003 and 0.036 p.p.m. respectively on airing for 48 hr.

Computed residue arising out of reversibly bound PH<sub>3</sub> is also found to be more in Robusta (0.186 p.p.m.) than in Arabica (0.121 p.p.m.), the same trend is observed in one-day aired samples of Robusta (0.084 p.p.m.) and Arabica (0.057 p.p.m.). Disappearance of computed residue during storage from Robusta and Arabica follows the same pattern as those by determinable free PH<sub>3</sub> residue. As observed in the case of cereals and their milled products<sup>11,14</sup> oxidation of PH<sub>3</sub> is predominant in coffee seeds. Increases in inorganic phosphorus over that of crop control during the storage of pre-aired and one-day aired samples of Robusta are 32.9 and 22.0 per cent while the corresponding values in Arabica are 19.7 and 7.2 per cent respectively. Since there is no increase in phosphorus contents of Robusta (1.22 to 1.28 mg/100 g) and Arabica (0.94 to 0.95 mg/100 g) during aeration for one

day, oxidation of phosphine mostly takes place during storage. Although the reported<sup>15</sup> phosphorus content in coffee is 130 to 165 mg/100 g, many samples examined by the method reported<sup>6</sup> show a mean value of 51.6 and 60.09 mg/100 g in Robusta and Arabica, respectively. Oxidation of PH<sub>3</sub> in the side tube containing detector strip<sup>16</sup> is negligible.

It is concluded that coffee species can be safely fumigated with aluminium phosphide preparations at 3 tablets/ton without having any perceptible free or bound PH<sub>3</sub> residues in the product.

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## ***In Vitro* Binding of Bile Salts with Plant Fibres**

VAISHALI AGTE AND SADHANA JOSHI

Maharashtra Association for the Cultivation of Science  
Law College Road, Pune - 411 004, India.

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***In vitro* binding of sodium cholate and sodium taurocholate with 5 and 10 m mole by 27 dietary fibres from leafy vegetables, other vegetables, pulses and cereals commonly consumed in India was studied at pH 8.0. Analysis of variance indicated that these plant fibres behave similarly within a group but differ from each other as a group ( $P < .01$ ). Leafy vegetables showed the highest per cent binding for both the bile salts followed by pulses, cereals and other vegetables. Binding response of both the bile salts showed a correlation of 0.92, 0.65 at 5 and 10 m mole concentrations. Percent binding was higher at 5 than at 10 m moles indicating that available binding sites were nearly saturated at 5 m mole concentration. Multiple regression analysis of components of fibre indicated that binding of bile acids is mainly contributed by hemicellulose component of plant fibres.**

The importance of dietary fibre came into focus for research due to the hypothesis put forward by Eastwood<sup>1</sup> and Cleave<sup>2</sup> that dietary fibre may be a protective factor in human diseases. The physiological consequences of fibre are partly predictable on the basis of its physico-chemical properties such as water holding capacity, viscosity, ion exchange capacity and binding of bile salts<sup>2</sup>. The components of dietary fibres such as cellulose, lignin and non-cellulosic polysaccharides are capable of binding bile acids and carcinogens. Adsorption of the bile acids is dependant on the composition of the fibre, the chemistry of the sterol, the pH and osmolarity of the surrounding medium<sup>3,4</sup>. Several views are put forth to explain the hypocholesteremic effect of certain foods. Some of these hypotheses suggest that the bile acids are adsorbed by the dietary fibre. Bile salts are then excreted through the faeces and they will be replenished by the metabolism of cholesterol in the liver<sup>5,6</sup>. Thus, the increase in faecal elimination of cholesterol in the form of bile acids will result in decrease in the serum cholesterol level. Plant foods are composed of substantial amounts of complex polysaccharides. Only a few studies have reported about dietary fibres from Indian foods and there is a need for a systematic investigation for their usefulness in disease and health. *In vitro* methods could form the basis of techniques that would enable screening of novel and processed fibres before undertaking time consuming, expensive and tedious studies in animals and humans<sup>7</sup>.

In the present paper, *in vitro* experiments are described to screen fibre residues from 27 commonly consumed Indian foods for their ability to bind cholate and taurocholate at 5 and 10 m moles. An attempt is also made to find association of the binding of bile salts with the composition of fibre residues.

### **Materials and Methods**

The fibrous residue of 27 food materials commonly consumed in the Indian diet was prepared by the simulation of physiological conditions<sup>8</sup>. For this, each food item was first cooked homogenized, dried and powdered. This was then defatted and treated with alpha amylase (Sigma F.C.3.2.11 Activity 90 units per mg) and pepsin (Loba 19780 1:10,000) at pH 4 using citric acid-HCl buffer. The residue was then suspended in phosphate buffer of pH 8 and treated with pancreatin (Loba 19137 Pig pancreas Activity eq to NF) and trypsin (Loba 14697 Activity 2000 units per g). The residue left was then dried and ground into a fine powder to pass through sieve of 0.5 to 1 mm size. This powder was then washed in cold water for 24 hr and then twice in 100 ml hot water (70 - 80°C). It was further treated with hot ethanol for 2 hr which resulted in the removal of pigments. The powder was then washed with 100 ml acetone and stored in a plastic bag. The purity of fibre residues was checked by the analysis of the protein and carbohydrate contents of the residues.

The binding experiment of vegetable fibre and bile salts was conducted as per the procedure of Eastwood *et al*<sup>9</sup>. The bile salt of 5 and 10 m mole concentration was prepared in phosphate buffer of pH 8 and 25 ml of this solution was then added to the test tube containing 500 mg of the sample. The tubes were then agitated for 2 hr at room temperature on a shaker. The period for bile acid binding and shaking time being so chosen that it is in keeping with the time the bile salts will be exposed to the fibre in the intestine. After shaking, the solution was centrifuged and the supernatant was estimated for bile acid concentration. Binding was expressed as mg per cent. Samples were run in triplicate and the coefficient of variation of the measurements was 1.0 per cent. The Pettenkoffer reaction was used to estimate both the bile

acids. For this purpose, independent standardisation of both bile salts was done.

For the analysis of the fibre components, the foods were dried in an oven at 40°C and powdered. The modified Van Soest<sup>10</sup> detergent system (AACC 1981) using alpha amylase pre-treatment to reduce the interference of starch was used for the analysis of Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and lignin. Hemicellulose and cellulose are both calculated by difference. Samples were analysed in duplicates and expressed as grams per 100 g fresh weight.

Analysis of variance, correlation analysis and paired t-test were also carried out.

### Results and Discussion

Table 1 shows the per cent binding of sodium cholate and sodium taurocholate. It was maximum for the class of leafy

vegetables followed by other vegetables, pulses and cereals. The per cent binding within a class was comparable.

Sharma<sup>11</sup> has reported that fenugreek leaves when given to rats resulted in significant reduction of serum cholesterol level. He has further reported the beneficial effect of some Indian pulses such as Bengal gram and rajmah. Our *in vitro* results are in agreement with these results. Further, they indicate that pulses like cowpea, green gram, lentils and red gram show comparable per cent binding with Bengal gram and rajmah, indicating that they may be of equal value. In our earlier studies<sup>12</sup>, response of 4 adult human volunteers was observed in two long term energy balance experiments. The diets in the two experiments were comparable in all the nutrients but differed in the source of fibre. When subjects were changed to a leafy vegetable and rajmah based diet (B) from cauliflower and green gram based diet (A) the faecal fat excretion was increased indicating reduction in fat digestibility

TABLE 1. BINDING (PER CENT) OF SODIUM CHOLATE AND SODIUM TAUROCHOLATE

Vegetable fibre	Sodium cholate		Sodium taurocholate	
	5 m mole	10 m mole	5 m mole	10 m mole
<b>Leafy vegetables</b>				
Radish leaves ( <i>Rhaphanus sativus</i> )	66.02	35.60	53.70	20.60
Onion leaves ( <i>Allium cepa</i> )	61.26	39.35	51.76	30.52
Spinach ( <i>Spinacia oleracea</i> )	60.68	42.92	60.95	43.09
Shepu ( <i>Peucedanum graveolens</i> )	65.58	21.94	52.64	30.80
Ambat chuka ( <i>Rumex vesicarius</i> )	57.72	40.15	51.92	34.02
Colcasia leaves ( <i>Colocasia antiquorum</i> )	37.80	14.60	49.28	37.71
Amaranth ( <i>Amaranthus gangeticus</i> )	49.20	36.14	49.64	24.94
Lettuce ( <i>Lactuca sativa</i> )	54.43	46.24	49.10	37.83
Chavli ( <i>Amaranthus viridis</i> )	58.07	37.35	51.58	41.39
Fenugreek ( <i>Trigonella foenum graecum</i> )	53.71	31.92	55.65	19.02
<b>Other vegetables</b>				
Cluster beans ( <i>Cyamopsis tetragonoloba</i> )	57.88	55.49	60.94	30.48
Capsicum ( <i>Capsicum annum</i> var. <i>grossa</i> )	16.96	6.70	24.63	9.46
French beans ( <i>Phaseolus vulgaris</i> )	23.69	5.45	33.38	13.13
Cauliflower ( <i>Brassica oleracea</i> )	29.34	27.94	37.97	15.06
Cabbage ( <i>Brassica oleracea</i> Var <i>Capitata</i> )	64.21	24.34	52.64	28.91
<b>Pulses</b>				
Bengal gram ( <i>Cicer arietinum</i> )	34.19	19.21	37.95	12.77
Moth bean ( <i>Phaseolus aconitifolius</i> )	19.38	13.71	28.09	11.27
Green gram ( <i>Phaseolus aureus</i> )	47.05	34.57	44.45	28.52
Lentil ( <i>Lens esculenta</i> )	29.29	25.29	33.77	28.12
Peas ( <i>Pisum sativum</i> )	25.42	19.37	18.11	14.94
Red gram dhal ( <i>Cajanus cajan</i> )	24.90	18.39	29.34	19.93
Rajmah ( <i>Phaseolus vulgaris</i> )	39.71	34.74	31.01	18.56
Cowpea ( <i>Vigna catjang</i> )	34.59	33.79	36.82	24.38
<b>Cereals</b>				
Wheat ( <i>Triticum aestivum</i> )	14.99	14.37	24.87	12.20
Jowar ( <i>Sorghum vulgare</i> )	17.77	4.84	29.19	14.88
Bajra ( <i>Pennisetum typhoides</i> )	27.14	23.46	27.73	12.21
Ragi ( <i>Eleusine coracana</i> )	27.46	12.11	29.87	17.88

ANOVA for % binding between different class (F = 17.66, 7.88 p < 0.01) within class (p > 0.1)

TABLE 2. BILE ACIDS BOUND PER 100 Gm FRESH FOOD MATERIAL

Vegetable fibre	Sodium cholate		Sodium taurocholate	
	5 m mole	10 m mole	5 m mole	10 m mole
<b>Leafy vegetables</b>				
Radish leaves	86.56	75.40	105.54	75.63
Onion leaves	90.40	93.99	114.20	125.98
Spinach	60.92	69.69	91.59	120.83
Shepu	87.12	47.37	104.48	115.46
Ambat chuka	32.78	37.01	44.40	53.62
Colocasia leaves	73.70	44.46	151.72	217.81
Amaranth	111.72	132.88	158.81	158.05
Lettuce	51.08	67.87	63.74	100.10
Chavli	96.28	125.40	133.66	200.49
Fenugreek	112.47	141.86	181.37	122.98
<b>Other vegetables</b>				
Cluster beans	125.02	243.01	193.99	194.37
Capsicum	14.49	11.68	31.31	23.94
French beans	17.06	7.07	35.71	37.92
Cauliflower	30.54	59.15	58.51	46.09
Cabbage	71.21	73.92	87.57	89.90
<b>Pulses</b>				
Bengal gram	369.93	426.12	611.90	408.90
Moth bean	313.52	313.52	495.56	376.66
Green gram	256.05	377.56	647.96	785.63
Lentil	237.56	411.23	732.19	1152.28
Peas	146.70	237.60	172.04	268.27
Red gram dal	141.16	210.44	449.47	576.77
Rajma	440.00	808.20	535.03	637.09
Cowpea	418.42	875.02	731.12	915.58
<b>Cereals</b>				
Wheat	160.45	311.98	417.24	386.90
Jowar	140.26	78.02	343.25	348.90
Bajra	255.80	471.30	406.48	355.67
Ragi	107.96	96.21	183.54	207.87

by 2 per cent. This may be due to the increased binding of lipids from the fibre residues of the B diet.

The cereal residues in general show 5 to 27 per cent binding. Similar results are reported by Eastwood *et al.*<sup>9</sup> when the husks of wheat, oat, barley flours were tested for *in vitro* binding with sodium cholate and sodium taurocholate and the per cent binding was found to be 20 and 39 respectively. The cereal fibres were generated in our study by simulating gastro intestinal conditions and therefore represent more natural conditions. Among other vegetables, residue from cluster bean, (guar) showed comparable binding with the leafy vegetables. Although there is no direct report on the residue of cluster beans, the beneficial effect of guar gum is well known. Our results indicate that guar gum can be substituted by guar itself.

Binding was also expressed in terms of mg of bile salt bound per 100 g fresh food material. It was observed that pulses

showed the highest binding followed by cereals, leafy vegetables and other vegetables. Leafy vegetables contain more moisture and therefore the dry fibre residue per 100 g fresh weight of food ingredients is low in leafy vegetables than pulses and cereals (Table 2). This explains the lower value of binding for leafy vegetables using this index.

The per cent binding at 5 m mole was significantly greater than at 10 m mole concentrations for both sodium cholate and taurocholate ( $t = 11.41, 6.49$  d.f. = 26  $p < .01$ ). These two concentrations are considered to represent the normal range of bile acid pool in the body. Results indicate that the available binding sites get almost saturated at 5 m mole with no further increase in binding (Table 1).

TABLE 3. COMPOSITION (G PER 100 G OF FRESH WEIGHT) OF NEUTRAL DETERGENT FIBRE (NDF) ACID DETERGENT FIBRE (ADF), LIGNIN, CELLULOSE AND HEMICELLULOSE IN INDIAN FOODS

Food material	NDF	ADF	Lignin	Cellulose	Hemi-cellulose
<b>Cereals</b>					
Jowar	9.37	7.37	2.64	4.73	2.00
Rice	4.69	2.88	1.38	1.50	1.81
Wheat	6.94	5.21	1.14	4.07	1.73
Ragi	9.08	6.08	4.17	1.91	3.00
<b>Pulses</b>					
Bengal gram	23.01	14.12	2.16	11.96	8.89
Cow pea	13.24	8.19	0.87	7.32	5.05
Green gram	11.40	4.42	1.74	2.68	6.98
Peas	14.11	8.59	0.92	7.67	5.52
Rajmah	23.96	12.72	2.38	10.34	11.24
Red gram	9.08	5.64	1.42	4.22	3.44
Lentil	11.75	6.43	1.75	4.68	5.32
Moth bean	14.48	8.24	1.62	6.62	6.24
<b>Leafy vegetables</b>					
Amaranth	2.90	1.57	0.11	1.46	1.33
Ambat chuka	1.04	0.33	0.12	0.21	0.71
Cabbage	1.80	1.02	0.17	0.85	0.78
Colocasia leaves	4.80	2.81	0.36	2.45	1.99
Fenugreek leaves	2.53	1.73	0.30	1.43	0.80
Lettuce	1.55	0.65	0.23	0.42	0.90
Raddish leaves	2.04	1.36	0.19	1.17	0.68
Onion leaves	2.66	2.05	0.22	1.83	0.61
Spinach	1.84	1.45	0.17	0.68	0.39
Shepu	2.60	1.72	0.23	1.49	0.93
Chowli	2.10	1.24	0.13	1.11	1.07
<b>Other vegetables</b>					
C. flower	1.88	1.42	0.09	1.33	0.46
Cluster beans	3.47	0.44	0.06	0.38	3.03
French beans	2.06	1.11	0.14	0.97	0.95
Capsicum	1.38	1.00	0.11	0.89	0.38

When the responses of two bile salts were compared at each concentration, it was observed that both the bile salts behave similarly. This is further supported by significant correlation between the per cent binding by cholate and taurocholate at each of the 5 m mole and 10 m mole concentrations ( $r = 0.92, 0.65$ ) indicating strong association in this response of binding to fibre residues. This suggests that the common structural groups in both the compounds are the main active sites for binding.

To further understand the binding of bile salts with fibre, the components of fibre residues were tested as independent factors for their ability to bind each of the bile salts using multiple regression analysis. This indicated that the binding with hemicellulose was most effective, followed by cellulose while lignin did not bind bile salts appreciably (Table 3).

In conclusion, it is felt that fibre residues from pulses and leafy vegetables are of promise as hypocholesteremic agents. The binding of tauroconjugated bile acids also indicates their usefulness in reducing the risk of colon cancer. The binding of bile acids is mainly contributed by hemicellulose component of dietary fibre. The similarity in the response of cholate and taurocholate indicates that the binding sites are situated in the common structural groups of the two bile salts.

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## Nutrient Intake by Punjabi Women with Special Reference to Iron Availability

MALKIT NAGI AND SUKHWANT K. MANN  
Department of Foods and Nutrition  
Punjab Agricultural University  
Ludhiana, India.

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Ninety young women in the age group of 16-20 years were classified into three groups of thirty each on the basis of their haemoglobin and food habits i.e. anaemic lacto-vegetarian (ALV) anaemic non-vegetarian (ANV) and non-anaemic (NA) groups. The average haemoglobin (Hb) levels of the subjects of ALV, ANV and NA groups were  $10.4 \pm 0.1$ ,  $10.7 \pm 0.13$  and  $12.5 \pm 0.10$  g/dl respectively. The ionizable iron accounted for 5.97 to 7.06% of the total iron in the diets consumed by all the subjects in both the seasons and was comparatively higher in the diets of ANV group. The mean dietary iron was inadequate while protein, calcium and ascorbic acid were adequate in all the groups and were significantly higher ( $P < 0.05$ ) only in the NA group. However, the intake of tannins during winter was significantly higher ( $P < 0.05$ ), than in summer in all the groups; while the intake of dietary fibre, phosphates, phytates and oxalates was almost the same in all the groups.

The nutrition of young women residing in University hostels depends largely on the quantity and quality of food served there. Nutrition surveys revealed that the diets of young women are mainly based on cereals and deficient in protective foods. The low intake of protective foods results in nutritional disorders. Iron deficiency anaemia is the first and foremost problem of women in the reproductive age group. The literature report indicates that 50 to 70 per cent women suffer from nutritional anaemia due to iron deficiency<sup>1</sup>. Iron deficiency appears to be caused mainly by the low dietary intake and poor availability of iron from the cereal based vegetarian diets coupled with additional amounts required by women. The iron absorption rate from such diets is less than 5 per cent<sup>2</sup>. The absorption of iron is inhibited by dietary phosphates, phytates, fibre, tannins and oxalates<sup>3</sup>, of these tannins appear to be an important factor inhibiting iron absorption from the habitual Indian diets<sup>2</sup>. On the contrary, protein and ascorbic acid promote iron absorption. The present investigation was undertaken to determine the adequacy of diets and factors affecting iron availability.

