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Spectrophotometric Determination of Some Permitted Food Colours Using Aliquat 336

U. VIPLAVA PRASAD AND C. S. PRAKASA SASTRY
Department of Chemistry, Andhra University, Waltair-530 003, India

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A new spectrophotometric procedure for the determination of microgram quantities of some permitted food colours (Amaranth, Ponceau 4R, Fast Red E, Carmoisine, Sunset yellow FCF, Tartrazine and Erythrosine) based on their ability to form water insoluble and chloroform soluble complexes with aliquat 336 (methyl tricaprylyl ammonium Chloride) has been proposed.

Schemes for the extraction and spectrophotometric determinations[1-3] of permitted colours in food stuffs have been described by various workers. The proposed method is based on the ability of food colours to form water insoluble and chloroform soluble complex with aliquat 336 (methyl tricaprylyl ammonium chloride). The method is advantageous as it involves direct extraction procedure for the photometric determination of single colour in food-stuffs.

Materials and Methods

Reagents: All the chemicals and reagents used were G.R. grade. Food colours and aliquat 336 were received from Idachem Industries Pvt. Ltd., Bombay and general mills chemicals, Illinois respectively and the purity of food colours were determined[5,6].

Preparation of solutions: A chloroform solution of aliquat 336 (4×10^{-2}M) was diluted to obtain various strengths.

Aqueous solutions of food colours (Amarnath, Ponceau 4R, Fast Red E, Carmoisine, Sunset yellow FCF, Tartrazine and Erythrosine) were prepared by dissolving 250 mg of each in 250 ml of distilled water and diluted to obtain the desired strengths.

General Procedure: Appropriate quantity of aliquat 336 and chloroform were placed in a 125 ml separating funnel to make organic phase 15 ml. A 5.0 ml quantity of food colour solution and distilled water were added so as to make aqueous phase 15 ml. The separator was shaken vigorously by hand for a min. The nonpolar and polar phases were allowed to separate. The chloroform layer extract was run into a conical flask containing 2 g fused sodium sulphate. Its absorbance was measured at appropriate wavelength against a reagent blank prepared in a similar manner. The food colour concentration was read from a standard calibration curve prepared with the same compound under identical conditions.

Recovery Experiments From Juices: To justify the suitability of the proposed method for fruit juices, etc., a known amount of single food colour was added to the known volume of the fruit juice squeezed freshly from the fruits. Suitable volume of the juice was taken in a 125 ml separater and extracted initially with chloroform (2×15 ml) to eliminate soluble natural pigments. The general procedure was then adopted for the determination of the colour and compared with the recommended method[2]. The results are included in Table 2.

Results and Discussion:

The λmax (characteristic of the food colour) in aqueous and organic phase, the experimental conditions established for complete extraction of each food colour into organic layer, Beer's law limits, Sandells sensitivity, standard deviation and molar composition of the complex by slope-ratio method and log-log method are shown in Table 1. Comparision of the results obtained in the estimation of single food colour in juices using proposed and reported methods reveal their agreement within 2 per cent. The results obtained with the present method are in good agreement (within 2 per cent) with those obtained by titrimetric methods[5,6] in the case of bulk samples.

The slight difference in λmax may be due to the solvent shift and complex formation between food colour and aliquat 336. In the formation of these chloroform soluble compounds azo and carbonyl groups in food colours are probably bound to the quaternary nitrogen of aliquat 336[4].

Interferences: It was found that the following range of folds with respect to one fold each dye concentration of ions or compounds usually present in

TABLE 1. CHARACTERISTICS OF THE FOOD COLOURS

Food colour	C.I. No.	Max absorbance		Colour stability in organic phase (min)	Concn of aliquat (10^{-3} M) (ml)	Beer's law limits in organic phase (μg)	Complex ratio dye: aliquat	Sandell's sensitivity μg/ml/0.001 absorbance	% Relative std deviation
		Aq. phase (nm)	Organic phase (nm)						
Amaranth	16185	520	525	5- 20	3	60–604	1 : 1	0.03329	0.6
Ponceau 4R	16255	505	515	5- 35	4	60–604	1 : 1	0.03329	0.8
Fast Red E	16045	505	520	5- 50	4	50–600	1 : 1	0.03347	0.3
Carmoisine	14720	510	530	5–100	3	37–401	1 : 1	0.02726	0.7
Sunset yellow FCF	15985	480	490	5- 45	3	45–585	1 : 1	0.03591	0.6
Tartrazine	19140	430	440	5- 30	6	160–695	1 : 1	0.03949	0.4
Erythrosine	45430	520	535	5- 20	5	88–792	1 : 1	0.04292	0.5

TABLE 2. RECOVERY STUDIES FROM FRUIT JUICES

Sample	Food colour	Amount added (μg)	Amount obtained from graph in 15 ml (μg)	% Recovery	
				Reported method	Proposed method
Lemon	1 Tartrazine	133.6	132.85	92	99.44
	2 Tartrazine	133.6	133.34	92	99.81
Orange	1 Sunset yellow FCF	113.1	113.89	92	100.70
	2 Amaranth	151.1	149.70	90	99.08

fruit preparations (given in parenthesis) did not cause deviation of more than ± 2 per cent in absorbance: glucose (115-275), sucrose (90-275), sulphate (115-275), phosphate (110-200), citric acid (55-200), tartaric acid (110-190), ascorbic acid (60-250), sulphur dioxide (170-280), (exceptions with tartrazine (35) and erythrosine interfered), benzoic acid (2-6) (exceptions-sunset yellow and erythrosine interfered) and saccharin (1-2.5).

Glucose, sucrose, sulphate, phosphate, citric acid, ascorbic acid, tartaric acid and sulphur dioxide do not interfere even when they are present in large excess. Benzoic acid and saccharin do not interfere when they do not exceed the limits prescribed in PFA Act. However, sodium nitrite, sodium nitrate and salicylic acid interfere even when they are present in less than one fold of the dye concentration.

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Incidence of *Klebsiella* in Foods and Water

D. VIJAYA RAO AND K. R. GOPALA RAO
Defence Food Research Laboratory, Jyothi Nagar, Mysore-570 011, India

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A variety of raw and processed food materials and water from rivers, wells and city supplies were analysed for standard plate counts (SPC), coliforms and *Klebsiella*.

***Klebsiella* along with high SPC and coliform counts was present in all tested samples except the processed samples which had relatively lesser numbers. Pasteurised milk was heavily contaminated with *Klebsiella*.**

From a survey of 523 samples, 382 isolates were identified as *K. pneumoniae*, *K. ozaenae* and *K. oxytoca*. *K. pneumoniae* was the most preponderent species.

Klebsiella pneumoniae, a drug resistant organism has been recognised as an opportunistic one involved in human pathogenesis[1-8] and bovine coliform mastitis[9]. It has been isolated from a wide variety of sources[10-17] and is a normal inhabitant of the gastro-intestinal tract[2,18].

Materials and Methods

Surface waters, raw milk and foods with gravy were collected in sterile wide mouthed glass bottles while solids were collected in ultraviolet light sterilized polythene pouches.

Food materials: Filtered raw milk (courtesy: the city milk dairy), pasteurised milk from the vending stall were analysed within 1 hr after collection.

Raw mutton samples were purchased from the market. Fresh eggs were sampled in the melange form (15 batches), each time made from several hundred eggs which had been steeped and scrubbed clean in chrorinated (5 ppm) water and rinsed in running water.

Varieties of fresh vegetables sampled at random from bulk quantities (10 kg or more in 25 lots) obtained for processing, were washed in running tap water free of coliforms.

Cloves, cinnamon, cumin, coriander, turmeric, red chilli, etc were obtained from retail stores and directly sampled.

Dehydrated foods containing cereals, vegetables and spices (HTST 160°-180°C for 6-10 min in a pneumatic drier followed by air drying in a fluidized bed at 60°-65°C for 2-2½ hr) egg powder, mutton chunks and spiced mutton mince prepared by freezing at-30°C followed by vaccum drying at a plate temperature of 55°C to a moisture level of 2-3 per cent in a freeze drier (Hull, USA), and pasteurised milk (HTST, 72°C for 30 sec) a commercial product were used: One third of the dehydrated instant mix were of commercial origin.

Water: Water samples (100 ml each, in triplicate) from rivers Cauvery and Kapila were collected at 6 points along their course through villages in Mysore and Mandya districts of Karnataka State in South India. Water samples from three wells located in different parts of Mysore city, and the city municipal water were collected and tested between July and December 1979.

Sampling of foods and water: Foods were sampled in the manner described earlier[15]. Water and milk were diluted in sterile distilled water. Processed foods were incubated at 30°C for 2 hr to allow recovery of sublethally injured bacteria prior to serial dilution.

Bacteriology: All bacteriological media[19] were prepared using bactograde ingredients of either Difco (Detroit, USA) or Oxoid (UK) origin. Chemicals used were of AR quality and all dyes used in the media were the products of British Drug House (India) or E. Merck (W. Germany).

Standard plate counts (SPC) were enumerated on plate count agar incubated at 30°C for 24-48 hr. Coliforms were counted on violet red bile agar (VRBA) incubated at 37°C for 18-24 hr.

Isolation and identification of Klebsiella: Greyish pink mucoid colonies picked from VRBA and showing no metallic sheen on eosin methylene blue agar were biochemically characterised[20,21] and differentiated[22] into species. Strains were maintained on soft agar.

TABLE 1. OCCURRENCE OF *KLEBSIELLA* AMONG TOTAL COLIFORMS IN VARIOUS RAW FOOD MATERIALS

Raw material	Samples tested (No.)	Std. Plate Count/g	Coliform/g	*Klebsiella* (as % coliforms)	% samples containig *Klebsiella*
Fresh milk	45	10^4-10^7	10^3-10^6	6-8	100
Fresh mutton	60	10^3-10^6	10^2-10^3	1-3	100
Egg melange	15*	10^2-10^3	10 -10^2	0-1	6.6
Spices and condiments	30	10 -10^4	5 -10^2	0-2	5.0
Vegetables	25**	10^5-10^7	10^4-10^5	3-6	80

*15 batches each having contents of 100 eggs

**25 batches contained 10 kg of mixed vegetables.

Results and Discussion

Random sampling of raw food materials (Table 1) revealed that vegetables, millk and meat contained the highest numbers (10^4-10^7 cells) of aerobic bacteria per gram. Coliform content of these three materials was also high (10^3–10^6 cells/g) compared to other materials such as egg melange and spices. *Klebsiella* was most abundant in raw milk (6-8 per cent of coliforms) and vegetables (3-6 per cent of coliforms). It was less frequently encountered in egg melange and spices, the coliform content of which was not generally more than 10^2 cells/g and comprised mostly of *E. coli*. Non lactose fermenting colonies from most samples belonged to pseudomonads and/or *Proteus* spp.