### Materials and Methods

Ninety young women in the age group of 16 to 20 years from the women's hostel of Punjab Agricultural University, Ludhiana were selected for the study. The subjects were classified into three groups of 30 each on the basis of their haemoglobin levels and food habits. The groups were anaemic lacto-vegetarian (ALV), anaemic non-vegetarian (ANV) and non-anaemic (NA) which consisted of both vegetarian and non-vegetarian subjects. The average haemoglobin levels of

the subjects of ALV, ANV and NA groups were  $10.4 \pm 0.11$ ,  $10.7 \pm 0.13$  and  $12.5 \pm 0.10$  g/dl respectively. Haemoglobin was determined by the cyanomethemoglobin method<sup>4</sup>.

The food consumption by the subjects was studied for 3 consecutive days using weighment method of cooked food consumed at each meal both during summer and winter. The meals consumed by the subjects were analysed for calculating the nutrient intake of the subjects. The food samples were stored in decor taminated airtight plastic containers in the deep freeze during the study period. Subsequently, these were homogenized in a blender with stainless steel blades using glass distilled water and the homogenates were dried in aluminium trays in a hot air drier at 70°C. The dried samples were ground in a stone pestle mortar to a fine powder and sifted through a 32 mesh sieve and stored in polyethylene containers for analysis.

Ionizable iron in the meals was determined by the method of Rao and Pabavathi<sup>5</sup> while total iron and calcium were analysed using atomic absorption spectrophotometer (GBC-902) after digestion with the diacid mixture<sup>6</sup>. Phosphorous was determined by the molybdc blue micro method<sup>7</sup> and phytic phosphorus was analysed by the modified method of Tara *et al.*<sup>8</sup>. Neutral Detergent Fibre (NDF) was determined by the method of Goering and Vansoest<sup>9</sup>. Tannins as well as protein analysis of the diets were carried out by AOAC methods<sup>10</sup>. Oxalate was determined by the modified method of Abeza *et al.*<sup>11</sup> while ascorbic acid content of the diets was estimated by Caraway method<sup>12</sup>. The results were statistically analysed<sup>13</sup>.



## Results and Discussion

**Food intake:** The difference in the cereal consumption between the three groups and in the two seasons were statistically non-significant ( $P < 0.05$ ). The mean daily intake of cereals (Table 1) by all the groups was inadequate when compared to the recommended dietary intake of ICMR<sup>14</sup> while the average daily consumption of pulses (Table 1) was adequate in all the groups as also reported by Menon and Rau<sup>15</sup>. The average consumption of pulses in winter by NA group was significantly higher ( $P < 0.05$ ) than ANV group. The mean daily intake of green leafy vegetables (Table 1) was grossly inadequate in all the groups. However, the consumption of roots and tubers was two to three times more than the recommendations of ICMR<sup>14</sup> but no significant differences were found amongst the three groups. The high consumption of roots and tubers in the hostel diets was due to the inclusion of potatoes in almost all the legume-vegetable preparations. The mean intake of other vegetables and fruits was higher than the recommendations in all the groups, the consumption was more in winter than in summer due to abundant availability at reasonable cost. The seasonal differences in the average intake was significant ( $P < 0.05$ ) in the ALV and NA groups. The intake of meat, fish and egg of ANV group was significantly higher ( $P < 0.05$ ) than NA group in the summer season and the intake was adequate as compared with the recommended dietary intakes of ICMR<sup>14</sup>. Similar finding has been reported by Sadasivam *et al.*<sup>16</sup> in the diets of South Indian College male hostellers. The mean daily intake (Table 1) of milk and milk products in all the groups was more than double the recommendations of ICMR for Indians and was significantly higher ( $P < 0.05$ ) of NA group in winter.

The overall range of sugar consumption in the present study was 21 to 29 g/capita/day (Table 1) which is marginally higher than the recommended daily intake. The seasonal differences

were significant ( $P < 0.05$ ) only in summer in the ANV and NA groups. The mean intake of fats and oils by all the groups both in summer and winter was more than adequate as compared to the recommended intake of 25 g. The differences in the consumption were significantly lower ( $P < 0.05$ ) of ALV group compared with ANV group in summer.

**Total and ionizable iron:** The mean daily iron intake (Table 2) of all the groups was much below the recommended daily intake of 32 mg for Indian women<sup>14</sup>. The low iron intake was attributed to insufficient consumption of cereals and green leafy vegetables. Fletcher *et al.*<sup>17</sup> also reported low iron content (10.6 mg) in the diets of women which is much lower than reported in the present study. The ionizable iron at pH 1.35 accounted for 27 to 29 per cent of the total iron in the diets consumed by the subjects of all the groups in both the seasons (Table 3). The reported values of 25.6 and 26.3 per cent in rice and wheat-based South Indian diets<sup>5</sup>, are very close to the results obtained in the present study. The mean dietary ionizable iron at pH 7.5 in the NA group was significantly ( $P < 0.05$ ) higher as compared to the ANV group in the winter. The ionizable iron at pH 7.5 accounted for 5.97 in 7.06 per cent of the total iron in the diets consumed by all the subjects in both the seasons (Table 3). Rao and Prabhavathi<sup>5</sup> reported 4.3 to 5.9 per cent ionizable iron in cereal-based vegetarian diets which is slightly lower in comparison to the ionizable iron percentage obtained in the present investigation. The main reason could be the higher intake of pulses and milk which are rich in ionizable iron as reported by Shah and Seshadri<sup>18</sup>.

**Nutrients facilitating iron absorption:** Protein was more in the NA group as compared to ALV and ANV groups in the winter, the differences being statistically significant ( $P < 0.05$ ). The protein intake was adequate in NA group and almost adequate in case of ALV and ANV groups when compared to recommendations of 45 g<sup>14</sup>. The average intake

TABLE 1. MEAN INTAKE OF VARIOUS FOODS (G/DAY) BY THE SUBJECTS DURING SUMMER AND WINTER

Foods	Anaemic group									RDI
	Lacto-vegetarian			Non-vegetarian			Non-anaemic group			
	Summer	Winter	Mean	Summer	Winter	Mean	Summer	Winter	Mean	
Cereals	158 ± 5.7	164 ± 5.1	161 ± 4.3	168 ± 4.6	159 ± 5.8	163 ± 4.2	176 ± 4.5	174 ± 4.5	175 ± 4.1	440
Pulses	64 ± 3.6	48 ± 2.6	56 ± 2.5	60 ± 3.5	42 ± 2.2	51 ± 2.2	60 ± 3.3	54 ± 1.9	57 ± 2.1	45
Leafy veg.	—	13 ± 0.4	6 ± 0.2	—	14 ± 0.4	7 ± 0.2	—	12 ± 0.4	6 ± 0.2	100
Roots & tubers	139 ± 0.4	151 ± 11.2	145 ± 5.9	144 ± 3.7	128 ± 4.6	136 ± 3.3	138 ± 1.1	142 ± 5.0	140 ± 3.0	50
Other veg.	61 ± 4.8	86 ± 7.3	73 ± 4.7	50 ± 4.7	72 ± 8.3	61 ± 5.2	56 ± 7.8	114 ± 5.9	85 ± 5.4	40
Meat, fish & egg	—	—	—	47 ± 4.8*	40 ± 4.0	43 ± 3.0	30 ± 3.5*	46 ± 4.6	38 ± 3.5	30
Milk & milk products	352 ± 8.2	354 ± 12.5	353 ± 7.9	350 ± 13.1	357 ± 8.3	354 ± 7.1	333 ± 6.7	376 ± 8.2*	355 ± 6.3	150
Sugar and jaggery	25 ± 0.9	27 ± 1.2	26 ± 0.8	21 ± 1.3*	28 ± 1.1	24 ± 0.7	23 ± 1.0*	27 ± 0.8	25 ± 0.8	20
Fats and oils	39 ± 1.2*	39 ± 1.5	39 ± 1.1	45 ± 1.5	40 ± 1.5	42 ± 0.8	43 ± 1.1	42 ± 0.9	42 ± 0.8	25

RDI = Recommended dietary intakes

30g of meat or fish or egg to replace 50% RDI of pulses for non-vegetarian.

The values are mean ± SE of three replications

The values are averages of 3 day's consumption of 30 subjects in each case. \* $P < 0.05$

TABLE 2. SEASONAL DAILY NUTRIENT INTAKES BY THE SUBJECTS

Nutrients	Anaemic group						Non-anaemic group		
	Lacto-vegetarian			Non-vegetarian			Summer	Winter	Mean
	Summer	Winter	Mean	Summer	Winter	Mean			
Protein (g)	42 ± 1.4	44 ± 1.2*	43 ± 1.1	45 ± 1.3	42 ± 1.2*	44 ± 0.9	44 ± 1.1	47 ± 0.9*	45 ± 0.7
NDF (g)	20 ± 0.9*	17 ± 0.6*	18 ± 0.7	22 ± 0.9	15 ± 0.4*	18 ± 0.5	22 ± 0.7*	17 ± 0.5*	20 ± 0.5
Calcium (mg)	605 ± 22.3*	723 ± 18.9*	664 ± 17.5	633 ± 22.8*	669 ± 18.6*	651 ± 13.3	617 ± 12.0*	741 ± 8.9*	679 ± 7.6
Total iron (mg)	13 ± 0.6	15 ± 0.7	14 ± 0.5	13 ± 0.5	14 ± 0.5	13 ± 0.4	13 ± 0.3	6 ± 0.4	15 ± 0.3
Phosphorus (mg)	815 ± 24.1	913 ± 31.4	864 ± 23.7	873 ± 24.1	892 ± 28.3	882 ± 20.0	870 ± 17.5	869 ± 21.4	869 ± 14.8
Phytin P (mg)	237 ± 9.1	237 ± 9.1	237 ± 7.5	263 ± 9.7	215 ± 7.2*	239 ± 6.1	254 ± 7.1	235 ± 8.6	244 ± 6.4
Tannins (mg)	367 ± 22.1*	596 ± 38.8	481 ± 23.9	302 ± 27.2	557 ± 36.4	430 ± 24.2*	324 ± 17.7	567 ± 20.1	445 ± 16.4*
Oxalate (mg)	6.6 ± 0.2	5.6 ± 0.2	6.1 ± 0.2	6.1 ± 0.2	5.7 ± 0.3	5.9 ± 0.2	6.4 ± 0.2	5.7 ± 0.1	6.1 ± 0.1
Ascorbic acid (mg)	20 ± 1.1	19 ± 1.1	20 ± 0.9	20 ± 1.0	21 ± 1.6	21 ± 1.0	20 ± 0.9*	27 ± 1.4*	23 ± 0.9

NDF: Neutral detergent fibre

The values are mean ± SE of 3 replications

The values are averages of 30 subjects in each case

\* P < 0.05

TABLE 3. IONIZABLE IRON IN THE SEASONAL DAILY DIETS OF THE SUBJECTS

Iron fraction	Anaemic group						Non-anaemic group		
	Lacto-vegetarian			Non-vegetarian			Summer	Winter	Mean
	Summer	Winter	Mean	Summer	Winter	Mean			
<b>At pH 1.35</b>									
Ionizable iron (mg)	3.60 ± 0.18	4.12 ± 0.18	3.86 ± 0.18	3.68 ± 0.16	3.76 ± 0.14	3.72 ± 0.15	3.59 ± 0.13	4.27 ± 0.09	3.93 ± 0.11
Ionizable iron % of total iron	28.00 ± 0.76	27.00 ± 0.51	28.00 ± 0.48	20.00 ± 0.81	28.00 ± 0.78	29.00 ± 0.63	28.00 ± 0.61	27.00 ± 0.38	27.00 ± 0.38
<b>At pH 7.5</b>									
Ionizable iron (mg)	0.83 ± 0.04	0.90 ± 0.03	0.87 ± 0.03	0.89 ± 0.03	0.87 ± 0.02	0.88 ± 0.02	0.81 ± 0.02	1.01 ± 0.03	0.91 ± 0.02
Ionizable iron % of total iron	6.44 ± 0.28	5.97 ± 0.14	6.21 ± 0.12	7.06 ± 0.19	6.60 ± 0.17	6.83 ± 0.14	6.33 ± 0.14	6.29 ± 0.15	6.31 ± 0.10

The values are mean ± SE of 3 replications

The values are averages of 30 subjects in each case

and almost adequate in case of ALV and ANV groups when compared to recommendation of 45 g<sup>14</sup>. The average intake of calcium was more in all the groups during the winter season (Table 2) because of increased intake of vegetables, fruits and milk products and was significantly more (P < 0.05) in winter among the subjects of ALV and NA groups than summer. However, the corresponding difference was nonsignificant among the subjects of the ANV group. The mean daily intake of calcium in all the groups exceeded the recommended allowances of 0.4 to 0.5 g<sup>14</sup>.

The daily mean intake of ascorbic acid was same (20 mg) in all the groups for the summer season (Table 2). The intake of ascorbic acid was significantly (P < 0.05) higher in the NA group as compared to other two groups in the winter. The corresponding difference was also significant in the two seasons in the NA group. The ICMR recommendation of 40 mg is based on the assumption that about half of the

ascorbic acid is lost during storage and cooking of vegetables. Accordingly, 20 mg may be considered adequate to meet the daily needs. The subjects of the NA group consumed citrus fruits more frequently during winter season as compared to ALV and ANV group which attributed to slightly better status of dietary ascorbic acid and hence iron in case of NA subjects due to enhancement of the non-heme (vegetable source) iron availability by ascorbic acid.

*Nutrients inhibiting iron absorption:* The differences in the mean intake of phosphorus were non-significant amongst the groups and between seasons. While in case of phytin phosphorus, the intake was significantly higher (P < 0.05) in winter than in summer for the ANV group only. However, no such difference was observed in the ALV and NA groups. The phytin phosphorus as per cent of total phosphorus ranged from 22.68 to 29.48 per cent with the mean values for ALV, ANV and NA groups being 25.75, 26.81 and 28.12 per cent

respectively which are not much different. It is a well known fact that phosphate especially phytates inhibit dietary iron absorption<sup>3</sup>.

The intake of neutral detergent fibre (NDF) was significantly more ( $P < 0.05$ ) in the summer in comparison to winter for all the groups (Table 2). According to the available reports, excess of dietary fibre inhibits the digestion and absorption of essential nutrients, at the same time sufficient quantity of the dietary fibre<sup>14</sup> (40 g) is required for the normal functioning of the gastro-intestinal tract.

The differences in the mean intake of tannins in the ALV, ANV and NA groups were significantly higher ( $P < 0.05$ ) during winter than summer. Tea was the main source of tannins among the subjects. The diets consumed in different parts of India have been reported to contain tannin contents ranging from 1.5 to 2.5 g/day<sup>2</sup> and is said to be major factor inhibiting iron absorption. Comparatively low tannin content of the diets in the present investigation could be due to total abstinence from coffee consumption by the subjects under study. The intake of dietary oxalates during summer and winter in the ALV, ANV and NA groups ranged from 5.6 to 6.6 mg/day. Results of some recent studies have confirmed the previous belief that oxalates may render dietary iron unavailable for absorption<sup>3,18</sup>.

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RESEARCH NOTES

DEVELOPMENT OF WET DEHULLER FOR  
BLANCHED SOYBEAN

R.T. PATIL, JASWANT SINGH, AND P.C. BARGALE

Soybean Processing and Utilization Project  
Central Institute of Agricultural Engineering,  
Nabi Bagh, Berasia Road, Bhopal - 462 018, India

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To remove hulls from blanched whole soybean, an unit based on the principle of rubbing and floatation was designed and fabricated. It is a batch type unit where hulls are removed from cotyledons by friction between two surfaces of wiremesh in the presence of water. The hull separation is achieved by difference in weight of hulls and cotyledons. The dehuller was tested by dehulling blanched soybean in the batch capacities of 1,2,4,5 and 8 kg. The rotor speed was between 72 and 96 r.p.m. It was found that hull separation efficiency increased with increase in capacity upto 4 kg/batch and thereafter it showed decreasing trend. Dehulling was found almost complete with about 50 % separation efficiency.

Most of the low cost processes developed to convert soybean into acceptable food products are wet type. Soybean is required to be blanched to remove the anti-nutritional factors<sup>1</sup>. The operation of hull removal can be done in both wet as well as dry condition. However, dry hull removal has been found to be easier as slight rubbing or shearing action between two plates can separate the hulls with splitting of soygrain. The equipment like Butt Mill<sup>2</sup>, roller concave dehuller<sup>3</sup> and concentric cylindrical dehuller<sup>4</sup> have been developed for this purpose. The dehulling percentages in these equipments range between 77 and 96 while 2 to 5 is the broken percentage. The splits are not 100 per cent whole but they are pieces bigger than 3/4 of split. This mechanical injury to the grain results in setting up of beany flavour during soaking in cold water. The beany flavour is due to lipoxy-genase oxidation in injured grain in moist atmosphere and therefore, pouring in of whole soybean in boiling water and removal of hulls thereafter has been suggested<sup>5</sup>. This process may also help in reducing the leaching losses during blanching due to coating of hull. However, very little effort has so far been made to develop a low cost machine to separate hulls after blanching as manual operation is tedious and time consuming.

The separation of hulls by floatation is also a tedious operation. So wet dehuller has two operations to be performed: i) the removal of hull from grain, and ii) separation of hulls from water. A batch type hull separator

having 100 kg/hr capacity has been reported by Anap *et al.*<sup>6</sup> It is mainly a hull separating unit where hull is removed on screen and water is circulated again. A composite unit was therefore developed and tested and the results obtained have been presented in this communication.

*Description and operation of the developed unit:* The dehuller (Fig.1) is a batch type unit which works on the principle of removal of hulls from blanched soybean by friction between two surfaces of wiremesh in the presence of water. The hull separation is achieved by difference in specific gravity of hulls and cotyledons. The equipment consists of a cylindrical container of diameter 400 mm and height of 600 mm. It is made of a MS sheet of 20 gauge. The upper 200 mm of this cylinder is relatively larger in diameter (445 mm  $\phi$ ). In the increased diameter, 4 openings each of 200 mm length and 30 mm width are provided. This is to facilitate the removal of separated hulls from the whole soybean. Each opening is provided with perforated small containers which retain the hulls and remove the water. The bottom of the cylinder and side upto 100 mm are covered with GI wiremesh of size 64. The central shaft is also provided with similar wiremesh covered disc raised from sides. The blanched soybean gets rubbed between these two wiremesh surfaces. As the central rotor is rotated in water, due to centrifugal force the lighter hulls floating on top surface of water reach to these openings and hulls are collected in these small containers. The Central rotor is manually operated and the power is transmitted at right angle through a bevel gear. This gear has 16 numbers

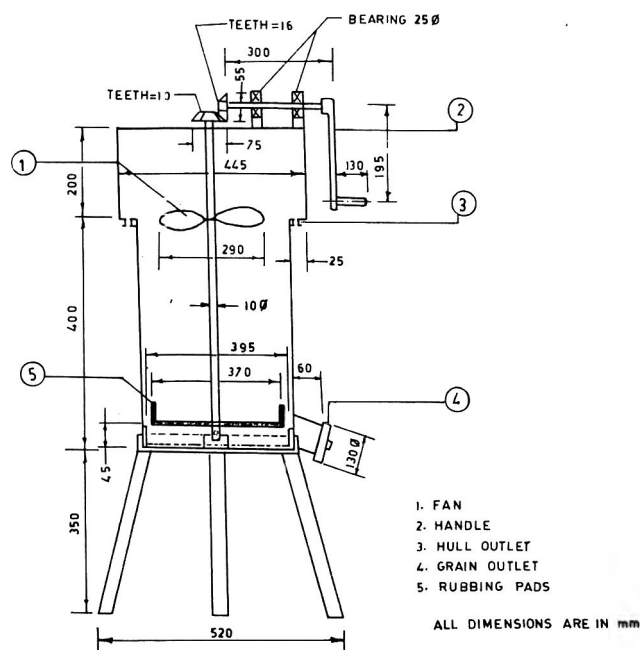


Fig. 1. Wet dehuller for blanched soybean

of teeth and rotates the central shaft of the rotor through suitable gear (with 10 teeth), at right angle. The other end of the central shaft is connected to a circular disc of diameter 395 mm fully covered with a wire mesh of 64 size. The bottom of the cylindrical container is also covered with the similar wiremesh. Thus, the blanched whole soybean when poured in water is rubbed and sheared between these two wiremeshed surface and shear is generated through rotating the upper surface and keeping the bottom layer of wiremesh as stationary. A frequent recirculating movement of whole soybean between these two layers makes the removal of hulls possible. The clearance between these two layers was made adjustable through four grooves and a bolt arrangement in lowermost portion of central rod. The four clearances which could be achieved were 35, 45, 55 and 65 mm. The clearance of 45 mm was fixed based on preliminary experimentation with the variety of soybean (JS-72-44) used for this study. An opening is provided (125 mm diameter) with a threaded lid just above the bottom of the container for removal of water and soysplits after the operation. A lever arm is provided for easier opening of the lid. The cylinder is fixed on a tripod for stability during the operation. The height of the unit is so kept that it is comfortable to operate for an operator with average height. The flat iron plate which supports the central shaft has been fixed through bolts at two ends and thus can be removed if needed for cleaning purpose. The wet dehuller was tested for dehulling blanched soybean in the batch capacities of 1,2,4,5 and 8 kg.

**Performance:** Soybean (variety 'JS-72-44') having an average size of 5.85 mm was used. The hull percentage for the samples used to test the dehuller was found to be 8.53 per cent. The speed of the rotation of handle at ease of manual operation during the experiments varied between 45 and 60 r.p.m. and corresponding speed of rotor ranged between 72 and 96 r.p.m. The hulls got removed due to rubbing action and came up floating in the water and were collected for a period upto 10 min at 2 min interval. The batch capacities were 1,2,4,5 and 8 kg and each experiment was replicated thrice. The data on cumulative hull removal with time for different batch capacities are shown in Fig. 3. The cumulative hull removal showed increasing trend with collection time. It is obvious because with longer residence time the hull removal also increased. However, the preliminary investigations had indicated that the hull recovery increased with time only upto 10 min of operation, beyond which the time input was much more than corresponding gain in the recovery of hulls. The final observations were, therefore, limited upto 10 min only. Another interesting observation on this Figure was that rate of recovery of hull was better at 4 kg per batch capacity. This was due to the fact that effective separation of hulls could be possible. Below this level, due to lower quantity of material between two plates, the friction of beans was not effective. Similarly at larger batch capacities, the stratification of cotyledons and

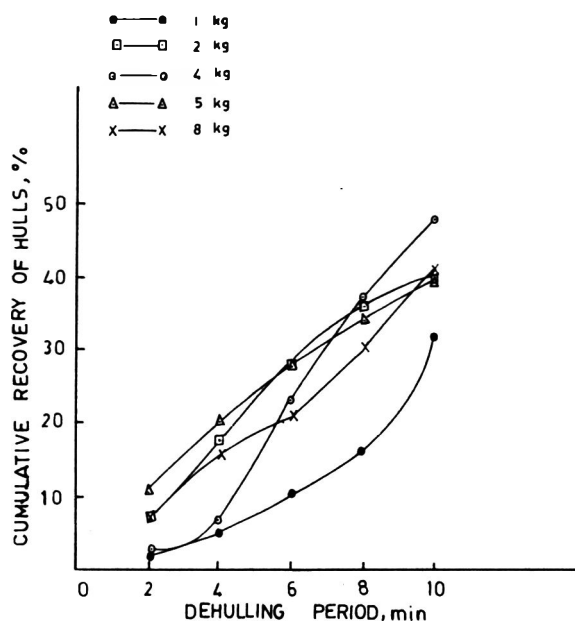


Fig. 2. Cumulative hull recovery corresponding to time of operation in soybean wet dehuller

hulls was not that effective, and hulls sunk in the water with split cotyledons.

Similar trend was seen when the hull recovery with corresponding batch capacity was plotted (Fig. 3). The hull removed increased from 27 to 49 per cent when the capacity was increased from 1 kg/batch to 4 kg/batch. However, further decrease at higher batch capacities may be due to the reasons explained earlier. Both the Figures indicated that optimum batch capacity was 4 kg/batch, though the operation was not perfect as desired. This was found mainly due to difficulty

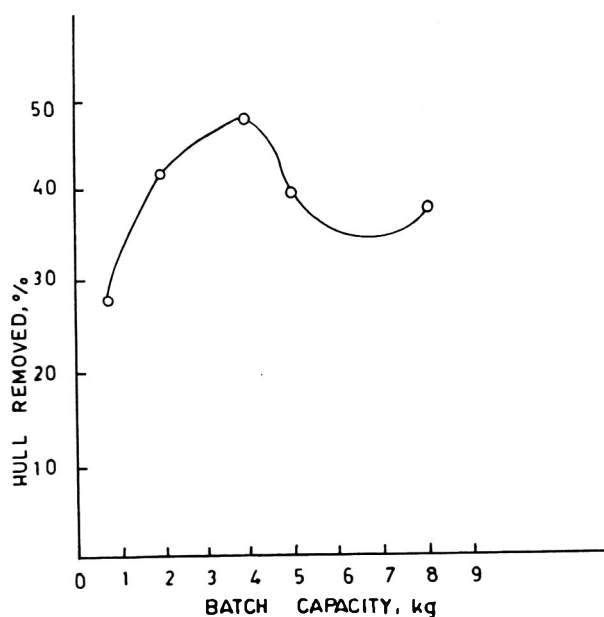


Fig. 3. Hull recovery corresponding to batch capacity in wet dehuller.

experienced in separation of hulls, though removal of hull due to friction between the wiremesh surfaces was satisfactory. The hulls get separated from cotyledons due to whirling action of the plate and start floating, which forms a strata; but when the rotation was stopped, the hull got stuck and sunk with cotyledons, when water gets filled into spherical hull it also has tendency to sink. The performance of unit indicated that though the design was feasible for intended operation, addition of a system to remove the hull water mixture and their separation outside will be essential, so that the water with hulls be removed without altering the required quantity of water for dehulling which could be recirculated after screening out the hulls. The main advantage of this unit is that due to blanching of whole soybean without cracking, the beany flavour problem could be completely eliminated. The leaching loss of dietary protein also could be avoided with the use of this device, as hull is impermeable membrane to the loss of soluble proteins from cotyledons during blanching.

A batch type wet dehuller was developed and tested for removal and separation of hulls from whole blanched soybean.

The performance of the unit indicated that optimum capacity of dehulling was 4 kg/batch with a separation efficiency (i.e. hull separation by floatation) of about 50 per cent. The unit offers the advantages such as elimination of beany flavour and reduced loss of nutrients while blanching the soybean.

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## NUTRIENT COMPOSITION OF SOME UNCOMMON FOODS CONSUMED BY KUMAON AND GARHWAL HILL SUBJECTS

S.S. PRAMILA, ANNAMMA KUMAR\* AND RITA RAGHUVANSHI

Department of Foods and Nutrition, College of Home Science,

G.B. Pant University of Agriculture & Technology,

Pantnagar - 263 145, India.

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The determination of the proximate principles and some of the essential nutrients in ten uncommon foods of Uttar Pradesh (India) had revealed that several of these foods contain high amounts of certain important dietary factors. These include protein and fat in Bhanjira (*Perilla frutescens*) and Chilu seeds (*Prunus armeniaca* Willd); calcium and  $\beta$ -carotene in Beng (*Basella alba* Willd) and Bichhu ghaas (*Urtica dioica* L.); carbohydrates in Gaderi (*Colocasia esculenta*), Gathi (*Dioscorea bulbifera* Linn), Bannda (*Alocasia macrorrhiza* Schott); ascorbic acid in Kunja (*Rosa moschata* Hermm), Mehal (*Pyrus pashia* Buch Ham ex O Don.) and Mongra (*Raphanus sativus* var *caudatus*).

A number of foods consumed by the people in the hill regions of Uttar Pradesh are not commonly eaten elsewhere in the country. Leaves of Beng (*Basella alba* Willd) are widely used in these hills as a substitute for spinach. Bichhu ghaas leaves (*Urtica dioica* L.) are also prepared in the same manner after boiling and throwing the water in order to destroy its irritant property. Bichhu ghaas is also said to have therapeutic properties in the treatment of certain diseases such as uterine

and nose haemorrhages, paralysis and rheumatism. The tubers of Gaderi (*Colocasia esculenta*) and Gathi (*Dioscorea bulbifera* Linn.) are similar to potato and colocasia respectively and are used as vegetables. The seeds of Bhanjira (*Perilla frutescens*) are used for thickening curries and also in tempering and chutneys. Chilu seeds (*Prunus armeniaca* Willd) as such are eaten like almonds and are also used for extracting oil for cooking purposes. Fruit of Mehal (*Pyrus pashia* Buch Ham ex O Don.) is eaten both as fresh fruit and dried fruit flour mixed with wheat or ragi (finger millet) flours. Mature fruits of Kunja (*Rosa moschata* Hermm) are eaten fresh. Mongraas (*Raphanus sativus* var *caudatus*) are plucked before fully ripe and prepared into a curry or pickle.

All the foods described above are consumed in the Kumaon and Garhwal regions in the winter season. Information on the nutrient composition of these foods is scanty. Goel<sup>1</sup> had earlier determined the nutrient composition of fifteen such foods eaten in the summer season. The present study was taken up to determine the nutrient composition of the ten foods described above. The fresh samples collected were preserved in a solution containing 5 ml acetic acid, 30 ml form-aldehyde and 30 ml ethyl alcohol in 50 ml distilled water. The components estimated were moisture, crude protein, crude fat, crude fibre, ash, carbohydrate, energy, iron, calcium, phosphorus,  $\beta$ -carotene, ascorbic acid, thiamine, riboflavin and niacin by standard procedures<sup>2,3</sup>. Only such foods were chosen for which the nutrient composition is not listed by Golapan<sup>4</sup> and in Wealth of India<sup>5</sup>. Samples from different plants of same kind (e.g., Bhanjira seeds from 5 Bhanjira plants) were collected and mixed together and from this mixture a small quantity was used for analysis in triplicate.

The proximate composition of the ten foods are presented in Table I and the mineral and vitamin contents are presented

TABLE I. PROXIMATE COMPOSITION (%) OF UNCOMMON FOODS CONSUMED IN KUMAON AND GARHWAL HILLS

Name	Plant part	Moisture	Crude protein	Crude fat	Crude fibre	Total ash	Carbohydrates (by diff)	Energy (kcal/100g)
Beng	Leaves	93.2 ± 0.075	1.48 ± 0.009	0.25 ± 0.002	0.74 ± 0.002	1.35 ± 0.001	3.40	21.90
Bichhu	Leaves	85.4 ± 0.017	3.82 ± 0.007	0.43 ± 0.001	0.50 ± 0.003	3.55 ± 0.002	6.50	42.80
Gaderi	Tuber	84.6 ± 0.138	1.61 ± 0.005	1.63 ± 0.002	1.63 ± 0.009	0.72 ± 0.003	0.93	60.32
Gathi	Tuber	74.8 ± 0.138	3.55 ± 0.022	0.38 ± 0.000	1.08 ± 0.002	0.89 ± 0.009	20.17	100.03
Banda	Tuber	62.8 ± 0.132	1.78 ± 0.004	0.33 ± 0.002	1.44 ± 0.001	1.20 ± 0.002	12.55	63.80
Bhanjira	Seed	6.01 ± 0.000	22.45 ± 0.107	39.75 ± 0.002	10.33 ± 0.003	3.71 ± 0.002	17.70	518.50
Chilu	Seed	4.81 ± 0.001	26.28 ± 0.080	46.52 ± 0.005	4.71 ± 0.002	2.51 ± 0.001	9.50	587.20
Mehal	Fruit	70.21 ± 0.039	1.04 ± 0.002	0.04 ± 0.001	3.23 ± 0.001	0.68 ± 0.002	16.40	84.90
Kunja	Fruit	65.51 ± 0.042	1.02 ± 0.007	0.62 ± 0.002	2.56 ± 0.000	4.15 ± 0.003	19.70	101.40
Mongra	Fruit	89.63 ± 0.029	1.26 ± 0.016	0.92 ± 0.002	1.52 ± 0.002	0.81 ± 0.001	4.56	42.15

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\*To whom all the correspondence should be addressed.

TABLE 2. MINERAL AND VITAMIN CONTENTS OF UNCOMMON FOODS CONSUMED IN KUMAON AND GARHWAL HILLS

Food (Name & part)	Iron (mg/100g)	Calcium (mg/100g)	Phosphorus (mg/100g)	$\beta$ -Carotene (mcg/100g)	Ascorbic acid (mg/100g)	Thiamine (mg/100g)	Riboflavin (mg/100g)	Niacin (mg/100g)
Beng (leaves)	11.04 $\pm$ 0.020	202.9 $\pm$ 0.020	38.2 $\pm$ 0.040	5606.6 $\pm$ 3.500	24.9 $\pm$ 0.040	0.016 $\pm$ 0.000	0.17 $\pm$ 0.002	0.112 $\pm$ 0.002
Bichhu (leaves)	2.13 $\pm$ 0.004	172.8 $\pm$ 0.320	48.3 $\pm$ 0.130	3132.1 $\pm$ 28.100	15.6 $\pm$ 1.220	0.027 $\pm$ 0.000	0.25 $\pm$ 0.005	0.86 $\pm$ 0.008
Gaderi (tuber)	3.13 $\pm$ 0.023	60.28 $\pm$ 0.050	42.3 $\pm$ 0.000	33.7 $\pm$ 0.700	4.03 $\pm$ 0.550	0.022 $\pm$ 0.002	0.041 $\pm$ 0.001	0.276 $\pm$ 0.005
Gathi (tuber)	6.23 $\pm$ 0.031	152.2 $\pm$ 0.105	65.7 $\pm$ 0.000	56.3 $\pm$ 0.790	5.27 $\pm$ 0.070	0.031 $\pm$ 0.001	0.084 $\pm$ 0.003	0.169 $\pm$ 0.000
Bannda (tuber)	2.5 $\pm$ 0.024	72.51 $\pm$ 0.024	56.3 $\pm$ 0.060	21.3 $\pm$ 0.480	2.35 $\pm$ 0.000	0.013 $\pm$ 0.000	0.054 $\pm$ 0.001	0.26 $\pm$ 0.006
Bhanjira (seed)	9.2 $\pm$ 0.050	200.6 $\pm$ 0.196	324.3 $\pm$ 0.070	209.1 $\pm$ 5.490	5.48 $\pm$ 0.830	0.145 $\pm$ 0.003	0.22 $\pm$ 0.060	0.324 $\pm$ 0.000
Chilu (seed)	6.5 $\pm$ 0.002	193.0 $\pm$ 0.330	232.7 $\pm$ 0.780	179.6 $\pm$ 0.000	6.12 $\pm$ 0.000	0.05 $\pm$ 0.000	0.16 $\pm$ 0.016	1.39 $\pm$ 0.020
Mehal (fruit)	0.71 $\pm$ 0.008	73.4 $\pm$ 0.144	26.3 $\pm$ 0.120	104.1 $\pm$ 1.000	57.3 $\pm$ 1.900	0.017 $\pm$ 0.000	0.045 $\pm$ 0.002	0.14 $\pm$ 0.001
Kunja (fruit)	0.52 $\pm$ 0.009	35.6 $\pm$ 0.210	51.2 $\pm$ 0.820	474.5 $\pm$ 5.000	97.6 $\pm$ 0.700	0.042 $\pm$ 0.001	0.063 $\pm$ 0.001	0.37 $\pm$ 0.000
Mongra (fruit)	1.87 $\pm$ 0.018	75.6 $\pm$ 0.460	108.9 $\pm$ 0.740	64.6 $\pm$ 1.240	61.92 $\pm$ 1.600	0.019 $\pm$ 0.000	0.046 $\pm$ 0.002	0.306 $\pm$ 0.0008

in Table 2. The protein, fat, energy and mineral values of the seeds are comparable to the values reported<sup>4</sup> for some of the commonly eaten seeds such as almonds and groundnuts which are already known to be rich sources of these constituents. Similarly, the mineral,  $\beta$ -carotene and riboflavin values of the leaves may be considered high as these values are similar to those of the well known good sources such as spinach. The fruits 'Kunja', 'Mahal' and 'Mongra' had ascorbic acid contents varying from 57 to 98 mg per 100 g. Considering that the daily recommended intake of the vitamin for Indians is only 40 mg<sup>1</sup> and these fruits are consumed in good quantities, the three fruits can be called good sources of vitamin C. Gathi, one of the tubers studied had a carbohydrate content (20.17 per cent) very close to the known rich sources, potato and colocasia which have 22.6 and 21.1 per cent, respectively.

The results of this investigation establish that these foods have promising values of nutritional constituents. However,

it may be necessary to determine the antinutritional factors and bioavailability of nutrients from these foods.

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## PUFFED RICE-DETECTION OF FRAUDULENT USE OF UREA

P.K. NAG, P.K. PAL, M.K. DAS  
Haringhata Farm, Nadia, West Bengal, India

AND  
T.S. BANDYOPADHYAY  
Science and Technology Department,  
Bikash Bhavan, Calcutta - 700091 India.

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**Alleged injurious use of urea in puffed or expanded rice has been investigated. It has been found in a model preparation that about 70% of 1% urea is retained in 'High temperature short time (HTST)' puffing. An analytical method based on the use of urease has been devised which is sensitive to 0.1% urea. The method is rapid and simple, and can be adopted to field or Kit tests. A number of commercial samples showed its presence and toxicological significance of urea injection has been discussed.**

*Muri*, a variety of puffed rice or rice crispy expanded by puffing is used extensively as a snack. It is distinguished from fried rice or puffed paddy. Of late, there are reports that urea is used during puffing of *muri* to have larger size than usual grains. Since urea is undesirable as a food additive on processing aid, the matter has been investigated specially to see whether appreciable residue still remains even after the 'High temperature short time' (HTST) puffing and to detect urea analytically.