The SPC of processed foods (Table 2) ranging between 10^1-10^4/g comprised largely of gram positive sporulating rods. Air dried foods yielded few coliforms. *Klebsiella* was more frequently encountered in commercial products. Coliform content of freeze dried meat was generally low but in 11 per cent of the samples, it was in greater numbers (10^2/g), 1-2 per cent of which was *Klebsiella*. There was lesser incidence of this genus in egg powder.

Pasteurised milk showed elevated SPC and coliform count. Only 3.3 per cent of samples were free from coliforms, *Klebsieila* incidence being 2-4 per cent of the latter.

SPC of water (Table 3) ranged between 10^3-10^6/ 100 ml and coliforms were present in the range 10^2-10^4/100 ml of which *Klebsiella* content was not more than 2 per cent. The majority of river water samples (13 out of 18 or 72 per cent) had high coliform numbers, of which 44 per cent had low levels of *Klebsiella*. Well waters had lower SPC and no coliforms except in one case where only coliforms were found. *Klebsiella* was recovered from this sample. The city water supply source had SPC of 10^2-10^4/100 ml. Coliforms were not more than 10^3/100 ml in 55.5 per cent of the samples. *Klebsiella* was found in one samples.

From several hundred presumptive *Klebsiella* colonies investigated, 382 were eventually confirmed as *Klebsiella*-347 from foods (100 from processed and 247 from raw materials) and 35 from water. Three species were identified.

K. pneumoniae occured most abundantly in both foods and water (Table 4). *K. oxytoca* was the next most preponderent species in foods but was not encountered in water. *K. ozaenae* was encountered more frequently in water than in foods.

The distribution pattern of the three species in the samples analysed is shown in Table 5. Raw as well as processed milk and mutton contained all three species of *Klebsiella* while the air dried instant mixes based on cereals (mostly rice), vegetables and spices contained only *K. pneumoniae*. Fresh vegetables showed the presence of both *K. pneumoniae* and *K.*

TABLE 2. BACTERIOLOGICAL QUALITY OF PROCESSED FOODS AS EXPRESSED BY SPC*, COLIFORMS AND *KLEBSIELLA*

Type of sample	Process	Source	Samples tested (No.)	Std plate count/g	Coliforms/g	*Klebsiella* (as % coliforms)	% of samples containing *Klebsiella*
Cereal and vegetable based instant mixes	Air dried	Laboratory	50	10^2–10^3	0–10	Occasional colony	5.1
–do–	–do–	Commercial	27	10^2–10^4	0–10		22.1
Egg power	Accelerated freeze dried	—	100	10 -10^2	0–10	–do–	4.7
Meat	–do–	—	70	10^2–10^3	10–10^2	1–2	15.7
Milk	Pasteurised	—	60	10^2–10^4	0–10^3	2–4	96.7

TABLE 3. BACTERIOLOGICAL QUALITY OF WATER FROM DIFFERENT SOURCES

Sl. No.	Sampling months	Source of water	Samples tested (No.)	Standard plate count	Coliforms	*Klebsiella*
1.	June–July	Cauvery river	6	10^4–10^5	10^2–10^4 (2) (4)	0–10 (3)
	Aug–Oct.	Kapila river	6	10^4–10^6	10^2–10^4 (2) (4)	0–10 (3)
	Nov–Dec.	Tributaries of Cauvery	6	10^4–10^6	10^2–10^4 (1) (5)	0–10 (2)
2.	June–July	Well (a)	2	10^3	0–10^3 (1)	0–10 (1)
		Well (b)	2	10^3	0	0
		Well (c)	1	10^3	0	0
3.	June-July	Municipal treatment works	6	10^2–10^4	0–10^3 (5)	0–20 (1)
	Aug–Oct.	,,	6	10^2–10^4	0–10^3 (3)	0
	Nov–Dec.	,,	6	10^2–10^4	0–10 (2)	0

Figures in parenthesis show the number of samples exhibiting the corresponding counts of SPC, Coliforms and *Klebsiella*.

ozaenae. *K. ozaenae* was detected in river water and treated waters.

Although studies have implicated *Klebsiella* in clinical conditions and histamine poisoning[23] its role as an indicator of pollution has not been seriously considered. A survey has shown that milk, mutton and vegetables harboured substantial numbers of *Klebsiella* which are contaminated at source[24], and since they are exposed to high temperatures during boiling and cooking the products are usually safe from viable bacteria. The risk thus of ingesting *Klebsiella* through these materials is minimal.

Broken eggs contaminated with the egg shell flora[25] were not a serious problem in our samples because of the elaborate surface washing given to the whole egg.

High levels of bacterial contamination of spices[26,27] consisting of *Klebsiella* or any other pathogen are specially noteworthy because of the additional use of spices for garnishing the cooked foods.

Washing of vegetables in tap water will not dislodge the contaminant as is evident in the recovery of *Klebsiella*. Salads[15,28] and garnishings made with uncooked or inadequately cooked vegetables expose the consumer to this and other bacteria.

Processing eliminated the bulk of vegetative bacteria except in the freeze dried meats were the inital contaminates could not be washed away.

TABLE 4. PERCENTAGE INCIDENCE OF *KLEBSIELLA* SPP IN FOODS & WATER

Type of sample	Samples tested (No)	*K.pneumoniae*	*K. ozaenae*	*K. oxytoca*
Foods-fresh & processed	347	65.7	8.9	25.4
Water	35	81.3	18.7	Nil

TABLE 5. OCCURRENCE* OF *KLEBSIELLA* SPP IN DIFFERENT MATERIALS

Klebsiella spp	Materials surveyed: Raw materials	Processed foods	Water
K. pneumoniae	Milk, mutton, vegetables, egg, spices	Milk, mutton, instant mixes, egg powder	River, well, treated water
K. ozaenae	Milk, mutton, vegetables	Milk, mutton	River, treated water
K. oxytoca	Milk, mutton	Milk, mutton	not detected

*Percentage incidence individually was not established as the strains were identified with source only.

Coliform contamination in milk was shown[29-31] to occur after processing. *K. ozaenae* and *K. oxytoca* species encountered in the study are ((D. V. Rao & K. R. Gopa Rao, *JFST*, 83, **20,** 268)) multiple drug resistant, psychrotrophic and a majority of the isolates showed positive response in the faecal coliform test, indicating pathogenic conditions[32]. *Klebsiella* in water may or may not be of faecal origin.

The present investigation has shown that *Klebsiella* species are found in several types of food materials and different water sources and that *K. pneumoniae* was the most abundant species in all. Whether this genus should be considered on par with *E. coli* in food and water sanitation may be open to debate but its carriage through these vectors is evident.

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Some Characteristics of *Klebsiella* Strains Isolated from Foods and Water

D. VIJAYA RAO AND K. R. GOPALA RAO
Defence Food Research Laboratory, Jyothi Nagar, Mysore-570 011, India

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Isolates of *Klebsiella* obtained from food and water samples were found to belong to three species. All the isolates exhibited good growth between 5-37°C and the majority of them also grew at 45°C. About 45% of the isolates gave positive reaction for the faecal coliform test. Isolates from food sources exhibited hydrolytic activity on starch, protein and fat. *K. pneumoniae* isolate produced 2 types of Klebecin. Erythromycin, sulphonamides, nitrofurantoin, ampicillin and furazolidone resistance was shown by 65-100% of the isolates. More than 85% of the isolates exhibited multiple resistance to 5 antibiotics. Four types of resistance patterns were noted for isolates from foods and water. The former responded to lower MIC (below 100 μg/ml) among the four antibiotics tested.

The presence of *Klebsiella*, potential pathogen and a member of the coliform group of organisms,[1,2] in processed foods may be due to post processing handling or inadequate processing. The incidence of *Klebsiella* as a food contaminant is significant from the point of view of general hygienic[3,4]. Some characteristics of several hundred isolates obtained from foods and water are reported in this paper.

Materials and Methods

Milk, mutton, eggs, vegetables and spices (107 samples from retail stores); processed foods (307 samples) like freeze dried mutton, egg powder (prepared in the laboratory), milk powder, pasteurised milk, dehydrated cereal and vegetable based instant mixes (commercial and laboratory samples), 41 water samples from three sources; river (Cauvery and Kapila), well and municipal water were investigated for the incidence of *Klebsiella*.

Identification of isolates: Mucoid greyish pink colonies picked from coliform enumeration plates were streaked on eosin methylene blue agar. Colonies showing no metallic sheen were confirmed as *Klebsiella* by IMViC and H_2S-ornithin-eurease tests.

Media were prepared as per standard methods,[5,6] and the strains were maintained on soft agar.

Growth characteristics: Nutrient agar plates and nutrient broth tubes inoculated with 18 hr growth of isolates were incubated at 5, 10, 30, 37 and 45°C. Growth on plates and visible turbidity in tubes was recorded from 18 hr to 7 days.

Hydrolysis of starch (1 per cent), casein (1 per cent) and of tributyrin (1 per cent) at 5 and 30°C were tested on Nutrient agar containing the various substrates[5,6].

Faecal coliform (FC) *test:* Isolates were grown in EC (Difco) broth at 44.5±1°C for 24 to 36 hr and cultures producing acid and gas were scored as FC positive.

Bacteriocin typing: A set of 15 klebecin producing and 13 klebecin indicator strains of *Klebsiella* were used to characterise *K. pnenmoniae* isolates. Klebecin was induced with mitomycin C (Sigma, 1 μg/ml) and identified by the procedure of Edmondson *et al.*[7], who supplied the 28 test strains used in this investigation.

Drug resistance: This was tested by the paper disc method[8] against ampicillin (A, 10μg), penicillin (P, 10 μg), tetracycline (T, 30 μg), neomycin (N, 30 μg,) compound sulfonamides (Su, 300 μg), furazolidone (Fx, 100 μg), nitrofurantion (Fd, 300 μg) nalidixic acid (Na, 30 μg), septran (BA, 30 μg) and erythromycin (E, 15 μg) by iso-sensitest agar[9] with a spread inoculum of 0.5 ml at a concentration of 10^6 cells/ml. *E. coli* strain 10418 (Public Health Laboratories, Colindale, UK) was used as standard.

Minimal inhibitory concentration (*MIC*): Minimal inhibitory concentrations of ampicillin, chloramphenicol, tetracycline and streptomycin were determined by the method of Kavanagh[10] over a concentration gradient of 40-100 μg/ml.