**Preparation of *muri*:** This method is representative of indigenous practices of *muri*. In a dish, take rice (about 200 g) and dip it in water for 15 min. Drain out the water and allow to dry slowly spreading at room temperature to an approximate moisture content of about 15 per cent which is achieved in six hours. Add about 8 g. salt to the rice and mix with a little water (This step can be dispensed with if unsalted product is intended). Place this dish on an air oven and wait till sparging begins. In the meantime, place a vessel with sand (about 500 g) on the burner and heat to 200°C. Pour treated rice from the dish on the sand and stir continuously; in about a minute the rice puffs and takes the shape of *muri*. Alternatively, rice spiked with urea instead of salt can be similarly converted to *muri*.

**Detection of urea in *muri*:** Take about 10 g of *muri* in a 250 ml beaker containing 50 ml distilled water and keep it for 10 min stirring at times. Filter and take 5 ml filtrate in a beaker, add 2 ml phosphate buffer (pH 7.0) and 150 mg of freshly prepared powder of soybean or arhar pulse and

warm on water bath (37°-40°C) for 10 min. Dip a litmus paper (pH 7-8.5), if the colour changes to alkaline urea is present. Phenolphthalein also can alternatively detect alkalinity.

It has been observed that *muri* prepared with urea has larger grains and shows a positive test of urea. On rough quantitative observation of the intensity of colour, it was found that only about 0.3 of 1 per cent urea is lost during the HTST preparation. The residue can be easily detected by this method as this can detect urea upto 0.1 per cent in *muri*. This method was applied to a survey collecting commercial samples of *muri* from Calcutta and suburbs. It has been observed that considerable number of samples of *muri* contain urea specially the *muri* coming from the district of 24-Parganas (South).

The method depends on the action of the enzyme urease on urea liberating ammonia<sup>1</sup>. Though *muri* preparation is still a home scale indigenous industry in India, it is known to have been mechanised in the advanced countries.

Urea is not recommended as an additive or processing aid<sup>2</sup>. Though there is no specific mention of prohibition of its use, the practice is to be condemned as urea is toxic and hence injurious to health attracting one of the definitions of adulteration. Urea is an ultimate catabolite of amino acids and extracted from blood through urine. Its accumulation in blood and tissues enhances burden on the kidney and is obviously undesirable. Some ailments can be ascribed to its excess. It is reported to be moderately toxic, skin irritant, a carcinogen and neoplastogen and has adverse effect on fertility and reproduction<sup>3</sup>. Data on its acceptable daily intake (ADI) are not available.

The probable intake of urea in the present situation is appreciable i.e. about 500-700 mg for a 100 g serving of treated *muri*. In villages, it has been ascertained, a man can even take 500 g. *muri* a day. Urea acts as a puffing agent by evolution of gas. When heated, it decomposes and emits its NO<sub>x</sub> fume which also is toxic. Hence its present use can also be an occupational hazard. The test is rapid and simple, and can be adopted to field tests and by housewives.

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## SURVIVAL OF PATHOGENIC BACTERIA IN YOGHURT AND DAHI

HITTU MATTA, M.S. KALRA AND AJIT SINGH  
Department of Microbiology, Punjab Agricultural University  
Ludhiana, India

Received 25 March 1990; revised 18 December 1990

The survival rates of *Salmonella typhimurium*, *Yersinia enterocolitica* and *Campylobacter jejuni* in yoghurt and dahi stored at 5-7°C or 37°C were determined. Changes in titratable acidity and pH were also measured in these fermented milks. During storage, the lactic acid percent increased from 0.6 to 1.1, in yoghurt and 0.46 to 0.95 in dahi after 48 hr. None of the pathogens survived in dahi/yoghurt stored at 37°C for more than 25 hr. However, a few survivors were still present even after 48 hr storage at 5-7°C. D values for all the pathogens ranged from 4.0 to 5.5 hr.

Fermented dairy products are inhibitory to both pathogenic and spoilage micro-organisms, yoghurt and dahi being the most effective<sup>1</sup>. Most of the pathogens gain entry into yoghurt and dahi after pasteurization, from sources such as diseased animals, contaminated utensils and milk handlers. It has been generally observed that the pathogens inoculated into yoghurt remain viable for a short period ranging from a few hours at summer temperature (30-40°C) to 1-5 days at refrigeration temperature. The anti-microbial activity of dahi has been attributed to production of lactic acid, H<sub>2</sub>O<sub>2</sub> and bacteriocin.

*Salmonella typhimurium*, *Yersinia enterocolitica* and *Campylobacter jejuni* are mainly concerned with enteric diseases and may cause enteric fever, gastro-enteritis, septicemia, diarrhoea, arthritis, pseudo-appendicitis, etc. These three pathogens have been isolated from raw and pasteurized milk<sup>2</sup>. There are limited reports of survival of these three pathogens in yoghurt and dahi. Kotz *et al.*<sup>3</sup> reported that *Escherichia coli* strains were killed in four to nine hr in yoghurt. It was reported that yoghurt inhibited *S. typhimurium* within three days when stored at 4°C. Mantis *et al.*<sup>4</sup> stated that *Y. enterocolitica* survived for six days in yoghurt with pH 4.6. Doyle and Roman<sup>5</sup> found that *C. jejuni* could not grow in high acid foods and at low pH of 3.0 or 3.5, the organisms were rapidly inactivated at all temperatures including 4°C.

The present study has been designed to evaluate the fate of the three pathogens during storage of yoghurt and dahi at 5-7°C and at 37°C. Raw cow's milk was obtained from the Department of Food Science and Technology, Punjab Agricultural University, Ludhiana (India). Cultures of *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Strepto-*

*coccus lactis* were procured from National Dairy Research Institute, Karnal (India) and subcultured fortnightly in litmus milk at 30/37°C for 24 hr and stored at 4°C. *S. typhimurium* was obtained from the College of Veterinary Science, Punjab Agricultural University, Ludhiana (India) and grown on Salmonella-Sh gella agar (Difco) at 37°C for 24 hr and the same medium was used for enumeration of viable cells. *Y. enterocolitica* strain B was procured from Dayanand Medical College, Ludhiana (India) and grown on soybean casein digest medium (Hi-media) at 23°C for 18-24 hr and for enumeration MacConkey agar medium was used. *C. jejuni* strain-35 was obtained from the Department of Microbiology, Post-Graduate Institute of Medical Education and Research, Chandigarh (India) and was subcultured after every 48 hr on blood agar medium at 42°C for 48 hr in an anaerobic jar supplied with 10 per cent CO<sub>2</sub> and 90 per cent N<sub>2</sub> and survival rate was studied on blood agar base No. 2 (Hi-media).

Cow's milk was heated in 4 oz wide-mouth sterile bottles at a pressure of 10 p.s.i. for 10 min. After cooling to about 40°C, milk was inoculated with *S. thermophilus* and *L. bulgaricus* in 1:1 ratio at the rate of 2 per cent of each starter organisms, for yoghurt preparation and *S. lactis* at 2 per cent rate, for dahi preparation. Milk containing yoghurt cultures was incubated at 42°C for 4 hr while that containing *S. lactis* was incubated at 30°C for 10-12 hr. After the preparation of yoghurt and dahi, they were stored at 4°C.

The three pathogens were grown in their selective broth media at their optimum temperatures for 18-24 hr and the initial count of inoculum ranged between 0.1 and 0.2 million cells/ml. These pathogens were inoculated into yoghurt and dahi separately at 2 per cent rate and were incubated at 5-7°C and 37°C. Aliquots were drawn on different time intervals and the counts were estimated using respective selective media. Titratable acidity was calculated in terms of lactic acid percentage according to APHA procedure<sup>6</sup>. Decimal reduction time (D value) of the three pathogens in yoghurt and dahi was calculated according to the method given by Stumbo<sup>7</sup>.

The growth pattern of *S. typhimurium* in yoghurt and dahi and changes in titratable acidity and pH of yoghurt and dahi are shown in Table 1. The initial titratable acidities in yoghurt and dahi were 0.63 per cent and 0.49 per cent lactic acid, respectively. But on storage at 5-7°C and 37°C, the values increased to 0.85 per cent and 1.07 per cent in yoghurt and 0.82 per cent and 0.94 per cent in dahi, respectively. pH values of yoghurt and dahi also decreased simultaneously. With the increase in lactic acid concentration in the fermentation product, there was substantial decrease in viable cells of the pathogens. No survivors were found in yoghurt stored at 37°C for 12 hr, dahi stored at 37°C for 24 hr and in yoghurt stored

TABLE 1. CHANGES IN TITRATABLE ACIDITY AND pH OF YOGHURT AND DAHI CONTAINING *SALMONELLA TYPHIMURIUM* DURING STORAGE

Storage period (hr)	Titratable acidity as lactic acid (%)		pH		<i>S. typhimurium</i> survived [CFU/ml ( $\times 10^2$ )]	
	5-7°C	37°C	5-7°C	37°C	5-7°C	37°C
<b>Yoghurt</b>						
0	0.63	0.63	4.82	4.82	80	80
2	0.65	0.69	4.66	4.48	62	46
4	0.67	0.70	4.56	4.25	42	32
6	0.69	0.73	4.48	4.13	30	15
8	0.72	0.74	4.39	4.06	23	9
10	0.74	0.78	4.32	3.92	15	5
12	0.76	0.81	4.26	3.84	11	4
24	0.81	0.94	4.08	3.73	7	0
48	0.85	1.07	3.91	3.64	3	0
<b>Dahi</b>						
0	0.49	0.49	4.85	4.85	74	74
2	0.52	0.57	4.76	4.60	53	32
4	0.54	0.59	4.70	4.56	35	14
6	0.57	0.62	4.66	4.51	18	5
8	0.62	0.64	4.50	4.43	11	3
10	0.64	0.67	4.46	4.42	7	0
12	0.71	0.74	4.37	4.03	6	0
24	0.74	0.82	4.28	3.96	3	0
48	0.82	0.94	4.09	3.52	0	0

Mean values are based on three trials. CFU = colony forming units

at 5-7°C for 48 hr. However, a few survivors were detected in yoghurt stored at 5-7°C for 48 hr. Rubin and Vaughan<sup>8</sup> reported that lactic acid was bactericidal for *S. typhimurium* at pH 3.85. Park *et al.*<sup>9</sup> observed that storage temperature and the nature of organic acids present in fermented milks were critical for the survival of *S. typhimurium*.

The changes observed in titratable acidity and pH of yoghurt and *dahi* when 0.1-0.2 million cells/ml of *Y. enterocolitica* were incorporated in yoghurt and *dahi* and stored at 5-7°C or 37°C for 48 hr are shown in Table 2. Increase in titratable acidity was higher at 37°C than at 5-7°C in both yoghurt and *dahi*. At 12 hr of storage at 37°C, no survivors were found in yoghurt but in *dahi* the complete inhibition was observed after 48 hr. But at 5-7°C, viable cells were observed in yoghurt and *dahi* even after 48 hr, i.e.  $2 \times 10^2$  cells/ml in yoghurt and  $9 \times 10^2$  cell/ml in *dahi*. Bimet<sup>10</sup> showed that *Y. enterocolitica* could survive three to five days in yoghurt if kept at 4°C, while Slavchev and Gogov<sup>11</sup> reported that *Y. enterocolitica* could survive for 24 hr during refrigerated storage of yoghurt.

The number of survivors of *C. jejuni* in yoghurt and *dahi* at both the storage temperatures and changes in titratable acidity and pH during storage of yoghurt and *dahi* are shown in Table 3. During storage at 37°C, no colony was observed from yoghurt after 12 hr, while there was no survivor in *dahi* after 24 hr. After 48 hr of storage at 5-7°C in yoghurt, no

colony was observed in yoghurt while  $2 \times 10^2$  CFU were observed in *dahi*. Cuk *et al.*<sup>12</sup> observed that none of the nine strains of *C. jejuni* studied, could survive for 25 min in yoghurt at pH 4.42 to 5.35. Christopher *et al.*<sup>13</sup> detected no survivors of *C. jejuni* after 24 hr of incubation at 37°C when pH of the medium was adjusted to 5.0 To reflect the relative resistance of pathogens in yoghurt and *dahi* at two storage temperatures 5-7°C and 37°C, D values were determined (Fig. 1), taking 't' equal to 8 hr.

All the three pathogens cannot utilize lactose increase in titratable acidity and decrease in pH on storage upto 48 hr at two different temperatures was mainly due to starter culture activity. Since test organisms did not multiply at all, lactic acid present in yoghurt and *dahi* may have inhibited the metabolic activity of the cells and resulted in inhibition of these three pathogens under study. However, the exact mechanism of lactic acid inhibition is not known but Weiner and Draskoczy<sup>14</sup> suggested that lactic acid might have terminated oxidative metabolism by inhibiting dehydrogenase activity. So, Gram-negative pathogenic microorganisms which may gain access into yoghurt and *dahi*, accidentally, rarely survive after 48 hr either at room temperature or refrigeration temperature. The antagonistic activity of these fermented milks against pathogens confirms therapeutic property of yoghurt and *dahi* and thus these are safer to consume as compared to milk.

TABLE 2. CHANGES IN TITRATABLE ACIDITY AND pH OF YOGHURT AND DAHI CONTAINING *YERSINIA ENTEROCOLITICA* DURING STORAGE

Storage period (hr)	Titratable acidity as lactic acid (%)		pH		<i>Y. enterocolitica</i> survived [CFU/ml ( $\times 10^3$ )]	
	5-7°C	37°C	5-7°C	37°C	5-7°C	37°C
<b>Yoghurt</b>						
0	0.60	0.60	4.95	4.35	39	39
2	0.63	0.73	4.76	4.48	29	27
4	0.67	0.73	4.52	4.35	23	20
6	0.72	0.75	4.42	4.26	18	15
8	0.76	0.79	4.29	4.14	15	11
10	0.77	0.82	4.20	4.09	11	4
12	0.82	0.85	4.14	3.92	8	0
24	0.89	0.90	3.94	3.63	5	0
48	0.92	1.01	3.87	3.52	2	0
<b>Dahi</b>						
0	0.47	0.47	5.10	5.10	43	43
2	0.54	0.57	4.99	4.34	37	32
4	0.57	0.60	4.95	4.76	32	26
6	0.59	0.63	4.87	4.74	27	22
8	0.62	0.68	4.80	4.52	25	17
10	0.64	0.73	4.74	4.41	20	10
12	0.68	0.76	4.52	4.35	17	6
24	0.75	0.88	4.21	3.82	14	1
48	0.84	0.95	4.00	3.75	9	0

Foot note as in Table 1

TABLE 3. CHANGES IN TITRATABLE ACIDITY AND pH OF YOGHURT AND DAHI CONTAINING *CAMPYLOBACTER JEJUNI* DURING STORAGE

Storage period (hr)	Titratable acidity as lactic acid (%)		pH		<i>C. jejuni</i> survived [CFU/ml ( $\times 10^3$ )]	
	5-7°C	37°C	5-7°C	37°C	5-7°C	37°C
<b>Yoghurt</b>						
0	0.60	0.60	4.81	4.81	70	70
2	0.63	0.68	4.75	4.49	52	48
4	0.65	0.70	4.64	4.41	38	32
6	0.68	0.72	4.57	4.39	25	24
8	0.70	0.77	4.42	4.21	18	16
10	0.72	0.79	4.39	4.18	15	6
12	0.76	0.82	4.25	4.12	11	0
24	0.82	0.94	4.13	3.87	5	0
48	0.89	1.10	3.93	3.57	0	0
<b>Dahi</b>						
0	0.48	0.48	5.00	5.00	90	90
2	0.52	0.51	4.97	4.87	68	64
4	0.54	0.56	4.91	4.77	52	44
6	0.57	0.59	4.80	4.69	43	30
8	0.60	0.66	4.77	4.54	32	21
10	0.62	0.68	4.68	4.42	25	12
12	0.65	0.72	4.42	4.14	20	3
24	0.74	0.83	4.18	3.90	9	0
48	0.81	0.90	4.08	3.78	2	0

Foot note as in Table 1

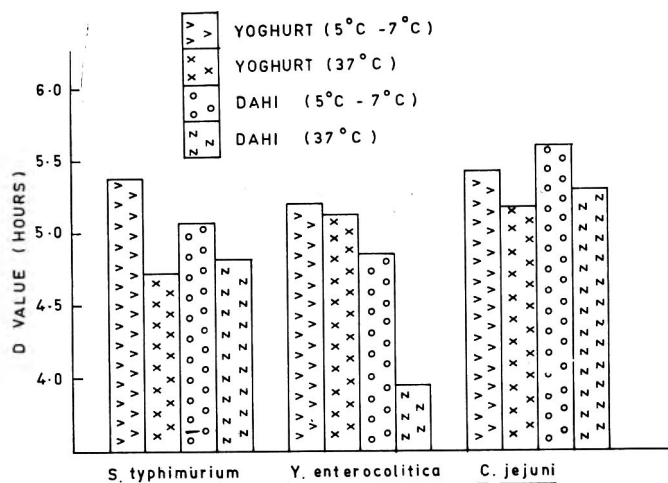


Fig. 1. Decimal reduction time (D value) of *S. typhimurium*, *Y. enterocolitica* and *C. jejuni* in yoghurt and dahi.

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## MICROBIOLOGICAL QUALITY OF RAW BEEF FROM IRRUA - NIGERIAN MARKET RETAIL TABLE

S.A. OKODUGHA AND L.E. ALIGBA

Dept. of Biological Sciences, Faculty of Natural Sciences,  
Bendel State University, P.M.B. 14, Ekpoma,  
Bendel State, Nigeria.

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A study of the microbiological quality of raw beef market samples was undertaken. Results showed that the total aerobic plate count (APC) ranged from  $\log_{10}$  6.11 - 6.65/g while the coliforms varied from  $\log_{10}$  3.43 - 5.95/g. Mould and yeast counts ranged from  $\log_{10}$  2.86 - 5.78/g. Bacterial genera isolated included *Micrococcus*, *Staphylococcus*, *Salmonella*, *Escherichia*, *Bacillus*, *Pseudomonas*, *Klebsiella*, *Proteus*, etc. The genera of moulds isolated were *Aspergillus*, *Penicillium*, *Sporotrichum* and *Mucor*. No yeasts were isolated from the raw meat samples. Incidence with which microorganisms occurred was 100% for *Pseudomonas*, *Salmonella*, *Aspergillus*, etc. The significance of some of the microorganisms isolated from the raw meat is discussed.

Slaughtering and dressing of meat animals and subsequent handling of the carcasses are done under poor sanitary conditions, in rural communities and small townships<sup>1</sup>, by several local butchers. Slaughtering and evisceration of smaller animals like goats, pigs, etc. are usually done on detached leaves while bigger animals like cows and bulls are done on wooden slabs in Irrua local market abattoir.

Because of shortage of portable water, it is sometimes purchased from 'tanker drivers' who fetch water from rivers, stream, etc. for sale. Consequently, the guts which are well cherished for 'pepper soup' making in most parts of Southern Nigeria, are not thoroughly washed before being displayed for sale. The water, whose wholesomeness cannot be guaranteed, is used in washing the carcasses and guts.

The meat is never aged; rather the hot carcasses are carried manually or in wheel barrows/trucks direct from the slaughter slabs to the retail tables<sup>2</sup>. The meat is also never packaged but is displayed sometimes side by side with the improperly washed guts on retail tables for sale. Consequently, the meat is further exposed to microbial contamination from the atmosphere and from finger feel of buyers and sellers and by environmental factors like high ambient temperature and high humidities<sup>1</sup> which favour microbial proliferation. The microbiological quality of meat is known to depend on several factors<sup>3,4</sup>.