Results and Discussion

Growth at 5 and 37°C was exhibited by all the strains of *Klebsiella* (Table 1), while the majority were capable of growing at 45°C as well. At lower temperature (5 and 10°C) the growth was slower (3-5 days) than

TABLE 1. PER CENT OF *KLEBSIELLA* SPP ISOLATED FROM FOOD AND WATER SHOWING DIFFERENT CHARACTERISTICS

Source	Growth Temp.		Hydrolysis			Klebecin type	
	5-37°C	45°C	Casein	Fat	Starch	P_1	P_2
Food (347)	100	95.2	5.9	7.4	4.7	3	3
Water (35)	100	97.0	0	0	0	0	0

TABLE 2. INCIDENCE OF *KLEBSIELLA* GIVING POSITIVE RESPONSE TO FAECAL COLIFORM TEST

Strains	Food			Water		
	No. tested	Positive response (No.)	(%)	No. tested	Positive response (No.)	(%)
K. pneumoniae	29	11	37.9	15	10	66.7
K. ozaenae	1	7	14.2	3	6	50.0
K. oxytoca	2	4	50	—	—	—

at higher temperatures (30-45°C). Protein, fat and starch hydrolysis were exhibited by 5.9, 7.4, and 4.7 per cent respectively of strains derived from foods only. Isolates from water were hydrolytically inactive.

Only two klebecins P_1I_1 and P_2I_2 were produced by 3 per cent of *K. pneumoniae* of food origin (Table 1). Other species and all isolates from water were not typable by the method employed.

In the faecal coliform test (Table 2) 14 out of the 40 (35 per cent) food isolates and 10 out of the 15 (66.7 per cent) water isolates gave positive response. Overall, positive response was given by 44.3 per cent of the strains tested which included three species of *Klebsiella*. Among *K. pneumoniae* strains 45 per cent showed a positive response to FC test. When the incubation period was extended to 36-48 hr. majority of this species and *K. ozaenae* from foods gave positive response. *K. oxytoca* which was encountered only in foods also gave 50 per cent positive response.

All strains were penicillin resistant (Table 3). Resistance to erythromycin, compound sulphonamides, nitrofurantoin, ampicillin and furazolidone were shown by 65-100 per cent of the isolates. Chloramphenicol, streptomycin and tetracycline resistance was comparatively of lower order among the food isolates. All strains isolated from water were sensitive to streptomycin and tetracycline. Septran resistant strain was not detected.

More than 80 per cent of the isolates were resistant to at least 5 antibiotics simultaneously. Of the six most common resistance patterns which emerged (Table 4) when resistance to only seven antibiotics rather than all the twelve studied was considered for case of categorisation, four patterns were common to isolates from both foods and water. Resistance to APSuE was exclusively present in those isolated from food while resistance to APSuFdNaE was represented by those isolated from water alone. However, the most preponderant resistace patterns APSuFdE and APSuFxFdE were exhibited by all the three species.

Tested over a concentration gradient, the minimal inhibitory concentrations of ampicillin and streptomycin (Table 5) were within 100 μg/ml for 56.9 and 83 per cent respectively of those isolated from food. For 37.2 per cent of these strains, chloramphenicol inhibition occurred at 50-80 μg/ml level, the remaining strains did not respond to the higher doses tested. Tetracycline inhibition at 100 μg/ml level was shown by a small fraction of food isolates. *Klebsiella* strains isolated from water were rather more resistant to ampicillin and chloramphenicol than those isolated from food.

Because of their capability to grow at different temperatures, *Klebsiella* can multiply even in refrigerated foods, if sufficient moisture available. Although only a small percentage of isolates was active hydrolytically, their growth over a wide temperature range can lead to spoilage of foods or even elaboration of some toxic compounds[11]. In water, perhaps sufficient nutrients are present to proliferate under the fluctuating temperatures.

TABLE 3. INCIDENCE OF RESISTANCE TO ANTIBIOTICS AMONG *KLEBSIELLA* STRAINS

Source	No. tested	Per cent strains showing resistance to indicated antibiotics											
		A	C	P	St	T	Neo	Sulpha	Fx	Fd	Na	Step	E
Food	347	65.0	19.4	100	8.9	5.9	4.7	94.0	40.3	86.6	5.8	nil	49.1
Water	35	100	6.2	100	nil	nil	31.2	87.0	62.5	100	6.2	nil	100

Symbols of antibiotics as mentioned in text

TABLE 4. MULTIPLE DRUG RESISTANCE PATTERNS IN *KLEBSIELLA* ISOLATED FROM DIFFERENT SOURCES

Antibiotics	Food (%)	Food Names of Strains	Water (%)	Water Names of Strains
		K. pneumoniae		*K. pneumoniae*
APSuFdE	25.4	*K. ozaenae*	31.3	*K. ozaenae*
		K. Oxytoca		*K. Oxytoca*
APSuF×FdE	7.5	-do-	18.8	*K. pneumoniae*
				K. Ozaenae
PF×FdE	11.5	*K. ozaenae*	6.2	*K. pneumoniae*
APNSuF×FdE	13.0	,,	12.5	,,
APSuE	11.5	*K. pneumoniae*	nil	
APSuFdNaE	nil		16.2	*K. pneumoniae*

Klebsiella isolates from the environment and clinical samples which were found to exhibit positive response to FC test were also associated with indole production and pectin liquefaction[7,8] although the *Klebsiellae* are indole negative. Further, such strains mostly of human and animal origin were unable to grow at 10°C, this being the characteristic of the majority of strains showing a negative response. Our studies indicate that considerable number grow at 10°C, is also positive for FC test. When a faecal coliform test is conducted on food samples it might be worthwhile to investigate to what extent *Klebsiella* spp are involved along with the *E. coli* in giving a positive response.

Attempt to categorise the food and water borne *Klebsiella* by Klebecin typing has not been successful. Mitomycin-C induction of klebecin did not always give reproducible results with the producer strains which may be one reason why with this method many strains could not be typed. On the other hand, there might be other distinct, but unrecognised differences between *K. pneumoniae* of food origin and those of clinical origin which preclude the application of this method of klebecin typing.

Significant in the ecological and epidemiological contexts, the occurrence of antibiotic resistant enterobacteria in the environment has been discussed by Richmond[12]. High levels of resistance to several antibiotics in *E. coli* and *Klebsiella* of food, water and clinical origin were reported from this laboratory.[13]

Subsequent investigations, part of which are reported here, show that the degree of resistance to many antibiotics and particularly to penicillin, sulphonamides and erythromycin remains unchanged, whereas resistance to ampicillin, tetracycline, streptomycin, chloramphenicol and septran has decreased. The similarity of resistance patterns among isolates from both sources may be indicative of a shared origin from common ecological niches.

Considering the dissemination possibilities of this organism coupled with the fact that drug resistance is genetically transferable, the multiple drug resistant *Klebsiella* achieve certain significance in food and clinical environments. The minimal inhibitory concentrations of the four broad spectrum antibiotics rising beyond 100 μg/ml, probably reacting levels as high as 320 μg/ml[13] for a large number of isolates, suggest that these antibiotics will be probably devoid of much therapeutic value in normal therapeutic doses in case of infections involving such strains.

TABLE 5. MINIMAL INHIBITORY CONCENTRATION OF SOME ANTIBIOTICS AGAINST *KLEBSIELLA* ISOLATES RESISTANT TO NORMAL TEST CONCENTRATIONS

Antibiotic	Concn. (μg/ml)	% inhibition of isolates Food	Water
Ampicillin	40	28.7	0
	60-80	14.2	60.0
	100	14.0	0
Streptomycin	40	17.0	*
	60-80	50.0	–
	100	16.0	–
Chloramphenicol	40	0	0
	50-80	37.2	0
	100	0	0
Tetracycline	50-80	0	*
	100	12	–

*No resistant strains found.

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Studies on the Hygienic Quality of Pork Products Sold in the Indian Market

MAHENDRA PRASAD, N. K. CHANDIRAMANI AND USHA MANDOKHOT
Department of Veterinary Public Health & Epidemiology, Haryana Agricultural University, Hissar-125 004, Haryana

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Nintynine samples of pork products comprising bacon (27), ham (35) and sausages (37) from retailers' shop were examined for microbiological status. Coliforms, *Streptococci*, *E. coli* and *Cl. perfringens* were isolated from the samples which were found to be of substandard quality. Enterotoxin producting *S. aureus* was isolated in large numbers from ready-to-eat pork products. *Salmonellae* were also isolated from a sample of ham and sausage.

With changing food habits of people, the ready-to-eat and ready-to-cook-food products like bacon, ham and sausages are becoming increasingly popular in urban and semi-urban areas of our country. These foods, however, are to be produced, maintained and sold under strict hygienic conditions, as otherwise microbial spoilage occurs, reduce the shelf life of the product and/or affects the consumer's health. While stright hygienic conditions are maintained in developed countries, in India, however, such measures are lacking. Investigations were undertaken to ascerain the hygienic quality of some of the commercially produced pork products such as bacon, ham and sausages sold in retail outlets and the results are presented here.

Materials and Methods

Collection of samples: Samples of bacon, ham and sausages were procured from retail shops in New Delhi. Eight sausage samples were also collected from Department of Animal Products Technology (APT), Haryana Agricultural University, Hissar. These are sold in brown or white paper wrapped packets or polythene bags. The samples were collected in the polythene bags and transported to the laboratory in ice-box, and stored in deep freeze at-25°C till processed, which was carried out within 12 hr of collection. Details of samples collected are presented in Table 1.

Preparation of sample for microbiological analysis: Seven grams of a sample were homogenized in 63 ml of sterilized normal saline from which further decimal dilutions were made for microbiological counts.

The method of American Public Health Association[1] was adopted to obtain standard plate counts (SPC) of mesophiles and psychrophiles.

Enumeration of numbers of coliforms, faecal *streptococci*, *S. aureus* and *Cl. perfringens* were carried out using violet red bile (VRB), Slanetz medium, Carter's and Iron Sulphite Agar, following the procedure of Nordic Committee on Food Analysis.[2] Colonies giving the characteristic fried egg appearance of

TABLE 1. SOURCE, NUMBER AND TYPES OF SAMPLES COLLECTED FOR BACTERIOLOGICAL EXAMINATION

Source	Bacon*	Ham*	Sausage**	Total
Shop A	9	7	8	24
Shop B	8	13	12	33
Shop C	10	15	9	34
APT	—	—	8	8

*Each sample comprised of three slices each of bacon or ham selected at random and pooled together.

**Four individual sausages pooled together formed one sample.

S. aureus on Carter's medium were further tested for the production of coagulase, thermonuclease[3] and enterotoxin[4]. *E. coli* was determined on Levine's Eosin Methylene Blue Agar[5] (EMB). Colonies revealing characteristic morphology and metalic sheen were further confirmed biochemically as suggested by Cowan[6]. Biochemically confirmed isolates were serotyped at Central Research Institute (CRI), Kasauli.

For isolation of *Salmonella*, about 10 g of samples was inoculated in Rappaport's medium[7] and incubated at 37°C for 24 hr. A loop full of this enriched culture was streaked on to the brilliant green agar[3] (BGA) plate and MacConkey's agar (MMA) plates. Colonies suspected to be of *Salmonella*, after isolation and purification, were tested biochemically as per the scheme given by Cowan[6]. Biochemically confirmed isolates were sent to I. V. R. I., Izatnagar and CRI, Kasauli for serotyping.

Results and Discussion

Psychrophilic counts: The minimum, maximum and average counts obtained per gram of bacon, ham and sausage samples from all the sources are given in Tables 2, 3 and 4. The countwise break up of samples is presented in Table 5. Average count of psychrophiles in different sausages per gram of bacon ranged between 10^7-10^8, 10^7-10^9 in ham and 10^6-10^8 in sausages. Minimum psychrophilic count in all the three products, irrespective of thier source was always above 10^4. Approximately 40 per cent samples of each product (Table 5) contained psychrophiles in the range of 10^7-10^8/g. Some samples revealed psychrophiles to the extent of 10^9/g. High numbers of psychrophiles would affect the shelf life. Only three samples, (one of ham and two of sausages) yielded psychrophiles as low as 10^4/g. These high counts, however, were not found to be associated with off flavours, though Peterson and Gunderson[8] have reported detectable off flavours, in chicken pie, at a level of 10^4/g of psychrophiles and a definite unacceptability at 10^5/g.

The average mesophilic counts obtained in ham, bacon and sausages were more or less equal to the averages obtained with the psychrophiles and in certain instances were even less (Tables 2, 3 and 4). Data presented in Table 5, again like those of psychrophiles reveal that majority (approx. 84 per cent) of the samples had the contamination of mesophiles at the level of 10^5-10^8/g. and some (approx. 7 per cent) had even as high as 10^9/g. Only few (approx. 8 per cent) had a count below 10^4/g.

Ham samples collected were ready-to-eat food products while sausages and bacon were ready-to-cook products. The suggested microbiological criterion (mesophilic counts) for these two kinds of foods vary between 2×10^2-10^6 and 5×10^5 per gram respectively[8–11]. In the present case about 90 per cent of the samples had SPC above 10^5/g, indicating the substandard hygienic condition either at the production line or in the retail shops.

TABLE 2. MICROBIAL COUNTS (RANGE) IN MARKET SAMPLES OF BACON

Sources	Psychrophiles	Aerobic mesophile	Faecal *Streptococci*	*S. aureus*	Coliform	*Cl. perfringens*
Shop A	3.1×10^5–3.5×10^8	3.8×10^5–3.7×10^7	0–3.7×10^5	0–2×10^4	0–3.3×10^2	0–50
Average	4.4×10^7	3.1×10^6	8.7×10^4			
Shop B	7.8×10^5–4.1×10^7	7.5×10^5–8.9×10^7	1.15×10^3–1.1×10^5	0	0	0
Average	1.2×10^7	1.8×10^7	3.1×10^4			
Shop C	5.9×10^5–1.03×10^9	1.5×10^6–2.83×10^9	4.2×10^3–1.09×10^6			
Average	1.4×10^8	7.2×10^8	3.5×10^5			

E. coli was absent

TABLE 3. MICROBIAL COUNTS IN MARKET SAMPLES OF HAM

Sources	Psychrophilic	Mesophilic	Faecal *Streptococci*	*S. aureus*	Coliforms	*E. coli*	*Cl. perfringens*
Shop A	3.1×10^5–3.7×10^7 (1.2×10^7)	3.7×10^6–3.9×10^7 (1.7×10^7)	3.2×10^3–1.26×10^5 (5.1×10^4)	0–8.2×10^4	0–9.4×10^2 (2.4×10^2)	0–1.6×10^2	*
Shop B	6.0×10^4–1.6×10^9 (1.3×10^9)	3.7×10^4–4.0×10^8 (2.8×10^7)	0–1.67×10^5 (2.9×10^4)	0–2.7×10^4	0–6×10^2 (5.3×10^1)	0	0–50
Shop C	1.1×10^6–3.9×10^8 (7.6×10^7)	6.1×10^5–2.89×10^9 (3.8×10^8)	3.1×10^4–2.79×10^7 (5.1×10^6)	0	0	0	0

*Uncountable
Figures in the parentheses indicate mean.

All the bacon samples except two from source A were negative for coliforms. The two positive samples yielded 100-330/g of coliforms. *E. coli* was not detected in any of the bacon samples. Contrary to the absence of coliforms and *E. coli*, faecal *Streptococci* were present in all but two samples from source A. The number of faecal *Streptococci* varied between 10^3 and 10^6 with the averages being 8.7×10^4, 3.1×10^4 and 3.5×10^5/g respectively (Table 2).

Samples of ham from source A and source B showed coliforms, but samples from source C indicated no coliforms (Table 3). *E. coli* was found in samples from source A (1.6×10^2/g). Faecal Streptococci were, however, present in all the samples but for two samples from source B. The averages for the three sources varied from 5.1×10^4 to 5.1×10^6/g.

Samples from APT Department were devoid of *E. coli* and faecal *Streptococci*. But the other three sources indicated coliform contamination, with the mean count ranging from 8.9×10^2 to 4×10^3/g (Table 4). Sixty two and half per cent of samples from source A yielded *E. coli* in numbers not less than 2×10^3/g; one third of samples from source B also yielded *E. coli* in numbers ranging from 1.2×10^2 to 9×10^3/g with a mean of 3.4×10^3 (Table 4). None of the samples from source C was found contaminated with *E. coli*. Faecal *Streptococci* were isolated from all the commercial samples.

Presence of coliforms, faecal streptococci and *E. coli* in food indicates the contamination reflecting the unhygienic conditions during various stages of processing. Goldenberg and Elliot[9] mentioned 500 coliforms/g in ready-to-cook foods as permissible maximum limit and 10 coliforms/g in ready-to-eat foods. *E. coli* could be allowed to some extent in raw foods, but not in the dehydrated, cooked and processed foods.[12-14] Therefore, suggested limits for *E. coli* are understandably narrower than the coliforms. The

TABLE 4. MICROBIAL COUNT OF MARKET SAMPLES OF SAUSAGES COLLECTED FROM SHOPS AND APT DEPARTMENT

Sources	Psychrophilic	Mesophilic	Faecal *Streptococci*	*S. aureus*	Coliforms	*E. coli*	*Cl. perfringens*
Shop A	9.8×10^6–3.0×10^9 (4.0×10^8)	3.5×10^7–1.7×10^9 (3.3×10^8)	2.2×10^5–1.0×10^6 (5.3×10^5)	0–1.6×10^5 (4×10^3)	0–1×10^4	0–2.1×10^4	*
Shop B	1.9×10^5–1.3×10^8 (2.1×10^7)	4.9×10^5–1.5×10^8 (2.5×10^8)	1.2×10^3–4.8×10^5 (7.7×10^4)	0–7×10^3	0–9.0×10^3 (3.4×10^3)	0–9.0×10^3	0–150
Shop C	3.4×10^4–1.7×10^9 (3.3×10^8)	3.5×10^6–2.9×10^9 (4.3×10^8)	3×10^3–3×10^5 (8.6×10^4)	0	0–5.0×10^3 (8.9×10^2)	0	0
APT	4.1×10^4–6.3×10^6 (2.0×10^6)	3.1×10^4–4.1×10^6 (9.5×10^4)	0	0	0	0	0

*Uncountable
Figures in the parentheses indicate mean.

TABLE 5. NUMBER OF SAMPLES HAVING DIFFERENT RANGE OF BACTERIAL CONTENTS

Types of organism	Range of counts	Kinds of pork products					
		Bacon (27)		Ham (35)		Sausage (38)	
		No. Positive	%	No. Positive	%	No. Positive	%
Psychrophilic	Upto 10^4/g	0	0	1	2.8	2	5.4
	10^5–10^6/g	14	51.8	20	57.1	18	48.6
	10^7–10^8/g	12	44.4	13	37.14		
	10^9/g	1	3.7	1	2.8	2	5.4
Mesophilic	Upto 10^4/g	0	0	3	8.5	5	13.5
	10^5–10^6/g	15	55.5	13	37.1	13	35.1
	10^7–10^8/g	9	33.3	17	48.5	17	45.9
	10^9/g	3	11.1	2	5.7	2	5.4
Faecal streptococcus	0	2	7.2	2	5.7	8	21.6
	10^2–10^3/g	8	28.8	9	25.5	8	21.6
	10^4–10^5/g	17	62.9	19	54.1	19	51.3
	10^6–10^7/g	0	0	5	14.2	2	5.4
Staphylococcus aureus	0	25	92.5	28	79.8	31	83.7
	10^2–10^3/g	1	3.7	4	11.4	2	5.4
	10^4/g	1	3.7	3	8.5	2	5.4
	10^5/g	0	0	0	0	2	5.4
Coliform VRB	0	25	92.5	28	79.8	13	35.1
	10^2–10^3/g	2	7.2	7	19.9	22	59.4
	10^4/g	0	0	0	0	2	5.4
E. coli	0	27	100	32	97.2	28	75.6
	10^2–10^3/g	0	0	1	2.8	8	21.6
	10^4/g	0	0	0	0.0	1	2.7
Clostridium perfringens	0	24	88.8	31	88.3	27	72.9
	20–150	3	11.1	4	11.4	10	27.1

Figures in parenthesis indicate total number of samples examined.

presence of *E. coli* in ham, bacon and sausages in numbers ranging from 1.2×10^2 to 2.1×10^4/g is quite alarming indicating the gross unhygienic conditions during processing. Sherikar *et al*[15] have also made similar observations with ready-to-cook pork products. Allowance of faecal *Streptococci* upto 10^3/g in frozen precooked foods and 10/g in ready-to-eat products has been suggested by Abrahamson[16]. In the present investigation, all the samples except four (2 ham, 2 bacon) yielded faecal Streptococci more than the suggested limit, confirming the standard sanitary conditions prevailing. The counts of *Cl. perfringens* present in bacon, ham and sausage samples ranged from 20 to 150/g which are in excess of the suggested limit of 10/g for ready-to-eat products[15].