It is against this background information that this preliminary study of the microbiological quality of raw meat

from Irrua market was conducted to determine the degree of contamination and the types of microorganisms associated with the raw meat.

Samples of raw beef were purchased, usually before 10.00 a.m., at different time intervals (ten days interval) from retail tables in Irrua open-market in Bendel State of Nigeria. These were collected aseptically in sterile containers and were immediately taken (under 7 min) to our laboratory for microbiological analysis.

A weighed quantity (10g) of each raw meat sample was homogenized in 90 ml of 0.1 per cent peptone water in sterilized blender for 10 min. Thereafter, serial dilutions of the meat homogenate were made. Pour plate procedure<sup>5</sup> was employed to determine the total aerobic plate count (APC) at 37°C, coliform count at 35°C and mould and yeast counts at 25°C using nutrient agar (NA, Oxoid), MacConkey agar (MA, oxoid) and violet red bile (VRB, DIFCO) and potato dextrose agar (PDA, DIFCO), respectively supplemented with 100 p.p.m. chloramphenicol to inhibit bacteria. Duplicate plates for each determination were made and incubated for 48 hr, 24 hr and 3-5 days for APC, coliforms and moulds and yeasts, respectively. Colonies observed were counted and reported as logarithms of the number of colonies observed per gram of raw meat.

Bacterial and fungal isolates were separated into groups and characterised on the basis of known methods<sup>5,6</sup>.

The microbial loads of raw beef samples are shown in Table 1. The mean microbial loads for the raw meat were  $\log_{10}$  6.34,  $\log_{10}$  4.95 and  $\log_{10}$  4.72 for APC, coliforms and moulds and yeasts, respectively.

Because the method of slaughtering and dressing of meat animals in Irrua market abattoir is local and somewhat unhygienic, the microbial loads (Table 1) were relatively different from those reported by previous workers<sup>7,10</sup>.

TABLE 1. MICROBIAL LOADS EXPRESSED AS  $\log_{10}$  COUNT/g OF MEAT OF RAW BEEF

Raw meat (Sample No.)	Total aerobic count at 37°C	Coliform count at 35°C	Mould and yeast counts at 25°C
1	6.11	5.48	5.70
2	6.63	4.95	5.60
3	6.15	5.70	5.48
4	6.48	5.48	5.78
5	6.45	4.11	4.54
6	6.65	4.51	3.08
7	5.92	3.43	2.86
Mean	6.34 ± 0.26	4.95 ± 0.87	4.72 ± 1.17

Values for each sample are average count of two determinations.

The coliform count for raw beef obtained in this study varied from  $\log_{10}$  3.43-5.95. Our observation of high coliform density for raw meat is not surprising since dressing and washing of the guts are done side by side with cutting up of the carcasses into meat parts by the same local butchers. Also, the improperly washed guts are sometimes displayed very close to the raw meat for sale. Consequently, faecal contamination of the raw meat is likely to have occurred. Besides, the stream or river water used in washing carcasses could have contributed to the coliform density of the raw meat. It has been reported that the control of microorganisms during primary processing of poultry and other meat animals plays an important part in determining the quality of the finished product<sup>11</sup>.

Although no yeasts were isolated from the raw meat samples, the high mould count of  $\log_{10}$  2.86 - 5.78 recorded may be due, at least in part, to the dusty nature of the local open-air market where the raw meat is displayed for sale.

In terms of APC and coliform density, Irrua raw meat is relatively of poor quality when compared to microbiological guidelines and standards for raw meat for some States of America reported by Wehr<sup>12</sup>.

Although some similar genera of microorganisms were isolated from the raw meat samples, their microflora were different. The genera of bacteria isolated included *Micrococcus*, *Staphylococcus*, *Salmonella*, *Escherichia*, *Bacillus*, *Pseudomonas*, *Klebsiella*, *Proteus*, etc. The isolation of some members of the enterobacteriaceae like *Escherichia*, *Salmonella*, *Pseudomonas* indicates faecal contamination of raw meat samples. Igene and Abulu<sup>9</sup>, Okodugha<sup>13</sup> and Bachhil and Jaiswal<sup>14</sup> isolated similar bacteria genera from raw meat. The genera of moulds isolated were *Aspergillus*, *Penicillium*, *Sporotrichum* and *Mucor*.

The isolation of *Salmonella*, *Bacillus*, *Staphylococcus*, *Aspergillus* and *Penicillium* from the raw meat is important not only from the point of view of meat hygiene but also public health since some strains of these microorganisms are very pathogenic (i.e. enterotoxigenic, possibility of occurrence of aflatoxin, etc.). The relatively frequent presence of *Salmonella* in some raw meat is of great concern because their toxigenic nature makes such beef unsuitable for human consumption. This is of particular significance since raw meat may serve as source of contamination (cross-contamination) of other foods especially those that are eaten raw such as fruits, vegetables and salad vegetables<sup>7</sup>. It has been reported that high heat resistance of *Bacillus* (*B. cereus*) spores, inadequate heating of the product and improper storage might result in heavy build up of *B. cereus* population in cooked meat products and must be considered a potential hazard in ready-to-eat meat products<sup>14</sup>.

The microorganisms that occurred most frequently were *Escherichia*, *Mucor*, *Bacillus*, *Penicillium*, *Aspergillus*, *Salmonella*, *Staphylococcus*, *Micrococcus*, *Pseudomonas*, etc. The high frequency of occurrence of *Pseudomonas*, *Escherichia* and *Salmonella* further confirmed faecal contamination of raw meat. Okodugha and Obanu<sup>1</sup> similarly reported a high frequency of occurrence of some bacterial and fungal species.

The data presented above show that the microbial loads particularly the coliform density of the raw meat were relatively high. Proper hygiene during slaughtering and dressing of meat animals and subsequent handling of the resultant carcasses as well as simple packaging of raw meat to avoid contamination during sale on retail tables are advocated. A study of the microbiological quality of water from the various sources is needed.

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## WHEAT HARDNESS : EFFECT OF MOISTURE ON PEARLING INDEX AND KERNEL HARDNESS

R. PAL SINGH AND A.K. BAKSHI

Department of Plant Breeding  
Punjab Agricultural University  
Ludhiana - 141 004, Punjab, India.

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Fourteen bread wheat varieties commercially grown in the region were studied for grain hardness using barley pearler and kernel hardness tester in the moisture range of 10-14%. The moisture level affected the pearling indices significantly when pearling time of 45 sec was used. However, its effect was non-significant when the pearling time was extended to 60 sec as also on the kernel hardness tester values. The increase in the pearling time had a greater effect on the softer varieties than the harder ones. The varietal differences were significant in all the cases. Kernel hardness tester is preferred over the barley pearler as the former is not affected by the factors which affect the latter.

Grain hardness is a commonly used characteristic in wheat classification and the hardness and vitreousness are used in the marketing of wheat, since they affect the appearance and milling and baking behaviour of grains<sup>1</sup>. Earlier the breeders have been using visual observations to screen/classify wheats in the absence of sophisticated methods for measuring wheat hardness. But in the past, various methods for evaluating kernel hardness have been developed and amongst those, Pearling Resistance Index (PRI) has been found to be useful as a screening method for breeding programmes<sup>2</sup>. However, the PRI is influenced by the size and shape of the grain, the resistance to abrasion offered by the outer layers and also the age of the Pearler<sup>2,4</sup>. Moreover, this bulk sampling method is suitable for homogeneous samples and not the mixtures of wheat classes<sup>3</sup>. Therefore, the single kernel evaluation of hardness has gained importance. The present study is aimed at evaluating the use of grain hardness tester against the Pearling resistance index values, as influenced by the moisture level of the grains.

Samples of 14 bread wheat cultivars recommended for cultivation in the State of Punjab representing a wide range of kernel hardness were obtained from the Punjab Agricultural University farm. The cleaned samples were conditioned to five different levels of moisture ranging from 10-14 per cent and kept for 48 hr in airtight containers to equilibrate the moisture in the grains. The pearling indices of the samples varying in moisture content were determined in duplicate in a barley pearler using 20 g sample and abrasion

times<sup>6</sup> of 45 and 60 sec, as being used at CIMMYT (personal communication). The hardness values on the grain hardness tester (Kiya Seisakusho Ltd., Japan) were recorded as the force (kg) required to crush the grain keeping the crease upwards. Ten representative grains were crushed from each sample and the average values were recorded. The data were analysed statistically using factorial design test and the correlations between different values were worked out.

The pearling index values of unconditioned samples using 60 sec pearling time ranged between 36.8 and 55.1 with a mean value of 43.4 (Table 1). Accordingly, the varieties K. Sona, 'WL 410', 'HD 2009', 'HD 2285', 'PBW 120', 'PBW 175' were classified as semi-hard and the varieties 'Sonalika', 'SKML-1', 'KSML-3' and 'PBW 54' and 'WL 711', 'WL 1562' and 'HD 2329' were classified as soft and very soft, respectively. Conditioning of the samples upto 14 per cent did not affect the pearling index values significantly. However, the differences amongst varieties and their interaction with the moisture levels was found to be statistically significant. This implies that the varieties interact with moisture differently and their overall effect is probably cancelled out.

The pearling index values using 45 sec pearling time were lower (Table 1) and the mean value obtained was 34.8 against 43.4 recorded with 60 sec of pearling. This reduction in pearling indices was non-uniform and ranged between 3.2 and 12.2 for different varieties. The minimum reduction was observed in case of 'PBW 138' a semihard variety and the maximum in 'WL 1562' a very soft variety on the basis of pearling index. On the whole, the reduction in the pearling index values increased with the increasing softness. Statistically, the effects of varieties and moisture levels as well as their interactions were found to be highly significant. This implies that the moisture level affects the pearling index values when lower pearling time as recommended by AACC is used whereas its effect is non-significant when the pearling time was 60 sec. Pooled analysis of the data for the two pearling times showed that changing the time from 45 to 60 sec affected the pearling indices significantly.

The kernel hardness values of the unconditioned samples at a moisture level of 10 per cent ranged between 7.33 and 11.00 kg (Table 2). Accordingly, the hardest variety was found to be 'KSML 3', categorised as soft on the basis of pearling index. The other varieties statistically at par with 'KSML 3' were: 'PBW 54', 'PBW 120', 'PBW 175', 'WL 711', 'SKML 1' and 'PBW 38'. Out of these seven varieties, 'KSML 3', 'PBW 54', 'WL 711', and 'SKML 1' were categorised as soft and very soft on the basis of pearling indices. The effect of moisture on the kernel hardness in the range of 10-14 per cent was found to be statistically non-significant but the differences due to varieties were significant. The correlation between the



TABLE 1. EFFECT OF MOISTURE CONTENT ON THE PEARLING INDEX OF WHEAT VARIETIES USING 60 SEC. AND 45 SEC. PEARLING TIME

Variety	Pearling index at indicated moisture contents (%)					
	10	11	12	13	14	Mean
K. Sona	37.67 (29.17)	36.06 (28.06)	35.73 (27.95)	35.73 (27.78)	36.16 (28.43)	36.27 (28.28)
Sonalika	48.51 (37.79)	47.59 (36.83)	47.45 (36.13)	46.63 (36.21)	45.18 (34.71)	47.07 (36.33)
WL 410	37.20 (28.12)	41.11 (30.25)	39.86 (31.14)	39.36 (29.87)	37.51 (28.76)	39.01 (29.63)
WL 711	52.33 (41.95)	50.00 (37.41)	49.81 (37.31)	49.99 (47.64)	46.63 (44.06)	49.75 (44.53)
WL 1562	55.10 (42.90)	57.29 (42.74)	56.79 (45.31)	57.77 (47.64)	57.90 (44.06)	56.97 (44.53)
HD 2009	39.03 (33.61)	42.65 (32.44)	43.97 (34.82)	41.05 (32.65)	39.93 (29.19)	41.33 (32.54)
HD 2285	39.75 (31.42)	39.46 (31.59)	39.86 (31.99)	40.18 (32.37)	37.82 (29.68)	39.41 (31.41)
HD 2329	54.19 (43.10)	54.32 (42.58)	52.69 (44.60)	57.06 (45.88)	57.79 (45.46)	55.81 (44.32)
SKML 1	42.75 (32.99)	34.13 (27.92)	41.33 (32.29)	39.94 (32.94)	44.39 (34.60)	40.51 (32.15)
KSML 3	47.64 (36.50)	47.31 (34.86)	45.51 (34.61)	42.28 (32.53)	40.42 (32.10)	44.63 (34.12)
PBW 54	40.18 (32.00)	38.13 (30.39)	39.06 (29.22)	38.22 (31.26)	37.87 (29.00)	38.69 (30.38)
PBW 120	37.19 (31.41)	36.70 (28.57)	36.63 (29.63)	36.92 (28.67)	37.78 (29.23)	37.04 (30.67)
PBW 138	39.13 (35.93)	37.57 (29.90)	38.19 (29.63)	38.36 (28.67)	37.53 (29.23)	38.15 (30.67)
PBW 175	36.81 (30.04)	37.99 (34.28)	39.01 (30.75)	38.10 (29.98)	37.11 (29.83)	37.80 (29.98)
Mean	43.39 (34.78)	42.88 (33.13)	43.49 (33.95)	42.97 (33.89)	42.43 (32.85)	

CD (45 Sec) : For varieties = 1.125, for moisture level = 0.672,

For varieties  $\times$  moisture level = 3.558.

CD (60 Sec) : For varieties = 1.433, for varieties  $\times$  moisture level = 4.533

Figures in parenthesis denote the values obtained by using 45 sec pearling time.

TABLE 2. EFFECT OF MOISTURE LEVEL ON THE KERNEL HARDNESS OF WHEAT VARIETIES

Variety	Kernel hardness (kg) at indicated moisture contents (%)					
	10	11	12	13	14	Mean
K. Sona	9.38	9.23	8.97	9.12	8.18	8.98
Sonalika	9.43	10.77	10.00	8.62	8.73	9.51
WL 410	9.75	10.80	10.83	9.23	10.67	10.26
L 711	10.40	8.55	9.67	12.02	9.55	10.04
WL 1562	9.47	9.02	8.78	9.00	9.45	9.14
HD 2009	9.78	7.83	10.05	8.65	8.42	8.95
HD 2285	7.33	10.58	8.73	9.10	10.03	9.16
HD 2329	9.90	9.72	9.87	9.37	10.58	9.89
SKML 1	10.20	9.28	9.77	7.12	7.52	8.78
KSML 3	11.00	9.93	10.10	10.50	9.63	10.23
PBW 54	10.90	11.57	10.22	10.02	8.87	10.31
PBW 120	10.72	10.32	8.95	8.48	9.32	9.56
PBW 138	10.15	10.20	9.70	10.13	10.08	10.05
PBW 175	10.60	9.10	11.22	10.48	10.80	10.44
Mean	9.93	9.78	9.77	9.42	9.42	

CD : For varieties = 0.991

kernel hardness and pearling index using either of the timings was non-significant. Kernel hardness tester should be preferred over the pearling resistance index test as it is not affected by the factors that affect the latter. However, in kernel hardness evaluations the number of replications should be fairly large so that the variation due to individual grains is averaged out.

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## STUDIES ON THE TRIBAL PULSE, *ENTADA SCANDENS* BENTH. : CHEMICAL COMPOSITION AND ANTINUTRITIONAL FACTORS

K. JANARDHANAN AND K. NALINI

Seed Physiology Laboratory, Botany Department  
Bharathiar University, Coimbatore - 641 046, India

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The seeds of *Entada scandens* Benth. are known to be consumed as a pulse by the Indian tribal sects, the Great Andamanese and Onges. The seeds contain fairly high amounts of crude protein, crude fibre and ash and minerals, like potassium, phosphorus, magnesium, iron, zinc, lead and manganese, when compared to common pulses of India and NRC/NAS recommended dietary allowance values. The seed protein fractions, albumins, globulins, prolamins and glutelins, occur in the ratio of 27:49:6:18, respectively. Both albumins and globulins form the major bulk of seed proteins, constituting 76% of the total seed proteins. The antinutritional factors, total phenols, tannins, trypsin inhibitor activity, haemagglutinating activity and L-DOPA (3,4-dihydroxy-phenylalanine) have also been detected.

*Entada scandens* Benth. (= *Entada phaseoloides* Merrill.) is a grain legume growing widely throughout sub-Himalayan tract from Nepal Eastward ascending upto 4,000 ft. Sikkim, Assam, West Bengal, Bihar, Uttar Pradesh, Orissa, monsoon forests of Western and Eastern Ghats and Andaman and Nicobar Islands. The tribal sects, the Great Andamanese and Onges of Andaman and Nicobar Islands consume the kernel of this legume after soaking in water followed by roasting<sup>1</sup>. The half-ripe seeds are ground to a paste and used as a substitute for coffee in South America. Also, a paste prepared from the seeds when applied locally is known to cure inflammatory glandular swellings<sup>2</sup>. In the present study, the results on proximate composition, total (true) proteins, starch, seed protein fractionation and mineral composition of the seeds are reported. The antinutritional factors like total free phenols, tannins and L-DOPA are quantified separately in kernel and seed coats; whereas the trypsin inhibitor activity and haemagglutinating activity are assayed from proteins extracted from the kernel flour alone.

Seeds of *Entada scandens* were collected from Siruvani Reserve Forest, Coimbatore district, Tamil Nadu. Bovine serum albumin fraction V and 3,4-dihydroxyphenylalanine (L-DOPA) were obtained from Sigma Chemical Co., U.S.A. Trypsin and benzoylarginine-p-nitroanilide (BAPNA) were received as a gift from Prof. Liener, Department of Biochemistry, College of Biological Sciences, Minnesota

University, U.S.A. Other chemicals were of analytical grade.

The dry seeds of *E. scandens* were powdered in a Willey Mill to 60 mesh size. Moisture was determined by drying split seeds in a hot-air-oven at 80°C for 24 hr. Total nitrogen was determined by micro-kjeldahl method and the crude protein (CP) content was calculated by  $N \times 6.25$ . The contents of ether extracts (EE), crude fibre (CF) and ash were determined by AOAC procedures<sup>3</sup>. The nitrogen free extractive (NFE) was calculated as follows: per cent NFE =  $100 - (\text{CP per cent} + \text{EE per cent} + \text{CF per cent} + \text{ash per cent})$ . The total (true) proteins were extracted following the method of Basha *et al*<sup>4</sup> and were spectrophotometrically quantified<sup>5</sup>. Starch content was determined following the method of Clegg<sup>6</sup>. The seed protein fractions, albumins, globulins, prolamins and glutelins were extracted in sequence and quantified as described by Janardhanan and Lakshmanan<sup>7</sup>. The minerals Cu, Zn, Pb, Mn, Mg, Fe, Ca, Na and K were estimated by using Perkin-Elmer, Model-5000 Atomic Absorption Spectrophotometer following the method of Issac and Johnson<sup>8</sup>. Phosphorus was determined in aqueous solution of ashed sample by colorimetric method<sup>9</sup>.