S. aureus were isolated in 2 out of 9 samples of bacon from source A and from 2 and 5 out of 7 and 13 samples of ham from source A and B respectively (Tables 2, 3 and 4). There numbers when present varied from 7.0×10^3 to 8.2×10^4/g. Similar observation were made in sausages isolated from 4 and 2 samples of source A and B respectively. *S. aureus* in raw foods is considered as indicator organisms, but in ready-to-eat foods as pathogen. Therefore, maximum limit suggested[9] for ready-to-cook foods is more (10^2/g) as compared to that suggested for ready-to-eat foods (not more than 10/g). These authors further suggested that products yielding more than 10^2 and less than 10^3 might be accepted but further investigations should be done and if 50 per cent of the repeat samples show more than 10^3/g of *S. aureus*, the production has to be stopped. In the present investigation,

the number of *S. aureus* isolated from ham (ready-to-eat product) or from bacon and sausage (ready-to-cook food) were more than 10^2/g. Further, it is observed that out of 15 *S. aureus* strains isolated, 8 are enterotoxigenic with one producing enterotoxin A and seven others producing enterotoxin B.

Salmonellae which should be absent in any food were also isolated from a sample of ham and a sample of sausage.

It could be concluded that the hygeinic standard of ham, bacon and sausages sold by the retailers are far from satisfactory. Though, it is difficult to identify from the present studies, the actual source of contamination, but still the conditions under which the products are handled and sold using their bare hands at the retailer shops should not be overlooked. Cross contamination of cressed chicken at the retailer's level had been observed by Panda *et al.*[17] To avoid such contamination, it is suggested that the manufacturers should supply these food items to retail shops in appropriate quantities, properly packed in sterile containers or bags.

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Fluctuations in the Quantitative Predominance of Bacterial Groups in Fresh and Stored Mutton

D. VIJAYA RAO, B. BHAGIRATHI AND T. R. SHARMA
Defence Food Research Laboratory, Mysore-570 011, India

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The aerobic bacterial flora of fresh mutton was largely made up of lipolytic or proteolytic psychrotrophs which increased 2-3 fold in mutton stored at 25±2°C. In mutton stored at 5±2°C, there was rapid increase in psychrotrophic and the proteolyitc groups initially, while in later stages lipolytic bacteria were predominant. At 20°C storage, there was gradual decrease in all types over a period of 3 months. Predominant aerobic bacteria were *Staphylococcus*, *Micrococcus*, *Bacillus*, three genera of coryneforms, enterobacteria, *Acinetobacter*, *Pseudomonas* and *Moraxella*. *Staphylococcus* were the dominant lipolytic bacteria followed by *Micrococcus*, whereas equal number of bacteria from both genera were proteolytic. In exposed mutton, stored at 25 and 5°C, gram positive bacteria were predominant, whereas in covered meat the number of gram negative bacteria were predominant. Moisture loss increased with the storage period. Meat developed off-odour when the total plate count was more than 10^{7-8} per gram, but the colour change was independent of TPC.

The Indian armed forces being one of the single largest procurers of meat in the fresh and processed form, we have been investigating the microbiological characteristics of meat and meat products and their spoilage aspects under different temperatures and manner of storage. Relevent results pertaining to the storage of fresh meat are presented in this paper.

Materials and Methods

Meat samples were collected from the butchers. Meat cuts (150 to 200 g) from the *longissimus dorsi* muscles of carcass of sheep (1-1½ year old, postmortem age 4-5 hr) were excised with a sterile knife under aseptic conditions. The samples were stored in a dust free room at ambient temperature (25°C), in a cold room (5±2°C) or frozen at-20°C and were examined at different time intervals for the microbial load.

The colour, odour and overall acceptability were judged by three judges.

pH was determined according to Newbold and Lee[1]. Moisture content was determined by heating 10 g samples at 100°C to constant weight.

Determination of lipolytic and the proteolytic population among the total aerobic bacteria: The relationships between TPC and lipolytics, between lipolytics and proteolytics and that between TPC and proteolytics were elucidated on fresh and spoilage samples by correlation test.

Six hundred isolates of aerobic bacterial flora of fresh mutton and a total of 800-900 isolates from samples held at ambient and low temperature were included in the analysis.

Sampling: Two 10 g pieces were aseptically excised from each replicate sample and macerated in 90 ml sterile 0.1 per cent (w/v) peptone water. This suspension was diluted 10 fold in peptone water. One millilitre portion of the various dilutions was used for enumeration in the appropriate medium.

Methodology adopted and the media used were as per standard prodedures[2-5], which are summarized below:

Test	Medium	Incubation Temp (°C)	Incubation Duration
Total Plate Count (TPC)	PCA	30	48 hr
Anaerobes	PCA	30	24-48 hr
Psychotrophs	PCA	5±2	7 days
Lipolysis	1% tributyrin agar	30	48-72 hr
Proteolysis	10% skim milk agar	30	18-24 hr
Coliforms	Violet red-bile agar (VRBA)	37	18-24 hr
Staphylococci	Baird-Parker agar	37	24-48 hr
Streptococci	Maltose-azide tetrazolium agar	35	48 hr
Lactobacilli	Rogosa agar (MRS)	30	2-3 days
Yeasts and moulds	Potato dextrose agar	25-28	3-4 days
Clostridia	Sulphite polymyxin sulphate agar	44	48 hr

PCA: Plate Count Agar

Identification of bacterial isolates: Isolates[2] were identified using the scheme of Newton *et al*[6]. and classified according to the scheme outlined by Cowan and Steel[7] and Edward and Ewing[8].

The isolates were not further identified to their species level except when the tests already carried out enabled them to be classified into species. Unidentified isolates were there, which exhibited variable and ambiguous biochemical reactions.

The non-spore forming gram positive rods were classified into *Corynebacterium*, *Kurthia* and *Arthrobacter* following the scheme of Cowan and Steel[7].

Results and Discussion

At ambient temperatures, the mesophilic aerobes, anaerobes, the facultative anaerobes, the lactic as well as the psychrotrophic bacteria, yeasts and moulds increased between 2 and 4 magnitudes in 48 hr (Tables 1 and 2). In all aerobic groups the increases followed similar trend in 36 and 48 hr of incubation. The *Lactobacilli* and *Clostridia* reached their near maximal levels in 36 hr, whereas the *Streptococci* and coliforms increased afterwards. At lower temperature, 5°C (Table 3), the psychrotrophs and the proteolytic bacteria increased more rapidly in the first 4 days as compared to the lipolytics. Afterwards the lipolytic and psychrotrophic numbers increased at the same rates until 21 days, while the proteolytics increased at a comparatively lower rate.

The pH fluctuated over a narrow range from the initial values in meat stored at different temperatures, while the moisture loss increased gradually with increasing storage time at all the three temperatures (Table 4). Off-odour development in 24-36 hr corresponded to a TPC of 10^8 cells/g at 25°C while at 5°C the TPC was 10^7/g when slight to distinct odour was detected after 13 days. No off-or rancid-odours developed in 3 months in frozen samples. The colour of meat ranging from the initial pink to the final deep red (at-20°C), reddish brown (at 25°C) or very dark brown (at 5°C) together with the surface dryness determined the overall acceptance when the off-odours were absent.

TABLE 1. RELATIVE INCREASE IN TOTAL MICROBIAL POPULATIONS OF FRESH MUTTON AT AMBIENT TEMPERATURES

Hr of postmortem	Total counts ($\times\log_{10}$/g)					
	Aerobes	Anaerobes	Psychrotrophs	Lipolytics	Proteolytics	Yeasts and moulds
3-4	5.0	3.6	4.5	4.3	4.0	2.1
24-36	8.0	5.0	7.1	7.6	7.1	4.1
48	8.9	8.2	9.3	8.1	8.0	6.6

TABLE 2. CHANGES IN THE FACULTATIVE AND OBLIGATE ANAEROBIC BACTERIAL POPULATIONS OF FRESH MUTTON AT AMBIENT TEMPERATURES

Hr of postmortem	Bacterial counts ($\times\log_{10}$/g)				
	Coliforms	*Staphylococci*	*Streptococci*	*Lactobacilli*	*Clostridia*
3-4	3.5	3.8	2.6	2.1	1.8
24-36	5.7	5.1	3.0	6.9	4.2
48	7.3	7.2	6.7	7.7	4.4

The levels of lipolytic and proteolytic aerobic bacteria in relation to TPC in fresh and spoiled mutton fell into 4 categories (Table 5). In fresh mutton where the TPC ranged between 10^3 and 10^5/g the relative populations of the lipolytic and proteolytic bacteria were significantly interdependent and could be grouped into 3 categories based on differences in counts amounting to one order of magnitude (columns 2 and 5 in Table 5). The lipolytic counts were equal to TPC in 57 per cent and lesser (by one order of magnitude) than TPC in 43 per cent of the samples. The proteolytic bacteria were equal to the lipolytic types and TPC in 28.5 per cent and equal to the former, but lesser than TPC in another 28.5 per cent of the samples. Category 4 of fresh mutton where TPC were the greatest followed by lipolytic and then proteolytic counts, was present in 14.5 per cent of samples.

TABLE 3. CHANGES IN POPULATIONS OF THE AEROBIC SPOILAGE FLORA AT LOW TEMPERATURES

Storage temp (°C)	Period after postmortem	Bacterial counts ($\times\log_{10}$/g)		
		Psychrotrophs	Lipolytic bacteria	Proteolytic bacteria
5±2	3-4 hr	4.4	4.4	3.4
	4 days	5.4	4.3	4.0
	7 days	5.6	5.0	4.5
	13 days	6.6	6.1	5.1
	21 days	7.5	7.1	6.4
−20	5-6 hr	6.6	6.3	5.4
	1 month	5.1	5.0	4.5
	2 months	4.0	4.9	4.0
	3 months	4.4	4.2	3.5

TABLE 4. EFFECT OF STORAGE TEMPERATURE ON pH, MOISTURE CONTENT, COLOUR AND ACCEPTABILITY OF RAW DEBONED MUTTON IN RELATION TO THE TPC

Temp. (°C)	Time postmortem	TPC (×log 10/g)	pH	Moisture (%)	Odour	Colour	Overall acceptability
25	3–4 hr	5.0	5.72	77.4	Nil	Pink	Yes
	24–36 hr	8.0	5.90	69.4	Nil or barely detectable	Deep red	Yes/No
	48 hr	8.9	5.80	66.6	Present	Dark red brown	No
5	3–4 hr	5.2	5.92	77.5	Nil	Pink	Yes
	4 d	5.1	6.01	67.8	Nil	Deep red	Yes
	7 d	5.4	5.72	65.3	Nil	Dark red brown	Yes/No
	13 d	6.3	5.65	43.7	Nil	Dark brown & dry	No
	21 d	7.4	5.92	26.7	Present	Dark dry	No
–20	5–6 hr	6.6	5.90	77.5	Nil	Pink red	Yes
	1 m	5.3	6.20	75.3	Nil	,,	Yes
	2 m	5.0	6.10	71.9	Nil	Deep red	Yes
	3 m	4.4	6.00	69.8	Nil	,,	Yes

d=Days
m=Months

In stored mutton which showed high total counts (10^6-10^8/g), the lipolytic bacteria equalled the TPC and were greater than the proteolytic bacteria in 40.6 percent of samples. Only in 15.6 per cent, all three groups were found to be in the same order of magnitude as opposed to the 28.5 per cent in fresh mutton samples. In 37.5 per cent, the lipolytic and proteolytic bacteria were equal to each other but lesser than TPC. In a small fraction of samples (6.2 per cent), however the proteolytic types were equal to TPC and greater than the lipolytic types.