Total phenols were extracted from the flour of the kernel and seed coats, separately with 80 per cent ethanol, filtered and quantified spectrophotometrically<sup>10</sup>. L-DOPA was extracted and estimated<sup>11</sup> and tannins were also extracted<sup>12</sup> and spectrophotometrically estimated<sup>13</sup>. Trypsin inhibitor activity (TIA) of kernel flour extract alone was measured<sup>14</sup>. One unit of activity corresponds to that amount of trypsin inhibitor which gave a 50 per cent inhibition of trypsin enzyme activity under the experimental conditions. Assay for haemagglutinating activity with erythrocytes from human blood groups A, B and O was carried out<sup>15</sup> and the albumin and globulin protein fractions (as obtained under seed protein fractionation) were used for carrying out the assay for haemagglutination.

The chemical composition of seeds is shown in Table 1. The data on total (true) proteins and different fractions of seed proteins are shown in Table 2. The data on antinutritional factors are shown in Table 3 and that of haemagglutinating activity in Table 4. The contents of crude protein, crude fibre and ash are higher than that of the most common pulses consumed in India<sup>16,18</sup>. Food legumes have been recognised as an important source of several minerals in Indian diets<sup>19</sup>. The minerals, potassium, phosphorus, magnesium, iron, zinc, lead and manganese occur in significant amounts compared to NRC/NAS recommended values<sup>20</sup>. The seed protein fractionation data are in agreement with the data of most common pulses<sup>21</sup>.

The usefulness of legumes is decreased by the presence

TABLE 1. CHEMICAL COMPOSITION OF MATURE SEEDS OF *ENTADA SCANDENS*

Moisture (%)	7.80 ± 0.12 <sup>a</sup>
Crude protein (N×6.25) (%)	23.37 ± 0.21 <sup>a</sup>
Ether extract (%)	3.11 ± 0.09 <sup>a</sup>
Crude fibre (%)	20.08 ± 0.24 <sup>a</sup>
Ash (%)	4.60 ± 0.11 <sup>a</sup>
Nitrogen free extractives (%)	48.84
Starch (%)	44.33 ± 0.31 <sup>a</sup>
Sodium (mg/100 g seed flour)	76.00 <sup>b</sup>
Potassium (mg/100 g seed flour)	3030.00 <sup>b</sup>
Phosphorus (mg/100 g seed flour)	338.00 <sup>b</sup>
Calcium (mg/100 g seed flour)	140.00 <sup>b</sup>
Magnesium (mg/100 g seed flour)	756.00 <sup>b</sup>
Iron (mg/100 g seed flour)	27.00 <sup>b</sup>
Zinc (mg/100 g seed flour)	19.00 <sup>b</sup>
Lead (mg/100 g seed flour)	10.00 <sup>b</sup>
Manganese (mg/100 g seed flour)	5.00 <sup>b</sup>
Copper (mg/100 g seed flour)	2.00 <sup>b</sup>

a - Values are mean of triplicate determinations

b - Values are of single determination

TABLE 2. PROTEIN AND ITS FRACTIONATION IN THE SEED FLOUR OF *ENTADA SCANDENS*

Protein fraction	(%)
Total protein	19.32 ± 0.19
Albumins	5.24 ± 0.10 (27.12)
Globulins	9.46 ± 0.14 (48.96)
Prolamins	1.21 ± 0.05 (6.26)
Glutelins	3.41 ± 0.08 (17.66)

Values are mean of triplicate determinations

Fig in parentheses are values in percent calculated taking seed protein as 100%.

TABLE 3. ANTINUTRITIONAL FACTORS

Antinutritional factors	Seed coat (g/100g)	Kernel (g/100g)
Total phenols	6.80 ± 0.12 <sup>a</sup>	0.56 ± 0.06 <sup>a</sup>
L-DOPA	2.55 ± 0.09 <sup>a</sup>	2.38 ± 0.08 <sup>a</sup>
Tannins	6.45 ± 0.21 <sup>a</sup>	Trace
TIA*	N.D	50.03 <sup>b</sup>

N.D = Not detected, a = Values are mean of triplicate determinations, b = Mean of duplicate determinations, c = Values of two independent experiments.

\*TIA = Trypsin inhibitor activity calculated as units/mg of extracted protein.

of toxic/antinutritional compounds associated with the large protein content in their seeds<sup>22</sup>. The antinutritional factors, phenols and tannins, are present in negligible quantities in the kernel. Trypsin inhibitor and haemagglutinins (lectins) present in the kernel are heat labile<sup>23</sup> and their potential hazards can be overcome by moist heat treatment or auto-

TABLE 4. HAEMAGGLUTINATING ACTIVITY OF ALBUMIN AND GLOBULIN PROTEINS

Protein fraction	Erythrocytes from blood group	Haemagglutinating activity
Albumin	A	-
Albumin	B	+
Albumin	O	+
Globulin	A	++
Globulin	B	++
Globulin	O	++

- = No clumping, pellet dispersed easily.

+ = Some clumping, pellet partially dispersed,

++ = No dispersion of pellet.

claving. The possible toxic/antinutritional properties of several non-protein amino acids including L-DOPA in legumes are meagrely understood. The L-DOPA content in the seeds of *E. scandens* is very low compared to that of another tribal pulse, *Mucuna utilis*<sup>7</sup>.

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## ADVERSE EFFECT OF MICROBIAL POLLUTANTS OF PADDY STRAW ON THE GROWTH OF PADDY STRAW MUSHROOM

A.V. SATHE AND SANGITA DIGHE  
 Maharashtra Association for the Cultivation of Science,  
 Research Institute, Pune 411 004, India.

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In the present study, out of the five pollutant microorganisms tested, two namely *Pseudomonas aeruginosa* and *Bacillus subtilis* were seen to exert the inhibitory effect over the culture of *Volvariella volvacea* which may be the cause of the variation in the yields of paddy straw mushroom during its commercial cultivation and it could be counteracted by maintaining proper hygiene of the mushroom beds.

The paddy straw mushroom - *Volvariella volvacea* (Bull. : Fr.) Singer - is the most popular edible mushroom of the tropics and subtropics. The commercial cultivation of this mushroom, however, suffers from the drawback of variations in the total yields of mushroom<sup>1</sup>. Such a variation has been shown to occur due to the competition between the paddy straw mushroom and weed fungi like *Coprinus* species<sup>2,3</sup>.

During the work on isolation and purification of wild mushrooms from South-West India, it was noted that certain bacterial contaminants exerted antagonistic and inhibitory effects on the growth of paddy straw mushroom. The present study aims to confirm this observation. The microorganisms tested were *B. subtilis* (Ehren.) Cohn., *B. firmus* Bred & Werner in Werner, *E. coli* (Migula) Cast. & Chad., *M. roseus* Fluegge, *P. aeruginosa* (Schroe.) Migula and *P. alkaligenes* Mon. The cultures of these microorganisms were maintained on appropriate media till further use. The respective microorganisms as test organisms and *Volvariella volvacea* were both aseptically inoculated on sterilized malt extract agar (2 per cent each) medium in one and the same petri plate at radially opposite ends. In the case of *E. coli*, however, nutrient agar medium was used. The interaction between *V. volvacea* itself was used as the control for comparison. The plates were then incubated at 30°C for seven days. The antagonistic effect, if any, was then recorded using the ratio of the radius of the colony of *V. volvacea* in the control plate over its radius in the test plate opposing the test colony as the indicator of suppression. The width of the inhibition zone was also recorded.

Out of the six test organisms used in the present experiments, only *P. aeruginosa* and *B. subtilis* were seen to exert an inhibitory effect over the paddy straw mushroom

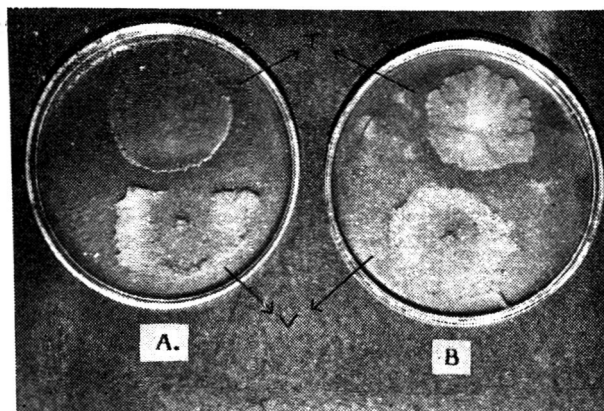


Fig. 1. Antagonism between *Volvariella volvacea* (V) and: IA: *Pseudomonas aeruginosa* (T) & IB: *Bacillus subtilis* (T)

TABLE 1. INHIBITION INDICES OF *PSEUDOMONAS AERUGINOSA* AND *BACILLUS SUBTILIS*

No.	Test organism	Inhibition zone (mm)	Inhibition index
1.	<i>P. aeruginosa</i>	6.13	2.00
2.	<i>B. subtilis</i>	8.18	1.25

Other test organisms showed no inhibition zone.

(Table 1 and Fig. 1). These two bacteria are reported to be common inhabitants of soil, air, water and skin and could easily gain access and thus contaminate the paddy straw<sup>4</sup>. Thus, not only the nutritional competition by weed fungi like *Coprinus*, but also the inhibitory effects of common bacterial contaminants like *B. subtilis* and *P. aeruginosa* are the possible factors responsible for causing the variation in the yield of paddy straw mushroom and use of the proper pasteurized paddy straw could preclude their inhibitory effects.

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## UTILIZATION OF COW MILK CASEIN IN RASOGOLLA PREPARATION\*

SURESH KUMAR AND C.M. KAPOOR  
 Department of Animal Products Technology,  
 Haryana Agricultural University,  
 Hisar - 125 004, India.

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Chhana can easily be replaced by 100% with casein obtained from skimmed cow's milk and vegetable oil without having adverse effects of the quality of the product. The acceptability of the product at 100% level ( $L_5$ ) was better than the product made from Chhana. The yield of Rasogolla was between 4.0 and 6.2 kg from 1 kg mixture of Chhana and casein. The yield was maximum at 100% ( $L_5$ ) and minimum at 50% ( $L_3$ ) level.

Rasogolla, is a famous Chhana based Bengal sweet, with complex physico-chemical characteristics. It is prepared from Chhana obtained preferably from cow's milk. Chhana is thoroughly kneaded along with the additives and then converted into small balls that are boiled in clarified sugar syrup. Many workers have reported the techniques of chhana and rasogolla preparation<sup>1,4</sup>, while others have studied the packaging, preservation and storage of the product<sup>4,5</sup>. Bureau of Indian Standards also brought out a specification (IS : 4079) on canned rasogolla<sup>6</sup>. Although preparation of rasogolla is largely in the hands of halwais, recently dairy industry has taken up its production on a limited scale.

In the present study, an attempt has been made to utilise casein (wet) obtained from skimmed cow milk with vegetable oil and in combination with Chhana obtained from cow's milk for the preparation of acceptable quality rasogolla.

Chhana was obtained from cow's milk standardised to 4 per cent fat by following the method described by De and Ray<sup>7</sup>. Casein (wet) was obtained from skimmed cow milk following the method described by N.D.R.I.<sup>8</sup> with some modifications of temperature of precipitation (70°C) and use of one per cent citric acid. Levels of chhana and casein for preparation of rasogolla were : chhana 100 per cent ( $L_1$ ); chhana 75 per cent + Casein 25 per cent ( $L_2$ ); chhana 50 per cent + Casein 50 per cent ( $L_3$ ); chhana 25 per cent + Casein 75 per cent ( $L_4$ ) and Casein 100 per cent ( $L_5$ ).

Rasogolla was prepared by following the method described by Soni *et al.*<sup>9</sup> with some modifications with respect to additives. Samples of chhana/casein and rasogolla were analysed for fat, protein, ash and total solids<sup>7,8</sup>. Total sugar was calculated by difference.

The samples of rasogolla were also analysed for Standard Plate Count (SPC) and coliform count<sup>6</sup>. The product was judged for its colour, appearance, body, texture and sponginess, taste and overall acceptability by a panel of 6 judges using 9-point Hedonic scale. Data obtained were analysed using Factorial Complete Randomized Design<sup>9</sup>.

The data on composition of milk, chhana and casein were in close agreement with the observations of different workers<sup>1,4</sup>.

The different recipes of rasogolla mix were standardized (Table 1). Refined vegetable oil was added to maintain the fat level. The average composition of fresh rasogolla is presented in Table 2. The fat percentage in rasogolla decreased significantly from  $L_2$  to  $L_4$  (CD = 0.2441). This variation may be due to the variation in fat holding capacity of different recipes. Bhattacharya and Des Raj<sup>2,3</sup> observed 7.5 and 8.42 per cent fat while the ISI<sup>6</sup> specifications stated minimum 5 per cent fat in the product. While significant increases in the protein and ash percentage were observed from  $L_2$  to  $L_5$  (CD = 0.0896) and  $L_1$  to  $L_5$  levels (CD = 0.0366),

TABLE 1. STANDARDIZED RECIPES OF RASOGOLLA

Levels	Constituents	Quantity (g)
$L_1$	Chhana	100.00
	Edible casein	0.00
	Arrowroot	11.50
	Vegetable oil (refined)	0.00
	Baking powder	2.13
$L_2$	Chhana	75.00
	Edible casein	25.00
	Arrowroot	12.50
	Vegetable oil (refined)	5.30
	Baking powder	2.25
$L_3$	Chhana	50.00
	Edible casein	50.00
	Arrowroot	12.75
	Vegetable oil (refined)	10.50
	Baking powder	2.50
$L_4$	Chhana	25.00
	Edible casein	75.00
	Arrowroot	13.10
	Vegetable oil (refined)	15.80
	Baking powder	2.75
$L_5$	Chhana	0.00
	Edible casein	100.00
	Arrowroot	13.50
	Vegetable oil (refined)	21.00
	Baking powder	3.10

\*Part of M.Sc. thesis work of first author, presented at Founder's Day of AFST, Hisar-Chapter, held on 16 Sept., 1989.

TABLE 2. COMPOSITION OF FRESH RASOGOLLA\*

Levels	Fat (%)	Protein (%)	Ash (%)	Total sugars (%)	Total solids (%)
L <sub>1</sub>	7.50	6.03	1.62	36.11	51.26
L <sub>2</sub>	7.60	6.11	1.72	35.19	50.65
L <sub>3</sub>	7.31	6.37	1.83	34.18	49.70
L <sub>4</sub>	6.96	6.50	1.94	35.06	50.46
L <sub>5</sub>	6.77	6.75	2.04	36.12	51.69
SEm	+0.0770	+0.0286	+0.0115	+0.0379	+0.0854
C.D. (1%)	0.2441	0.0896	0.0366	0.1214	0.2701

\*Average of three replication

TABLE 3. SENSORY EVALUATION OF FRESH RASOGOLLA\*

Levels	Colour	Appearance	Body texture & sponginess	Taste	Overall acceptability
L <sub>1</sub>	7.4	7.8	7.7	7.7	7.6
L <sub>2</sub>	7.1	7.3	7.2	7.3	7.2
L <sub>3</sub>	6.9	7.0	7.1	7.0	7.0
L <sub>4</sub>	7.4	7.4	7.6	7.4	7.4
L <sub>5</sub>	7.9	7.9	7.6	7.8	7.8
SEm	-0.1282	+0.1367	+0.2271	+0.0869	+0.0966
C.D. (1%)	0.4067	0.4330	0.7194	0.2751	0.3032

\*Average of three replication

respectively. This increase was because of higher protein level in casein which quantitatively increased from L<sub>2</sub> to L<sub>5</sub>. Increased ash is due to the fact that there was an increase in the baking powder content in recipes from L<sub>1</sub> to L<sub>5</sub> (Table 1). Total sugars decreased significantly from L<sub>1</sub> to L<sub>3</sub>, while total solids increased significantly from L<sub>3</sub> to L<sub>5</sub> (CD = 0.1214 and CD = 0.2701, respectively). The present findings in respect of fat, protein, total sugars and total solids are in close agreement with the observations of Bhattacharya and Des Raj<sup>2,3</sup> and ISI<sup>6</sup>.

There was non-significant increase of SPC (23 to 32 × 10<sup>3</sup> (CD = 3.4715) and coliform (14 to 20 counts/g in rasogolla from L<sub>1</sub> to L<sub>5</sub> level (CD = 4.1722). As per the ISI<sup>6</sup>, SPC in sugar syrup should not be more than 50 × 10<sup>3</sup>/g and free from coliform count. SPC count in this study was within the desirable limits. But in the present study, a low level of coliform count was observed in the product.

On the basis of overall acceptability, taking 6.0 as the minimum level in 9-point Hedonic scale, the product was found to be acceptable at all the levels. For colour, appearance, body texture and sponginess, taste and overall acceptability, highest score was obtained for L<sub>5</sub>, L<sub>5</sub>, L<sub>1</sub>, L<sub>5</sub> and L<sub>5</sub> respectively whereas minimum score was observed in L<sub>3</sub> for all parameters. (Table 3). Acceptability of L<sub>5</sub> level

was significantly higher than the other levels (CD = 0.3032). Results showed that acceptability of rasogolla prepared from casein (wet) obtained from skimmed cow's milk was better than rasogolla obtained from other levels of combinations.

The cost of rasogolla prepared from cow's milk casein is much cheaper than the product prepared from cow's milk chhana.

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## PREPARATION OF COCONUT MILK

K.K. AGRAWAL

College of Agriculture, Raipur (M.P), India

P.L. CHOUDHARY

College of Dairy Technology, Raipur (M.P), India.

AND

V.S. SHARMA

TISCO, United Republic of Tanzania, Tanzania

*Received 30 October 1989; revised 30 April 1990*

**Coconut milk is the product which is prepared by blending skim milk powder with coconut milk of freshly grated coconut and pasteurized at 70-72°C for 10 min. It contains 6% skim milk powder (SMP) and 9.65% total solids on sterilization and it can be utilized as coconut flavoured milk in food industry.**

Coconut milk is the product made from non-fat milk solids either of liquid or powder origin, in which vegetable fats or oils have been incorporated in approximately the same proportion as in the butter fat of fresh milk. Vitamins are normally added to make-up for those lost in the extracted fat<sup>1</sup>. The idea of preparing coconut milk is originally thought to have arisen due to the economic factor. Thus, in simple term, the preparation of coconut milk involves, basically a substitution of animal fat in milk by vegetable fat and manipulating the processing parameters to get a product similar to standard milk.

In the present work, an attempt has been made to prepare coconut milk obtained by pressing the freshly grated coconut. The materials used were, fresh mature coconut, skim milk powder, buffer salts and commercial crystal sugar.

*Preparation of pasteurized coconut milk:* Dehusked whole coconuts were broken into halves. The shell was peeled off from the kernel using a hand sheller. The brown testa was then removed from the coconut meat. The white meat was passed through a Krausmaffeii cutter which has a metal sieve plate (3 mm diameter holes) through which the shredded meat is forced out. The disintegrated material was squeezed by hand using a cheese cloth to press out the coconut milk.

The coconut milk was diluted with water. Skim milk powder (SMP), buffer salts and sugar were added to adjust the fat and solids-not-fat (SNF) contents and blended in a mechanical blender. The milk thus obtained was pasteurized at 70-72°C for 10 min.