The aerobic flora of fresh mutton with a TPC of 2.5×10^5/g (Table 6) consisted of 76.4 per cent Gram positive and 23.6 per cent Gram negative bacteria. The proteolytic bacteria (2×10^4/g) were present in approximately the same proportions (70 per cent Gram positive to 30 per cent Gram negative types). The lipolytic bacteria (8×10^4/g) were represented by an even

TABLE 5. CORRELATION COEFFICIENTS OF RELATION BETWEEN TPC (T), LIPOLYTIC (L) AND PROTEOLYTIC (P) BACTERIA IN 67 BATCHES OF FRESH AND STORED (SPOILING) MUTTON

Observed TPC range	Category	r values for T & L	r values for L & P	r values for P & T	% of samples	Explanation of categories (counts/g)
			Fresh mutton			
10^3, 10^4, 10^5	T=L=P	0.73	0.77	0.79	28.5	10^5T–10^5L–10^5P
10^4, 10^5	T=L>P	0.81	0.6*	0.54**	28.5	10^5T–10^5L–10^4P
10^4, 10^5	T>L=P	0.55**	0.79	0.65*	28.5	10^5T–10^4L–10^4P
10^5	T>L>P		–Not done–		14.5	10^5L–10^4L–10^3P
			Stored mutton (5°C and 25°C)			
10^7, 10^8	T=L=P		–Not done–		15.6	10^7T–10^7L–10^7P
10^6, 10^7, 10^8	T=L>P	0.91	0.78	0.85	40.6	10^7T–10^7L–10^6P
10^6, 10^7, 10^8	T>L=P	0.78	0.84	0.74	37.5	10^7T–10^6L–10^6P
10^7, 10^8	T=P>L		–Not done–		6.2	10^7T–10^7P–10^6L

All singnificant between 0.1 and 1 per cent level

*Significant at 5 per cent level
**Significant between 5 and 10 per cent level

TABLE 6. COMPOSITION OF THE AEROBIC BACTERIAL FLORA OF FRESH MUTTON

Genera and Gram nature	% representation of genera		
	TPC	Lipolytic	Proteolytic
Staphylococcus (+ve)	44.6	56.0	30.0
Micrococcus (+ve)	20.6	16.0	30.0
Aerococcus (+ve)	1.0	—	—
Bacillus (+ve)	4.2	—	10.0
Corynebacterium (+ve)	3.2		
Kurthia (+ve)	1.2		
Arthrobacter (+ve)	1.3	12.0	—
Microbacterium (+ve)	0.3		
Enterobacteria (–ve)	9.2	4.0	10.0
Acinetobacter (–ve)	3.2	2.0	2.5
Pseudomonas (–ve)	3.5	3. 0	3.5
Moraxella (–ve)	5.4	3.4	11.0
Unidentified	2.3	4.0	3.0

greater proportion (84 per cent) of Gram positive genera. Predominant among the lipolytic and the proteolytic types were *Staphylococcus and Micrococcus.* More enterobacteria, *Moraxella* and all *Bacillus* isolates were proteolytic, while the coryneform group was essentially lipolytic.

In mutton stored at room temperature, the total counts rose to nearly $1\text{-}6\times10^8$/g in both covered and uncovered samples and had off-odours. The proportion of the individual genera as compared to that in fresh mutton, varied (Table 7) in the two types of samples. *Staphylococcus* and *Micrococcus* were still the major genera in uncovered mutton, but in slightly lesser proportions than before. Other genera like *Kurthia* and *Arthrobacter* increased as did *Acinetobacter* and *Pseudomonas.* At lower temperature, the dominance of the Gram Positive species (82.5 per cent) and specially of *Micrococcus* was marked in the uncovered samples. However, a definite shift to the Gram negative types was noticed in covered samples held at either temperature although the proportion of Gram negative to Gram positive types was very much higher (93.3:6.6) at low temperature than at room temperature (49:51).

At the time of off odour development at 25°C and 5°C, the counts of all aerobic and some of the anaerobic groups were found to be at least 10^7/g whereas in pork and mutton[9,10] the spoilage odour corresponded to $10^8\text{-}10^9$/g of TPC, while the Enterobacteriaceae were 10^5/g.

The colour of meat apparently is not directly related to the bacterial numbers. The colour of meat darkened

TABLE 7. AEROBIC SPOILAGE FLORA OF MUTTON HELD AT 25±2°C AND 5±2°C

Genera and Gram nature	25°C		5°C	
	Exposed mutton	Covered mutton	Exposed mutton	Covered mutton
Staphylococcus (+ve) *S. aureus* *S. epidermidis*	40	12.5	10.0	3.3
Micrococcus (+ve)	10	12.5	57.5	2.0
Aerococcus (+ve)	2.5	—	2.5	—
Bacillus (+ve)	2.5	9.5	4.0	—
Corynebacterium (+ve)	(70.0)–	(51.0)8.5	(82.5)3.5	(6.6)—
Kurthia (+ve)	7.5	2.5	—	—
Arthrobacter (+ve)	7.5	5.5	—	—
Microbacterium (+ve)	—	—	5.0	1.3
Enterobacteria (–ve) *E. coli, Klebsiella* (+ve) *Enterobacter, Citrobacter,* (–ve) *Serratia,* (–ve) *Proteus* (–ve)	9.0	12.5	5.0	40.0
Acinetobacter (–ve)	6.0	12.0	4.0	40.0
(*A. anitratus, A. lowffi*)	(30)	(49)	(17.5)	(93.3)
Pseudomonas (–ve) (*P. flourescnes,* other spp.)	7.5	12.5	3.5	25.0
Moraxella (–ve)	2.5	5.0	2.0	3.3
Unidentified	5.0	7.0	3.0	—

Figures in parenthesis indicate the overall percentages of Gram positive and Gram negative flora.

after a preliminary bloom (deep red colour in 18-24 hr at 25°C and 2-4 days at 5°C) and became progressively darker to render meat unacceptable.

In fresh mutton, no single category is clearly dominant, 3 of the 4 identified categories being equally represented. But in spoiled mutton containing higher bacterial numbers ($10^7\text{-}10^8$/g), the flora were chiefly lipolytic. The proteolytics were never greater than the lipolytics in fresh mutton and only rarely so in spoiled mutton.

Data presented (Tables 1 and 3) show that at 5°C, the proteolytic bacteria increased more rapidly than the lipolytic bacteria, while at 25°C they showed similar increase. Nevertheless, the predominant flora at the time of spoilage at 5°C are still the lipolytic ones (Table 3), among which the Gram positive types are greater in exposed meats while the Gram negative types predominate in covered meats (Table 7). Similar changes in the proportion of Gram positive and Gram negative dominance were noticed by Banks[11] on croaker stored at 5°C. The refrigerator spoilage of

exposed and covered meat is distinct as opposed to its spoilage at 25°C as *Staphylococcus* was predominant at 25°C, while *Micrococcus*, *Acinetobacter* and *Pseudomonas* were predominant at 5°C.

The incipient spoilage became noticeable when the total counts were about 10^7/g. *Pseudomonas*, *Acinetobacter* and Enterobacteriace constituted the major spoilage flora under aerobic conditions at 20 and 30°C according to Gill and Newton[12]. Our observations on the other hand have shown that the spoilage flora at 25°C is made up of *Staphylococcus*, *Micrococcus* and Gram positive bacilli besides the three groups mentioned above.

It has thus been found that the initially predominant *Staphylococcus* of fresh mutton and of mutton exposed at 25°C was no longer the most abundant genus despite its proteolytic and lipolytic abilities. It may thus be concluded that the quantitatively predominant bacteria of fresh mutton are not the most important ones in spoilage under all conditions and manner of storage.

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Sorghum Quality Studies—Part I. Rolling Quality of Sorghum Dough in Relation to Some Physicochemical Properties

A. CHANDRASHEKAR AND H. S. R. DESIKACHAR
Central Food Technological Research Institute, Mysore, India

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***Roti* rolling quality of 45 varieties of sorghum (*Sorghum bicolor* Monech), in relation to the physicochemical characteristics of flours was studied. Varieties possessing good rolling quality showed lower gelatinization temperature, higher peak viscosities, higher set backs in the Brabender viscograph and higher water uptake at 70°C was correlated to starch damage in flour. Amylose, protein and prolamine content of flour had no relation to *roti* rolling quality.**

Sorghum is one of the important staple food grains of India and Africa. It is cooked into a dry baked unleavened pancake (*Bhakri*, *Roti*) or a dumpling (*Mudde*, *Kali*, *Ugali*)[1], for consumption. Some of the recently developed high yielding varieties have not become popular, as they are reported to possess poor *roti*/dumpling making qualities. As sorghum lacks gluten, the dough cannot be easily rolled into pancakes like wheat dough. However, it is known by experience, that if the dough is made with hot water, it gains adhesive property enabling it to be rolled. The present study is mainly concerned with the relative

rolling quality of sorghum dough from different varieties in relation to some physicochemical properties of sorghum flour.

Materials and Methods

Milling: Grains of 45 pure bred sorghum varieties, obtained from breeder's (International Crop Research Institute for the Semi-Arid Tropic and University of Agricultural Sciences, Dharwad) farm, were ground in a plate type grinding mill to pass through 60 BS mesh size.

Dough mixing and rolling: One hundred g of flour was added to 90 ml of boiling water, mixed thoroughly into a uniform dough, brought to a consistency by adding cold water, so that the dough could be rolled into *Roti* or *Chapathi*. Twenty-five g of dough was made into balls and rolled on a wooden plate into circular discs of 6 cm diameter and 2 mm thickness. The rolling quality of the dough was evaluated by its ability to roll without breaking, tendency to break at the edge and strength of the *Chapathi* to withstand handling. The rolling quality was evaluated subjectively assigning numbers, and grouped into poor rolling (Group 1), moderate rolling (Group 2) and good rolling (Group 3).

Subsamples (14 varieties) were also scored for rolling quality in a similar way, but the dough was prepared using different ratios of water and flour (1 to 1.4) and the average value recorded. Here the scores were recorded for individual varieties.