*Sterilized coconut milk:* The hot pasteurized coconut milk was homogenized by passing through a homogeniser (Taj Homoz) at 76°C operated at a pressure of 1500-2000 lb. The homogenized milk was filled hot into pre-sterilized bottles

(200 ml capacity) at a temperature of 70-72°C. The bottles were immediately crown corked, autoclaved at 14.223 POU 121°C for 30 min and cooled gradually. The sterilized milk was stored at room temperature (28-30°C) to see if there was separation of the fat emulsion or any other spoilage during storage.

The total solids (T.S.) and fat contents of the coconut milk were estimated by A.O.A.C. method<sup>2</sup>. The fat content of the fresh mature coconut was estimated by Soxhlet method and the total solids were calculated by hot air oven method<sup>2</sup>. The stored coconut milk was sensorily evaluated by experts of 6 judges by single sample (monadic) test. The statistical analysis was done for calculation of standard deviation.

The milk extracted had a fat content of 37-38 per cent and an average total solids content of 48 per cent. For the preparation of coconut milk, it was essential to dilute the coconut milk due to the high fat content in it. The diluted milk was used along with different amounts of S.M.P. for the preparation of coconut milk. The undiluted coconut milk coagulated on heating to a temperature of about 80°C. The diluted milk also showed phase separation on standing. Hence it was necessary to stabilize the emulsion.

It was observed that two stages (1st stage-2500 POU-63°C and second stage 500 POU-74°C) homogenization of the diluted milk gave a stable emulsion which did not separate on heating. It was further noticed that there was no phase separation when the homogenized, diluted coconut milk was subjected to centrifugation at 2500 r.p.m. for 10 min. This stabilization may be due to better emulsification on homogenization. S.M.P. was added for the preparation of coconut milk. Addition of skim milk powder helped in the stabilization of fat emulsion as well as providing body to the coconut milk. Hence S.M.P. was added at various levels ranging from 2 to 10 per cent to the diluted milk before homogenization. It was found that less than 6 per cent S.M.P. in the formulation did not provide sufficient body. The protein and fat contents of the coconut milk containing 6 per cent S.M.P. were 2.34 and 3.8 per cent, respectively. The total solids content of milk was 9.65 per cent on sterilization at 14.223 p.s.i. for 30 min and there was no coagulation or destabilization or thickening of the emulsion. The sterilized milk stored well for a period of 4 months without spoilage or thickening. Data on the fat and total solids contents of bottled coconut milk with different levels of added S.M.P. are given in Table 1.

Coconut milk is an emulsion of oil, water and proteins. Usually, the emulsion separates on heating and the proteins are coagulated.

The studies indicate that it is possible to prepare heat stable coconut milk by using of SMP because it has 35 per cent protein including heat stable casein protein. It is not necessary

TABLE I. BOTTLED COCONUT MILK

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SMP added (%)	Fat content (%)	Total solids (%)
0	3.7	5.20
2	3.6	5.42
4	3.7	7.47
6	3.8	9.65
8	3.7	11.35
10	3.6	13.14

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Replication = Three. Treatment = Six for both fat and T.S.  
S.E. = 0.272 (fat); S.E.D. = 0.233 (T.S.)

to use buffer salts in the formulation of an acceptable coconut milk; but it is necessary that the formulation should have 6 per cent S.M.P. The sterilized coconut milk can be kept at room temperature for more than four months, without any apparent thickening or coagulation, separation of phase or any other spoilage and it can be used for the preparation of flavoured milk.

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## LOW ALCOHOLIC BEVERAGES FROM CULLED APPLES

VISHAL SINGH BARWAL

Regional Fruit Research Station,  
Mashobra, Shimla - 171007 (HP), India

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To utilize culled apple produced from different cultivars in Himachal Pradesh in large amounts, an attempt was made to prepare apple cider and wine by fermentation of apple juice and pomace. Physio-chemical and sensory evaluations indicated that cider and wine prepared from apple (*Malus domestica* Borkh). cultivars 'Golden Delicious' and Rus Pippin were preferred most and were at par with 'Royal Delicious' (except wine) in all the attributes. Other cultivars differed significantly.

Apple is a principal horticultural crop of Himachal Pradesh. Its area and production have increased since 1960-61<sup>1</sup>. However, many times the production of apple in Himachal Pradesh is adversely affected by natural calamities like drought, hailstorm, and excessive rains, which lead to deformities in/on the fruit. Consequently, there is no market for such fruit (unfit for table purpose) and they usually go waste. As per the estimate, the loss is about 25 per cent of total produce<sup>2</sup>. The present study was, therefore, undertaken to utilize unmarketable apple for the preparation of apple beverages.

Apple pomaces is the residue left after extraction of juice and contains 9 to 22 per cent fermentable sugars depending on the variety<sup>3</sup>. These sugars are converted into ethyl alcohol by fermentation and utilized for ameliorating alcohol contents of apple wine to acceptable level. A good apple cider

and wine could be a viable alternative for the utilization of culled apple.

Culled apple (small, hailed, deformed and bruised) of 7 cultivars were collected on different dates depending upon the harvesting of cultivars. Fruits were sorted manually to discard rotten portion, washed in sufficient water to remove dust and cut into four pieces to extract juice in Electrocom juice extractor. Juice was clarified by filtration through muslin cloth and preserved by pasteurization.

Fresh pomace was boiled with four times water for 20 min and filtrate was inoculated with 5 per cent active culture of *Saccharomyces cerevisiae* yeast. Must was allowed to ferment completely at 25°C. After fermentation, it was distilled and redistilled to adjust alcohol contents to 40 per cent by weight.

Active culture of *Saccharomyces cerevisiae* yeast was added to apple juice of seven different apple cultivars at a rate of 5 per cent to initiate fermentation at 25°C. The fermentation was stopped by filtration and pasteurization, when total soluble solids (T.S.S.) fell to 6° Brix. Half cider produced from each cultivar was blended with pomace alcohol to produce wine containing 9 per cent alcohol by weight.

T.S.S. content was recorded by using (DBX-50, Atago) refractometer. Alcohol content was estimated by using alcoholometer (Gay Lussac-Cartier). Titrable acidity was measured by using N/10 NaOH. Apple beverages (juice, cider and wine) were evaluated for general appearance, taste and overall quality on a 5-point grading scale by a panel of 10 judges. The samples were coded and replicated thrice. Experiment was conducted three times. Statistical analysis of the data were done according to the randomized block design<sup>4</sup>.

Chemical characteristics of juice, cider and wine are presented in Table 1. Sensory scores for juice, cider and wine made from different apple cultivars are presented in Table

TABLE 1. MEAN CHEMICAL CHARACTERISTICS SCORES OF APPLE BEVERAGES FROM DIFFERENT APPLE CULTIVARS.

Varieties	Juice		Cider		Wine	
	TSS (°Brix)	Acidity (% MA)	Rate of fermentation (fall °B/- day)	Acidity (% MA)	Alcohol (% wt)	Volatile (% AA)
Royal Delicious	13.5	0.24	0.95	0.35	3.5	0.012
Starkrimson	12.0	0.21	0.83	0.28	3.0	0.015
Lord Lambourne	9.4	0.36	0.80	0.48	1.5	0.022
Red Gold	11.0	0.36	0.90	0.42	2.0	0.020
Golden Delicious	12.5	0.32	0.95	0.37	3.0	0.019
Rus Pippin	14.0	0.38	0.95	0.40	3.5	0.020
Granny Smith	13.0	0.60	0.83	0.73	3.0	0.24
CD at 5%	0.88	0.04	0.05	0.04	0.67	0.007

MA : Malic acid

TABLE 2. QUALITY OF APPLE BEVERAGES FROM DIFFERENT APPLE CULTIVARS AS JUDGED BY SCORING

Varieties	Juice			Cider			Wine		
	Appearance	Taste	Overall quality	Appearance	Taste	Overall quality	Appearance	Taste	Overall quality
Royal Delicious	4.8 <sup>ab</sup>	4.8 <sup>a</sup>	4.8 <sup>a</sup>	4.1 <sup>ab</sup>	4.7 <sup>a</sup>	4.3 <sup>a</sup>	4.1 <sup>ab</sup>	4.0 <sup>b</sup>	4.2 <sup>b</sup>
Starkrimson	4.9 <sup>a</sup>	4.8 <sup>a</sup>	4.8 <sup>a</sup>	4.2 <sup>ab</sup>	4.1 <sup>a</sup>	4.1 <sup>a</sup>	4.3 <sup>a</sup>	4.0 <sup>b</sup>	4.1 <sup>b</sup>
Lord Lambourne	4.1 <sup>c</sup>	4.0 <sup>b</sup>	4.2 <sup>b</sup>	2.5 <sup>c</sup>	3.5 <sup>b</sup>	3.6 <sup>b</sup>	2.5 <sup>c</sup>	3.5 <sup>bc</sup>	3.1 <sup>d</sup>
Red Gold	4.7 <sup>ab</sup>	4.1 <sup>b</sup>	4.1 <sup>c</sup>	3.6 <sup>b</sup>	3.2 <sup>b</sup>	3.5 <sup>b</sup>	3.7 <sup>b</sup>	3.4 <sup>c</sup>	3.5 <sup>c</sup>
Golden Delicious	4.6 <sup>b</sup>	4.7 <sup>a</sup>	4.7 <sup>a</sup>	4.4 <sup>a</sup>	4.5 <sup>a</sup>	4.5 <sup>a</sup>	4.3 <sup>a</sup>	4.7 <sup>a</sup>	4.7 <sup>a</sup>
Rus Pippin	4.1 <sup>c</sup>	4.6 <sup>a</sup>	4.4 <sup>b</sup>	4.0 <sup>ab</sup>	4.4 <sup>a</sup>	4.3 <sup>a</sup>	4.1 <sup>ab</sup>	4.8 <sup>a</sup>	4.6 <sup>a</sup>
Granny Smith	4.0 <sup>c</sup>	3.7 <sup>c</sup>	3.9 <sup>c</sup>	2.5 <sup>c</sup>	2.8 <sup>b</sup>	2.8 <sup>c</sup>	2.6 <sup>c</sup>	2.9 <sup>c</sup>	3.0 <sup>d</sup>

Sensory mean scores for individual quality attributes:

5 = Excellent, 4 = Very Good, 3 = Good, 2 = Fair, 1 = Poor.

Means with same superscript in each column do not differ significantly (P = 0.05)

2. The scores for appearance, taste and overall acceptability were highest for 'Starkrimson' and 'Royal Delicious' juice followed by 'Golden Delicious'. It could be due to high T.S.S./acid ratio. Similarly, the lower values depicted in Table 2, for other cultivars correspond to their T.S.S./acid ratios. The scores for taste and overall quality were highest and statistically non-significant for 'Royal Delicious', 'Golden Delicious' and 'Rus Pippin' cider. It could be because of the astringent taste developed due to formation of more ethyl alcohol in sweet cultivars<sup>5</sup>.

Grading of wine was highest for all characteristics for 'Golden Delicious' and 'Rus Pippin' cultivars. In subacidic and high T.S.S. cultivars probably ameliorated alcohol to 9 per cent (by weight) resulted in better grading for all attributes.

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## NUTRITIONAL AND TOXICOLOGICAL EVALUATION OF *PLEUROTUS* SPP.

S. MUKTA SINGH, R.N. VERMA

Division of Plant Pathology, ICAR Research Complex for NEH Region,  
Umroi Road, Barapani - 793 103, India.

AND

K.S. BILGRAMI

P.G. Department of Botany, Bhagalpur University, Bhagalpur 812 007, India.

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Two wild mushrooms viz. *Pleurotus djamor* and *P. platypus* collected from North Eastern Hill regions of (NEH) India and cultivated on paddy straw, were analysed for their proximate composition and tested for their edibility. The sporophores of *P. djamor* and *P. platypus* contained 14.10 and 16.86% crude protein and 2.75 and 2.59% crude fat respectively. Feeding the dry powder of the sporophores as supplement to Swiss albino mice led to a significantly higher body weight in case of *P. platypus*. However, no toxic effect of the test fungi either on the morphology or histology of the vital organs of the animals were noticed.

The North Eastern Hills of India abound in wild fleshy fungi, many of which are edible and consumed by the local inhabitants. No information is however, available on their taxonomy, edibility and nutritional composition. An attempt was therefore, made to identify the common edible species of fungi and to study the nutritional composition of those which could be cultivated. Several of the edible species have earlier been reported to contain cardiotoxic protein such as Volvatoxin, Flammutoxin isolated from *Volvariella volvacea* and *Flemmulina velutipes* respectively<sup>1,2</sup>. It is, therefore, essential to be vigilant so that types which could produce harmful toxic protein may not enter the chain of commercial propagation<sup>3</sup>. During the present study, therefore, feeding of two *Pleurotus* spp viz. *P. djamor* and *P. platypus* was carried out with Swiss albino mice (*Mus musculus*) with a view to ascertain whether these wild species had any toxic effect on the test animals.

Pure cultures of the test fungi were obtained from freshly collected fruit bodies by tissue culture technique. The cultures were maintained on potato dextrose agar. Maize grain spawn was used for inoculation of paddy straw substrate. Modified Till's cube culture method of *Pleurotus* cultivation was employed for growing the fungi with paddy straw as substrate. All the optimum cultural practices were followed. Fruiting occurred within 15 days of spawning. Mature fruit bodies were harvested and dried in a hot air oven at 50°C. Total nitrogen was determined by microkjeldahl method and factor 4.38 was

used to calculate crude protein. Total ash, crude fibre and crude fat were determined by AOAC methods<sup>4</sup>.

To test their edibility/toxicity, dried mushroom powders were given to Swiss albino mice (*Mus musculus*). Three to four weeks old healthy mice (both male and female) each weighing 7-15g were randomly divided into 3 groups of six animals each and kept in separate cages. The stock was received from the Institute of Veterinary Biologicals, Animal Husbandry Department, Khanapara, Guwahati. The normal diet (cooked rice and soaked Bengal gram) for the first and second groups was supplemented with dry mushroom powder of *P. djamor* and *P. platypus* respectively. The 3rd group fed, on normal diet served as control. All 3 groups of animals were put on normal diet for one week before starting the experiment. Each animal was given 10g of soaked Bengal gram in the evening everyday. On the first day, the diet, of the first and second groups was supplemented with 3.3 per cent of dry mushroom powder. It was increased to 4, 7.5 and 12.5 per cent on the second, third and fourth day, respectively. From 5th day onwards, the diet was supplemented with 20 per cent dry mushroom powder for one month. The diet was also adequately supplemented with minerals and vitamins at weekly intervals<sup>5</sup>. All the animals were allowed free access to water. Body weight of the animals were recorded at 15 and 30 days after feeding. The animals were slaughtered on the 31st day and their vital organs such as brain, lungs, liver, kidney and heart were examined for any morphological changes before fixing in 4 per cent formalin solutions. For histological studies, the organs were washed in running water overnight and processed and mounted in paraffin blocks. Sections (6 µ m) of the organs cut with a rotary microtome were stained in haematoxylin and eosin and observed critically for any histological changes.

Results (Table 1) indicated that both the test fungi were of medium nutritional composition as compared to other fleshy fungi studied earlier<sup>6,9</sup>. Of the two, *P. platypus* was richer in protein and less fibrous than *P. djamor*. Data in Table 2 indicated that the test animals fed on *P. platypus* attained

TABLE 1. PROXIMATE ANALYSIS OF THE SPOROPOHORES OF *PLEUROTUS* SPECIES

Constituents	<i>P. djamor</i>	<i>P. platypus</i>
Moisture	91.70	92.69
Crude protein (N × 4.38)	16.86	14.10
Crude fat (ether extract)	2.75	2.59
Crude fibre	13.60	7.79
Ash	9.12	8.62

All data (average of three replicates) presented as % of dry wt except initial moisture content (% of fresh wt).

TABLE 2. BODY WEIGHTS OF THE TEST ANIMALS AFTER FEEDING WITH *PLEUROTUS* SPECIES

Treatments	Body wt (g) during indicated feeding period		
	0 Day	15 Days	30 Days
Control	9.32	22.82	31.50
<i>Pleurotus djamor</i>	10.17	23.78	31.83
<i>Pleurotus platypus</i>	11.28	22.83	33.67
Source	S.E.	C.D. at 5%	
Day	0.70	2.01	
Mushroom	0.70	2.01	
Interaction	1.21	3.49	

significantly higher body weight than on *P. djamor* and control after 30 days. This obviously indicates that the mushroom supplemented feed did not have any adverse effect on the test animals rather it proved more nutritious than the control diet, at least in case of *P. platypus*. The same conclusion could be derived from the results of the morphological and histological examinations of the vital organs, since no abnormality was recorded in any of the organs of the test animals. Similar increase in body weight and non-toxic effects were recorded by other workers also<sup>10-11</sup>.

Authors are thankful to the Director, ICAR Research

Complex for NEH Region, Shillong for laboratory facilities and ICAR, New Delhi for financial assistance.

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### Prof. J. V. Bhat — Eureka Forbes Award

Prof. J.V. Bhat Memorial Committee organised the award function at the UDCT, Bombay on 29th June 1991. The Award amount is Rs.10,000 and it is for excellence in Research in Microbiology conducted in Indian Laboratories over the last five years (1986-1990). The award was shared by Dr. L.V. Venkataraman of the Central Food Technological Research Laboratory, Mysore and Dr. S.K. Apte of the Bhabha Atomic Research Centre, Bombay.

*Radiation preservation of fish and fishery products: Technical Reports Series No. 303*: International Atomic Energy Agency, Vienna, PP: 139; 1989, Price: Not indicated.

This book presents the work conducted under a coordinated research programme on radiation preservation of fish and fishery products in several Asian countries supported by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. It contains research summaries on irradiation disinfestation of dried and semi processed fish, combination treatment of irradiation and other processes on fishery products and economic feasibility of radiation preservation of dried fish. An overview is also given highlighting the essential observations of the studies. Shelf life extension by radiation of fresh fish under ice is not, however, included in this publication.

A sizeable amount of fish landed in tropical countries is preserved by drying, curing and smoking. However, due to high ambient temperature and humidity prevalent in these countries, the processed fish is susceptible to rapid spoilage not only by insects but also by moulds. While mould growth could be controlled by reducing the moisture level to about 20%, insect infestation during processing is a major problem that limits the shelf life of these products even if held under ideal packaging during storage. Exposure of such packaged fish to low doses of gamma radiation can eliminate the eggs and larvae of insects, reduce microbial load and give enhanced shelf life for the products. Chapter 2 by Dollar provides information on storage losses associated with current processing practices, packaging, transportation and retailing of dry fishery products in tropical countries and economic feasibility of disinfestation of the products by gamma irradiation. Energy input for radiation treatment is less as compared with chemical fumigation.

Detailed economic analysis on radiation preservation of dried and cured fishery products and also of onions and potatoes with respect to Bangladesh has been given by Ahmed and colleagues (Chapter 3). Another group led by Ahmed (Chapter 6) also examined the radiation disinfestation of sun dried fish in the country. A dose of 0.3 KGy could control infestation of dried mackerel by earwigs, hide beetles and copra beetles. The exhaustive data collected by the authors establish the usefulness of gamma irradiation to control perishability of the commodity in Bangladesh. While the process is economically feasible in the country, with international acceptance of guidelines on process and storage parameters, as well as quality standards, trade in irradiated dry fish may become a reality.