Dough Texture: Texture of dough prepared at three different water flour ratios was measured in a General Foods Texturometer using flat plate and plunger. Average value for each sample has been recorded. The texture was measured as a ratio of peak in the first bite of plunger (P_1) (resistance to flow of dough) to dip in the same bite (S_1) (stickiness of dough). A clearance of 2 mm was maintained between plunger and plate.

Viscographs and water uptake: Amyloviscography was carried out on the flour sample using a Brabender Amyloviscograph according to the method described by Halick and Kelly[2]. Instead of whole meal flour, 60 mesh flour from polished sorgum was used for ensuring reproducibility and to avoid interference by husk, at a 10 per cent (w/w) slurry condition. Water uptake of flours was determined by stirring 1 g of flour (1 g-30 ml water) and holding at different temperatures ranging from 50 to 70°C for 3 hr, centrifuging at 3500 rpm for 15 min and determining moisture content in the sedimented residue by drying at 105°C for 16 hr.

Starch damage: Starch damage in the flours was assessed by the method of Williams and Fegol[3] using 200 mg flour. The values are expressed as optical density (OD). Starch damage was also determined by the enzymatic method of Barnes[4] and that of Chiang and Johnson[5] with slight modifications by extending the incubation for 16 hr. The reducing sugar liberated by enzyme was determined by the Dinitrosalicylic acid method of Bernfeld[6].

Analysis: Amylose[7], total protein[8], prolamine[9], starch[5,6] were determined in defatted flours.

Results and Discussion

Gelatinization of the starch which gives adhesive properties to the dough depends on the nature of the starch constituents, granule structure, time and temperature of heating and the extent of its damage while grinding the grains. These parameters were, therefore,

TABLE 1. VISCOAMYLOGRADHIC AND TEXTURAL PROPERTIES OF SORGHUM IN RELATION TO DOUGH ROLLING PROPERTIES

Group	Rolling quality	Amylose (%)	Starch damage (Williams & FeGol) (OD)	Viscoamylographic data: Gelatinization temp. (°C)	Viscoamylographic data: Peak viscosity (BU)	Viscoamylographic data: Setback (BU)	Dough characteristics: Water absorption (%)	Dough characteristics: Adhesiveness (P_1/S_1)
I	Poor (1)	30.01±1.03(9)	0.51±0.70	74.8±1.5(9)	218±19(7)	425±40(6)	103±2.2(8)	3.53±0.3(9)
II	Moderate (2)	30.81±0.55	0.51±0.05	72.4±0.8(18)	266±13(16)	572±21(16)	107±1.7(10)	4.63±0.8(8)
III	Good (3)	30.61±0.83	0.63±0.06	70.7±0.6(18)	396±18(13)	630±32(13)	106±1.5(11)	5.32±0.5(18)
Test of significance								
Group I vs III				*	**	*		
Group I vs II				NS	NS	*		
Group II vs III				NS	**	NS		

All figures are mean±S.E.; Figures in parenthesis indicate number of varieties tested.
*Significant at 1% level; **Significant at 0.1% level: NS=Not significant

determined in the samples and the data are presented in Table 1.

Rolling quality of the dough was found to correlate with the gelatinization temperature. Varieties having low gelatinization temperature gave adhesive doughs with good rolling quality. The peak viscosity and the setback values also showed good correlation with the rolling quality of the dough. The data show that the rolling quality is not correlated with the amylose content of the flour while starch damage improved the rolling quality, though this was not statistically significant (Table 1). A significant correlation was established between the texturometer values (P_1/S_1) and the rolling score. Similar relation between pastiness and texturometer reading of cooked rice has also been reported[9].

When equal quantities of flour and boiling water were mixed to make the dough, the resultant temperature was found to be in the range of 65-70°C which is in the gelatinization temperature range of some sorghum starches. Varieties with low gelatinization temperatures will undergo more gelatinization resulting in better adhesive/plastic doughs enabling it to be rolled into thin sheets for *Chapathis*, which is evident from the results obtained.

Varieties possesing good rolling are—'M-35-1', 'CSB-8', 'M-64-77', 'CSH-6', 'M-35688', 'IS-9327', 'E-116', 'IS-11025', 'CSH-5', 'SB-1103', 'SB-1066', 'Local' (Group-III).

The varieties with moderate rolling quality are—'BP-53', 'GFR-370', 'A-2283', 'M-35602', 'Moti', 'IS-1584', 'MY-35625', 'MY-3599', 'CS-3541', 'SB-1103', 'SB-1066', 'Local' (Group-II).

The poor rolling varieties are — 'Patcha Jonna', 'HY-2259', 'MY 316-5', 'E-35-I', 'Patancheru', 'B-721', 'SPV 105', 'SB-1079' (rain soaked), 'CS 3544', (Group-I).

Detailed studies on the water uptake of 15 varieties of sorghum at temperatures ranging from 50-70°C were also carried out and consistent varietal variations were found at 70°C. As can be seen from Table 2, the water uptake values at 70°C could be correlated with the rolling quality scores, significant at 1 per cent level. No correlation was observed between the rolling score of dough and the corresponding water uptake values at 60°C.

There was no significant correlation between the amylopectin/amylose ratios and rolling quality. There was no significant correlation between total protein or prolamine with rolling quality, but varieties with very

TABLE 2. RELATION BETWEEN ROLLING SCORE, CHEMICAL COMPOSITION, STARCH DAMAGE AND WATER UPTAKE OF FLOUR IN SORGHUM VARIETIES

Variety	Rolling score	Amylopectin / Amylose	Starch damaged (enzymic) (%)	Total protein (%)	Prolamine (%)	Water uptake at 60°C (g/g)	Water uptake at 70°C (g/g)
SB 2401 K	2.5	1.91	48	10.59	2.96	2.00	3.80
SB 1079 R	3.0	1.46	51	10.65	4.55	2.86	4.50
SB 2401 R	3.5	1.95	68	9.34	4.40	2.44	4.73
SB 1079 K	1.5	1.60	47	10.82	3.93	2.32	3.45
SB 1066 R	4.0	1.51	45	9.56	3.92	2.72	6.02
CS 3544	1.0	1.66	60	9.01	3.53	2.70	3.75
SB 1085 K	3.5	1.81	71	10.29	4.17	2.88	4.95
SB 1103	4.0	1.53	72	9.29	4.59	2.40	4.51
LOCAL	4.0	1.53	71	9.28	3.71	2.71	4.45
SB 1079 K	1.0	1.70	61	10.97	4.26	2.46	—
SB 901	3.5	1.55	57	8.19	2.90	2.29	4.44
SB 1066 K	4.0	—	73	7.84	4.19	2.10	5.04
CSH-5	4.0	1.38	63	10.34	3.88	2.40	4.36
CS 3541	4.0	1.58	69	—	4.26	2.63	4.36
SB 1085 R	3.0	—	55	8.98	—	2.22	4.76

Correlation coefficient: Rolling Score vs Water uptake 70°C*
Water uptake 70°C vs Enzymic starch damage*
*Significant at 1% level.

The other values are not significantly correleated with rolling score or among themselves.

high prolamine and total protein contents tended to have better rolling quality. This may be due to higher hardness of grains with high prolamine contents as established in maize[11]. This needs further confirmation by using larger number of samples and grinding under different defined conditions. There was some correlation between enzymatic determination of starch damage and water uptake at 70°C.

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Effect of Aging on the Fatty Acid Composition of Some Indian Varieties of Brown Rice

N. RAMARATHNAM AND P. R. KULKARNI

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

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A study on the changes in fatty acid composition of ten varieties of Indian brown rice indicated that there was slight increase in linoleic acid, while palmitic and oleic acid levels declined during storage for 120 days.

It is a common practice to age the freshly harvested rice at least for six months, before consumption. It has been reported that certain changes occur in the rice grain during storage[1]. Spherosomes of the embryo and the endosperm of brown rice contains lipoproteins.[2-5]

In addition, the starch granules contain bound lipids, mainly phospholipids, particularly lysolecithin[5-6]. The crude fat content of brown rice has been reported to be between 1.8 and 4 per cent [7-10]. The fatty acid composition of brown rice lipids shows that palmitic, oleic and linoleic acids are the major constituents.[11-14]

The rice lipids are liable to oxidation and/or hydrolysis during storage and thereby contribute to the flavour characteristics of the stored rice. In the present work, an attempt has been made to investigate the changes in fatty acid composition of lipids of some of the Indian rice varieties during storage.

Materials and Methods

Rice: Ten varieties of paddy including 'Basmati' and 'Ambemohor' were collected at the harvest time from the Rice Research Station, Konkan Krishi Vidyapeeth, Karjat, fumigated and stored in loosely tied gunny bags, at room temperature for four months.

Extraction of the lipids: The paddy varieties were dehulled using Rensington Shearing Machine and the brown rice was then ground to 60 mesh. One hundred gram of flour was extracted thrice with four volumes of chloroform: methanol mixture 2:1 (v/v)[15] in a Soxlet extractor at 70°C for 6 hr. The combined extracts were evaporated to dryness in a rotary eva-

porator. The residue was dissolved in similar solvent mixture and washed with water to obtain purified total lipid[15].

Esterfication of the lipids: Two hundred and fifty mg of the lipid was refluxed at 110°C for 6 hr in 25 ml of 5 per cent HCL in methanol[16], cooled, transferred to a separating funnel and shaken with 20 ml water and 30 ml petroleum ether (b. p. 40-60°C). The aqueous layer was reextracted twice with 20 ml of ether. The combined ether extracts were washed with water until free of acid, dried over anhydrous sodium sulphite and concentrated under reduced pressure in a rotary evaporator.

The methyl esters were separated from unsaponifiable matter by the silicic acid (Acme Synthetic Chemicals, India) column (2.5 cm×60 cm) chromatographic method of Luddy et al.[17] and eluted with petroleum ether (b. p. 40°-60°C): diethyl ether (99:1 v/v) in about 2 hr at a rate of 2 ml per min. The solvent was evaporated from the eluate under reduced pressure in a rotary evaporator.

Similar extraction and esterification of lipids from stored paddy was carried out at regular intervals of four weeks.

Analysis of fatty acid composition of the methyl esters of lipids by GLC: The methyl ester obtained from the lipids of freshly harvested stored rice samples were analysed on a Tracor-560 model gas chromatograph equipped with a flame ionisation detector. A glass column (2 mm×180 cm) packed with 20 per cent diethylene glycol adipate supported on acid washed chromosorb-w (80-100 mesh) was used for the analysis. The oven temperature was maintained isothermally at 190°C, while both the detector and injector port were maintained at 300°C. The carrier gas (nitrogen) flow rate was 20 ml/min. The identification of the fatty acid ester was made on the basis of retention time of the standard derivatives. Quantitative estimation of the fatty acid composition was done by the peak area method.