Combination treatments involving dip in potassium sorbate, dehydration followed by gamma irradiation could extend the

shelf life of fishery products. One such item well examined is fresh water fish, rahu (*Labeo* sp.) which could be preserved up to 6 months at room temperature when given a dip treatment in 2% sorbate, dehydration to 40% moisture content, irradiation at 0.5 to 0.75 KGy and packaging in polyethylene pouches (Hussain *et. al.* Chapter 4). Similar processes could also be applied with suitable modifications to enhance the shelf life at room temperature of salted chub mackerel (15 weeks) boiled chub mackerel (5 weeks) and smoked milk fish (20 days) as observed by Maha and his group (Chapter 5). Moisture content in these products is a critical parameter that can influence their safety and shelf stability. While a moisture control of about 20% can control microbial proliferation, higher moisture level may jeopardise the microbial safety of the products by favouring growth of not only radiation survived moulds but also of bacteria including pathogens. Thus, bacterial proliferation was observed in irradiated dried mackerel having moisture contents varying from 41 to 48% when stored at  $30 \pm 2^\circ\text{C}$  (Guevara *et al*, Chapter 1).

This publication will serve as a reference book for commercialisation of radiation processes for preservation of dried fish and fishery products.

V. VENUGOPAL  
B.A.R.C., BOMBAY

*Omega - 3 Fatty Acids in Health and Disease*: Ed. Robert S. Lees and Marcus Karel Marcel Dekker, Inc., 1990. PP: 328; Price: \$99.75 (US & Canada) \$119.50 (all other countries).

This book is part of a vast enterprise to define the role and functions of omega -3 fatty acids in health and disease. It discusses several major aspects of the relationship between fish oil and human health.

The first part of this book deals the impact of dietary fat on human health, citing the experimental and clinical evidences. The beneficial and damaging effects by consumption of fish or fish oil have been vividly elaborated. Dietary intake of omega-3 fatty acid is shown to (a) reduce the death rate from coronary artery disease (b) retard the development of experimental coronary atherosclerosis (c) reduce the plasma triglycerides in patients with hypertriglyceridemia (d) reduce blood pressure in patients with hypertension (e) decrease joint pain and increase the mobility in patients with rheumatoid arthritis (f) increase bleeding time (g) impair the stimulation of insulin in diabetic patients. Further the book explores the epidemiological evidences concerning the consumption of fish and health with reference

to low incidence of cardiac vascular disease and cancer in Greenland Eskimos, Kitasoo Indians and Okinawa Japanese.

The second part of this book deals with science, technology, economics and the delivery of omega-3 fatty acids to the consumers. Further, it presents the sources and the amount of this fatty acid in common fishes and shellfishes within the traditional dietary sources. Microalgae is suggested for tapping this acid by enhancing the productivity through genetic manipulations. The methods for producing purified fish oil, the problems with standardization and preservation of the relatively unstable omega-3 fatty acid and the strategy for the regular supply to the consumers are well documented.

In conclusion, there are informative contributions in this book. The topics covered are up-to-date and the objectives are set defining its contents. The style is lucid and readable. The book presents the current knowledge very comprehensively with good references. The book can serve as a useful guide to food scientists and technologists, biochemists, chemists, nutritionists, pharmacologists, toxicologists and medical scientists.

R. SELVAM,  
UNIVERSITY OF MADRAS, MADRAS.

'Recent Achievements in Engineering - Cryoprague 86'  
Supplement of International Institute of Refrigeration -  
Proceedings of Meetings of Commission A 1/2 held at  
Prague during 8-12 September 1986, PP.215, Price: not  
mentioned.

The proceedings have been classified into four themes, covering a total of 30 paper presentations.

#### I. Magnetic separation using superconducting magnets:

Ten papers are published under this theme covering various aspects of superconducting magnetic separation and its application in ceramic and mining industries. Cryogenic system for cooling of the superconducting windings, use and handling of liquid helium, liquid nitrogen, mathematical modelling of superconducting systems, their advantages etc. have been discussed.

#### II. Cryogenic aspects of the NMR imaging and other medical applications:

Three papers are dealt under this theme, covering specifications for superconducting magnets, helium cryostat, biological and technical side effects, examples of NMR imaging superconducting magnet designs and methods. Cryosurgical appliances for the treatment of tumorous diseases by local undercooling - viz. surgery, radiotherapy, chemotherapy, thermotherapy, cryotherapy besides special ways of handling cryogenic products in medicines and biological research have been discussed.

#### III. Refrigerants (Including miniature and magnetic refrigerators)

Three papers are published under this theme, giving an overview of the magnetic refrigeration which has wide scope for future application. Wave expansion of air and helium at low temperatures and helium expansion turbines have been discussed.

#### IV. Other new developments and achievements in cryo-engineering below 20 K:

Fourteen papers are discussed under this theme concerning R&D on cryogenics and large superconducting magnets associated with nuclear fusion projects. Thermal and magnetic correlation of apparent strain in Karma strain gauges in cryogenic environments, application of SQUIDS for bio-magnetism, geophysics, NMR and squid, properties of insulating materials at 4.2 K and also super conductors have been discussed at length.

The supplement gives an insight into the role of cryogenics as an adjunct to various industrial and medical applications for attaining high degree of economy and efficiency. The book provides advanced information on cryogenics application for those who are engaged in the R&D work in the field.

H. KRISHNA MURTHY  
C.F.T.R.I., MYSORE

*Chemical Senses. Vol.2 Irritation:* (Eds) Barry G. Green, J. Russel Mason and Morley R. Kare, Marcel Dekker Inc, 270, Madison Avenue, New York, N.Y. 10016, 1990; PP: 361, Price: US \$ 99.75 (US and Canada) \$ 119.50 (All other countries).

The publication is the outcome of the first scientific meeting devoted exclusively to the discussion of sensitivity of the oro-nasal region to chemical irritants held at Monell Chemical Center during June 1988. The effort marks the beginning of documenting the understanding of the reaction of human system to chemical irritants. Studies on chemical irritations are very sparse. Only some investigations on the sensitivity of the skin and mucous membranes to chemical irritations have been sporadically reported. The present publication cuts across the areas of neuroanatomy, neurophysiology, respiratory physiology, psychophysics, animal behaviour and food science, the diversity reflecting the broad relevance.

The scientific perspectives from neurophysiology to psychophysics have been brought together. Discussions are devoted exclusively to chemical irritants and the study of the sensitivity of the oro-nasal regions to the various irritants. Outcomes of studies on anatomy and pharmacology of nerve fibres and the psychology of acquisition of preference have been lucidly presented. Reports on relationships between chemical nociception, respiratory function and airway



irritation have been included. Attempts have been made to describe individual differences in chemical nociception. The need for development of specific terminology for describing the chemical sensitivities has been highlighted. The role of nociceptors in the detection of endogenous and exogenous chemicals have been reported. The psychophysical perception of the irritants have been described and the causal relationships explored.

The first eight chapters deal mainly with the sensory physiology aspects viz. afferent and effector functions of peptidergic innervation of the nasal cavity, physiology factors in nasal trigeminal chemoreception, perceptual characteristics of nasal irritation, evidence for interactions between trigeminal afferents and olfactory receptor cells in the amphibian olfactory mucosa, trigeminal vs olfactory input for laryngectomized patients, responses of normal and anosmic subjects to odorants, brain responses to chemical stimulation of the trigeminal nerve in man, and, capsaicin,

irritation and desensitization. The next six chapters highlight the psycho-physical perception and the last one potential uses of irritants as bird repellents. The psychophysical perceptions dealt with are - the effects of thermal, mechanical and chemical stimulation on the perception of oral irritation, differences between and interactions of oral irritants, personality variables in the perception of oral irritation and flavour, acquiring a preference for irritants, nasal sensation for the benefit of industrial workers and consumers. The last chapter is on the effectiveness of potential irritants on the food consumption of birds and their repellent effects.

On the whole, the book contains a lot of information and provides food for thought in the rapidly growing field of chemoreception. It is an excellently brought out publication which should be of interest to the multidisciplinary users.

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

#### SEMINAR

A seminar on 'Prospects of Potato Processing Industry in India' will be organised during September 1991 by the Calcutta Chapter of the Association.

For details contact: Dr. Pratap Chakraborty, Hon. Secretary, AFST(I) Calcutta Chapter Secretariat: Department of Food Technology and Biochemical Engineering, Jadavpur University, Calcutta - 700 032.

#### CONFERENCE ON FOOD INGREDIENTS EUROPE

VI Food Ingredients Europe, Exhibition and Conference on 'Ingredients, Additives and Services related to the Food Industry' will be held from October 8-10, 1991.

For details, contact: Judith J. Markus, Expoconsult, P.O. Box 200, 3600 AE Maarsse, The Netherlands.

#### ANNOUNCEMENT

A National Symposium on "SURVEILLANCE, PREVENTION AND CONTROL OF FOOD CONTAMINANTS" will be held at CFTRI, Mysore during 3-5 December 1991. Interested scientists may contact Dr. Badri N. Saxena, Senior Deputy Director General, Indian Council of Medical Research, Post Box 4508, Ansari Nagar, New Delhi - 110 029.

## ANNUAL GENERAL BODY MEETING 1990-91

The Annual General Body Meeting of the Association for the year 1990-91 was held on 11th June 1991 at the CFTRI, Mysore. The membership (including all categories) of the Association at the end of the year 1990 was 1916.

World Food Day was celebrated on 16th October 1990. A seminar on 'Processed Foods and Institutional Catering' was also arranged.

A National Convention on 'Application of Biotechnology in the Development of Food Processing Industries' was held from 10th-12th June 1991. It was attended by more than 250 delegates from all over the country. In the IXth IcFOST, 178 posters presentations were displayed by the delegates on various topics of Food Science and Technology.

## AFST(I) Fellowships

The Fellowship of the Association was conferred on the following persons:

1. Dr. L.V. Venkataraman, Past-President, AFST(I), Mysore.
2. Dr. S. Ranganna, Senior Scientist (Retired), CFTRI, Mysore.
3. Mr. V.B. Oberoi, Fruit Technologist, New Delhi,
4. Mr. H.C. Bhatnagar, Consultant, Mysore.
5. Prof. Sunit Kumar Mukherjee, Jadavpur University, Calcutta.

## AFST(I) Awards for the year 1990

Prof. V. Subrahmanyan Industrial Achievement Award was given to Dr. V.H. Potty, Deputy Director, CFTRI, Mysore.

Laljee Godhoo Smarak Nidhi Award was given to Dr. S.C. Basappa, Scientist, CFTRI, Mysore.

The recipient of Young Scientist Award was Mr. Beliram Thakur, Scientist, DFRL, Mysore.

Gardener's Award has been awarded jointly to C. Arumughan, A. Sundaresan, K.V.S.V. Prasad, A.D. Damodaran and K.U.K. Nampoothiri, Scientists, Regional Research Laboratory, Trivandrum for their paper entitled 'Studies on the Extraction and Evaluation of Raw Palm Oil for Edible Use' published in the *Journal of Food Science and Technology* 1989, Vol. 26, No.5, pages 277-282.

Best Student Award was received by Mr. Wasesh Abdul Kadar, Student, M.Sc., (Food Tech), CFTRI, Mysore.

## Publication of Journals

The Association continued the publications of (1) Journal of Food Science and Technology and (2) Indian Food Industry during the year. Consequent on the increase in publication costs, the subscription rates of JFST have been increased to Rs.300/-, US\$ 100 via Surface Mail and US\$ 125 via Air Mail

for Indian and Foreign subscribers respectively. For IFI, the corresponding subscription rates are Rs.180/- and US\$ 60 by Surface Mail and US\$ 80 by AIR Mail.

## AFST(I) Education and Publication Trust

The Trust met twice during the year and decided to encourage publications of monographs beneficial to schools, colleges, industrial establishments and research institutions.

## AGBM Discussion

During the discussion that followed several suggestions were made, which included establishing contact with developing countries to improve the number of subscribers, to arrange training programmes for young students by the AFST(I), inclusion of reports from all chapters in Secretary's report and furnishing more details about the activities of the Trust. The Amendments to the Constitution were not taken as these were not circulated 15 days earlier among the members as per the Rules. The suggestion for increasing the tenure of office of President for two years was referred to CEC. Enhancement of the membership fee of all categories as suggested by CEC was discussed and it was decided to take the opinion of all the chapters before taking any decision in this respect.

The following office bearers for 1991-92 were inducted:

President	:	Dr. P.J. Dubash
Vice-President (HQ)	:	Dr. S.C. Basappa
Vice-Presidents	:	Dr. S.K. Roy (New Delhi) Dr. Pratap Chakraborty (Calcutta) Mr. N. Ibrahim (Madras) Mr. S.V. Krishnaswamy (Bombay)
Hony. Exec. Secretary	:	Dr. M.S. Prasad
Hony. Jt. Secretary	:	Dr. M.N. Krishnamurthy
Hony. Treasurer	:	Mr. G.A. Krishna

The meeting ended with a vote of thanks by the Secretary.

## Hisar Chapter

The Annual General Body Meeting was held on 28th January 1991. The following office bearers were elected for the year 1991-92:

President	:	Dr. B.M. Chauhan
Vice-President	:	Dr. B.S. Yadav
Hony. Secretary	:	Dr. Salil Sehgal
Hony. Treasurer	:	Dr. S.S. Dhawan

**Trivandrum Chapter:**

The Annual General Body Meeting was held on 8th May 1991. The following office bearers were elected unanimously for the year 1991-92:

President : Dr. C.S. Narayanan  
Vice-President : Dr. Saharia Omman  
Hony. Secretary : Dr. M. Gopalakrishnan  
Hony. Jt. Secretary : Dr (Mrs) P. Prema  
Hony. Treasurer : Mrs. Sankarikutty

**Pune Chapter:**

The Annual General Body Meeting was held on 30th April 1991 and the following office bearers were unanimously elected for the year 1991-92:

President : Ms. V.A. Gangolli  
Vice-President : Ms. L. Pradhan  
Hony. Secretary : Ms. S.S. Nayak  
Hony. Jt. Secretary : Ms. K.S. Redoy  
Hony. Treasurer : Ms. V. Vartak

**Bombay Chapter**

The following office bearers were elected at the Annual General Body Meeting held on 5th July 1991.

President : Dr. C.L. Nagarsekar  
Vice-Presidents : Dr. H.R. Adhikari  
Dr. J.S. Pai  
Hon. Secretary : Dr. S.V. Padgaonkar  
Hon. Jt. Secretary : (Mrs) Rekha Singhal  
Hon. Treasurer : Dr. A.S. Gholap

# INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (in triplicate) should be typewritten in double space on one side of bond paper. They should be complete and in final form. The paper should not have been published or communicated for publication anywhere else. Research Notes should clearly indicate the scope of the investigation and the salient features of the results. Only *invited* review papers will be published.
2. The typescript should be arranged in the following order: Title (to be typed in capital and small letters for Research Papers and all capitals for Research Notes), Authors' names (all capitals) and Affiliation (capitals and small letters). Also give a short running title not exceeding 10 words as a footnote.
3. **Abstract:** The abstract should indicate the principal findings of the paper and typed in single space. It should not be more than 200 words and in such a form that abstracting periodicals can readily use it.
4. Use names of chemical compounds and not their formulae in the text. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Footnotes especially for text should be avoided as far as possible.
5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables should be typed on *separate* sheets. Nil results should be indicated and distinguished clearly from absence of data, which is indicated by '—' sign. Tables should not have more than *nine* columns.
6. **Illustrations:** Graphs and other line drawings should be drawn in Indian ink on tracing paper or white drawing paper preferably art paper not bigger than 20 cm (OY axis) × 16 cm (OX axis). The lettering should be twice the size of the printed letter. Photographs must be on glossy paper and must have good contrast; **three copies** should be sent.
7. **References:** Names of all the authors along with title of the paper should be cited. Abbreviations such as *et al.*, *ibid*, *idem* should be avoided. References should be serially numbered as superscripts in the order they are cited in the text and the same order should be maintained in the reference list. The titles of all scientific periodicals should be abbreviated in conformity with the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.

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

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

# JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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## Research Papers

HEAT TRANSFER DURING CONCENTRATION OF WHEY IN THIN FILM SCRAPED SURFACE HEAT EXCHANGER by *V. Kapil, A.K. Dodeja and S.C. Sarma.*

INHIBITION OF GROWTH OF PATHOGENIC BACTERIA DURING PRODUCTION AND STORAGE OF ACIDOPHILUS MILK by *C.D. Khedkar, J.M. Dave and S.S. Sannabhadri.*

EFFECT OF VARIETY, PARBOILING AND AGING OF RICE ON THE TEXTURE OF IDLI by *C.M. Sowbhagya, L.K. Pagaria and K.R. Bhattacharya.*

STUDIES ON THE UTILIZATION OF SUNFLOWER KERNELS IN BAKERY PRODUCTS by *K. Leelavathi, P. Haridas Rao and M.C. Shamanthaka Sastry.*

STUDIES ON THE STORAGE CHARACTERISTICS OF KHAKRA by *Maya Prakash, Sarojani K. Dastur and Suwendu Bhattacharya.*

EFFECT OF STORAGE TEMPERATURES ON SENSORY, CHEMICAL AND RHEOLOGICAL CHARACTERISTICS OF MOZZARELLA CHEESE by *Bikash C. Ghosh and S. Singh*

A STUDY OF CERTAIN FUNCTIONAL PROPERTIES OF CHICKEN AND DUCK EGGS by *L. Satyanarayana Reddy, M. Sreenivas Reddy and S.M. Siddiqui*

EFFECT OF FROZEN STORAGE AND EXTENDERS ON THE QUALITY OF MEAT TIKKAS FROM CULLED HENS AND BROILER BREEDER MALES by *K.S. Sekhon and A.S. Bawa*

EFFECT OF MAIDA, POTATO AND TEXTURED SOYA AS BINDERS ON THE QUALITY OF CHICKEN AND MUTTON KABABS by *Mir Salahuddin, N. Kondaiah and A.S.R. Anjaneyulu*

STUDIES ON THE QUALITY CHARACTERISTICS OF BUFFALO SKELETAL, OFFAL MEATS AND THEIR COMBINATIONS by *K.R. Krishnan and N. Sharma*

FLOW BEHAVIOUR OF PEACH AND APRICOT PULPS AND CONCENTRATES OF SOME INDIAN VARIETIES by *G.H. Shah and G.S. Bains*

NUTRITIONAL EVALUATION AND COOKING QUALITY OF DRY COWPEA (*VIGNA SINENSIS* L.) GROWN UNDER VARIOUS AGRICULTURAL CONDITIONS. EFFECT OF SOAKING AND COOKING ON THE CHEMICAL COMPOSITION AND NUTRITIONAL QUALITY OF COOKED SEEDS by *A.A. Bakr and R.A. Gawish*

## Research Notes

THE MICROBIOLOGICAL QUALITY OF ICE CREAMS SOLD IN BANGALORE CITY by *M. Sarada and J. Mushtari Begum*

SCREENING FOR POPPING QUALITY IN POPCORN by *R.P. Singh, K.L. Sehgal and A.K. Bakhshi*

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