Results and Discussion

The total lipid content of different varieties of brown rice extracted with chloroform-methanol (2:1) during different periods of storage are listed in Table 1. This data are in agreement with those in other varieties[7-10]. It is observed that the total lipid content of the brown rice in all the varieties remained almost constant during the period of storage, which is similar to those reported for milled rice.[12,18,19]

The major fatty acids of lipids of freshly harvested and stored rice varities are presented in Table 2. Only

TABLE 1. TOTAL LIPID CONTENT OF BROWN RICE DURING STORAGE

Variety	Lipid content (%) during different days of storage				
	0	30	60	90	120
Taichung Native-1	2.37	2.36	2.35	2.35	2.30
Karjat-13-21-2	2.55	2.55	2.54	2.53	2.53
IR-8	2.15	2.15	2.10	2.10	2.10
Ratnagiri-24	2.47	2.46	2.46	2.45	2.44
Karjat-7-3-A	1.63	1.62	1.62	1.61	1.60
Jaya	1.95	1.94	1.94	1.93	1.92
Pankaj	2.29	2.28	2.27	2.27	2.26
Basmati-257	2.38	2.37	2.36	2.36	2.35
Ambemohor	2.36	2.36	2.35	2.35	2.35
Pusa-33	2.47	2.47	2.47	2.46	2.44

*Results average of three experiments

TABLE 2. FATTY ACID COMPOSITION (PER CENT) OF BROWN RICE VARIETIES AT HARVEST AND DURING STORAGE

	At harvest			30 days			60 days			90 days			120 days		
	Pal.	Oleic.	Lino.	Pal.	Oleic.	Lino.	Pal.	Oleic.	Lino.	Pal.	Oleic.	Lino.	Pal.	Oleic.	Lino.
Taichung Native-1	25.7	41.8	32.3	25.6	41.8	32.5	25.5	41.6	32.8	25.4	41.4	33.1	25.4	40.9	33.6
Karjat-13-21-2	28.5	46.1	25.3	28.4	45.9	25.6	28.2	45.8	25.9	28.1	45.6	26.1	28.0	45.4	26.5
IR-8	35.4	44.1	20.4	35.3	43.9	20.7	35.1	43.7	21.1	34.9	43.4	21.5	34.8	43.2	21.8
Ratnagiri-24	25.2	46.2	28.5	25.1	46.0	38.7	24.9	45.9	29.1	24.8	45.6	29.5	24.6	45.4	29.9
Karjat-7-3-A	27.5	47.1	25.2	27.4	47.0	25.5	27.3	46.7	25.9	27.1	46.4	26.3	27.0	46.3	26.6
Jaya	29.5	44.3	26.1	29.4	44.2	26.3	29.1	44.0	26.7	29.0	43.8	27.1	28.8	43.6	27.5
Pankanj	32.2	46.1	21.6	32.0	46.0	21.9	31.8	45.8	22.3	31.6	45.6	22.7	31.4	45.8	23.0
Basmati-257	26.4	48.7	24.8	26.3	48.5	25.1	26.1	48.0	25.7	25.9	47.7	26.2	25.7	47.4	26.7
Ambemohor	25.3	45.6	29.0	26.1	45,4	29.4	24.9	45.0	30.0	24.6	44.8	30.5	24.4	44.6	30.8
Pusa-33	28.1	44.3	27.5	27.9	44.1	27.8	27.8	44.0	28.1	27.6	43.8	28.4	27.5	43.6	28.8

Pal—palmitic acid; Lino—linoleic acid.

TABLE 3. MINOR FATTY ACIDS IN BROWN RICE AT HARVEST AND ON STORAGE

Variety	Freshly harvested					60 days					120 days				
	C	L	M	LO	A	C	L	M	LO	A	C	L	M	LO	A
Taichung Native-1	+	+	+	+	+	+	+	+	+	+	—	—	+	—	—
Karjat-13-21-2	+	+	+	+	+	+	+	+	+	+	—	—	+	—	+
IR-8	+	+	+	+	+	+	—	+	+	—	—	—	+	—	—
Ratnagiri-24	+	+	+	+	+	+	+	—	+	—	—	—	—	—	—
Karjat-7-3-A	+	+	+	+	+	+	+	+	+	—	—	—	+	+	—
Jaya	+	+	+	+	+	+	+	+	+	—	—	+	+	—	—
Pankaj	+	+	+	+	+	+	+	+	+	—	+	+	+	—	—
Basmati-257	+	+	+	+	+	+	+	+	+	—	+	+	—	—	—
Ambemohor	+	+	+	+	+	+	+	+	+	—	+	—	—	—	—
Pusa-33	+	+	+	+	+	+	+	+	—	—	—	—	+	—	—

C—Capric acid; L—Lauric acid; M—Myristic acid: LO—Linolenic acid and A—Arachidic acid.
+: detected, —: absent or not detected.
Results are average of three experiments

palmitic, oleic and linoleic acids were determined quantitatively. It is observed that 'Basmati-257' had the maximum oleic acid content. Palmitic acid content in all the varieties were low compared to the unsaturated fatty acids (oleic and linoleic) contents. The values reported here are in agreement with those reported for brown rice[11,14] and milled rice[12-14]. During storage, the levels of palmitic acid and oleic acid decreased while those of linoleic acid increased in all the varieties. However, the extent and the period of storage at which they occured differed in individual varieties. It has been reported that some other varieties of old rice contained less palmitic and linoleic acids and higher oleic acid levels.[12,18]

In addition to the three major fatty acids, traces of capric, lauric, myristic, stearic, linolenic and arachidic acids are also present in these varieties (Table 3) at harvest time. Except in 'Karjat-13-21-2', in all the varieties, arachidic acid was found disappear on storage for 120 days. Similarly at the end of 20 days storage, lauric acid could be detected only in 'Jaya', 'Pankaj' and 'Basmati-257' and capric acid could be detected in 'Karjat 13-21-2', 'Pankaj', 'Ambemohar' and 'Basmati 257'.

These changes in different fatty acids, content during storage are reported to influence the levels of carbonyl compounds responsible for the "cooked flavour" of aged rice[20]. The present observations suggest that in all the varieties tested, oleic acid and linoleic acid may play a major role.

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Variabilities in the Dehulling Characteristics of Pigeon Pea (*Cajanus cajan* L.) Cultivars

N. RAMAKRISHNAIAH AND P. P. KURIEN
Central Food Technological Research Institute, Mysore-13, India

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Milling (dehusking and splitting) characteristics of eighteen cultivars and one commercial variety of pigeon pea were studied. There were variations in dehusking characteristics, which were independent of size or husk content of the grain, but was influenced by other varietal factors such as adherence of husk to cotyledons and moisture content. The yield of dehusked split *dhal* and pearled grains also do not depend on the size of grains or the proportion of the cotyledons, but are influenced by splitting and scouring losses in dehusking machines. Splitting during dehusking appears to be a varietal character which is also influenced by moisture. Moisture has an adverse effect on dehusking. The moisture level at which there is maximum dehusking is specific to each variety and is usually below the normal moisture level. Appropriate pre-milling heat treatment enhances dehusking.

Pigeon pea (Tur, Arhar, *Cajanus cajan* L.) is the second largest pulse crop of India forming about fifteen per cent of the total pulse production[1]. The entire annual production of about two million tonnes of this grain legume is converted to *dhal* (dehusked split form) for consumption. Several methods are adopted for dehusking the pulse[2], which may be classified as wet (water soaking, sun-drying and milling) and dry (oil/water application sun-drying and milling) methods. The processing is usually done in two steps: the first step for loosening the husk by wet and/or dry methods and the second step for dehusking and splitting using suitable machines.[2,3]

Variations in milling characterstics of pigeon pea as influenced by variety and agro-climatic factors are recognised in milling circles, but not fully understood[2]. Millers use different techniques like, water soaking, oil and water application and sun-drying for varying periods or even treatments with alkaline solutions to impart adequate loosening of husk[3]. In addition to these pre-milling treatments, the difficult-to-mill varieties are scoured repeatedly in dehulling roller machines to obtain complete dehusking. Recently, it is reported that the husk is attached to the cotyledons through a layer of gums, the amount, chemical nature and level of hydration of which affect its aherence[4,5]. As pigeon pea is consumed in India as *dhal*, its dehusking characteristics is a major factor for *dhal* recovery in the processing stages.

It has been reported that when pigeon pea is heated

and tempered (conditioned) under controlled conditions to a 'critical moisture level', the husk is loosened and becomes brittle so that it can be removed in an abrasion machine[6]. Under appropriate processing conditions, adequate loosening of husk may be achieved in different cultivars[7]. The present study is aimed at assessing the influence of varietal factors on the extent (degree) of dehusking and yield of split/unsplit dehusked grains.

Materials and Methods

Eighteen varieties of pigeon pea obtained from Agricultural Research Stations (International Crop Research Institute for Semi-Arid Tropics, Hyderabad and Indian Agricultural Research Institute, New Delhi) and one commercial variety from Uttar Pradesh were cleaned, size-graded and kept for moisture equilibration in closed containers for three weeks and were used for the studies.

Thousand grain weight was determined by counting out one thousand grains and weighing them, in replicates for average value.

Husk, germ and cotyledon contents: was done manually for each variety by separating the husk from 20 g. of the grains by a pin and scalpel, and extracting the germ after splitting the kernel. Husk, cotyledons and germ were weighed seperately for calculating their percentages.

Degree/extent of dehusking: This was determined for each variety after conditioning and dehusking the grains as described below and then separating the husked and unhusked split *dhal* and pearled grains (after dehusking) by hand and weighing them separately. Grains/*dhal* with more than 75 per cent husk removed are considered dehusked. Degree of dehusking $= \frac{\text{wt. of dehusked split dhal and unsplit grains}}{\text{wt. of total split dhal and unsplit grains}} \times 100$

Yields of dhal: yields were calculated as 'apparant' and 'true'. The apparent yield is the percentage of split *dhal* and unsplit grains (both husked and unhusked) from a weighed sample of pulse after dehusking and aspiration.

$$\text{i.e., } \frac{\text{wt. of split } dhal \text{ and unsplit pulse}}{\text{wt. of grains used for dehusking}} \times 100$$

The true yield was determined by deducting the weight of the remaining husk from the unhusked split *dhal* and whole grains (after determining the degree of dehusking) and calculating that weight as percentage of grains dehusked.

$$\text{i.e., } \frac{\text{Wt. of dehusked } dhal \text{ and pulse} + (\text{wt. of unhusked } dhal \text{ and pulse} - \text{wt. of husk})}{\text{Wt. of grain used for milling}} \times 100$$

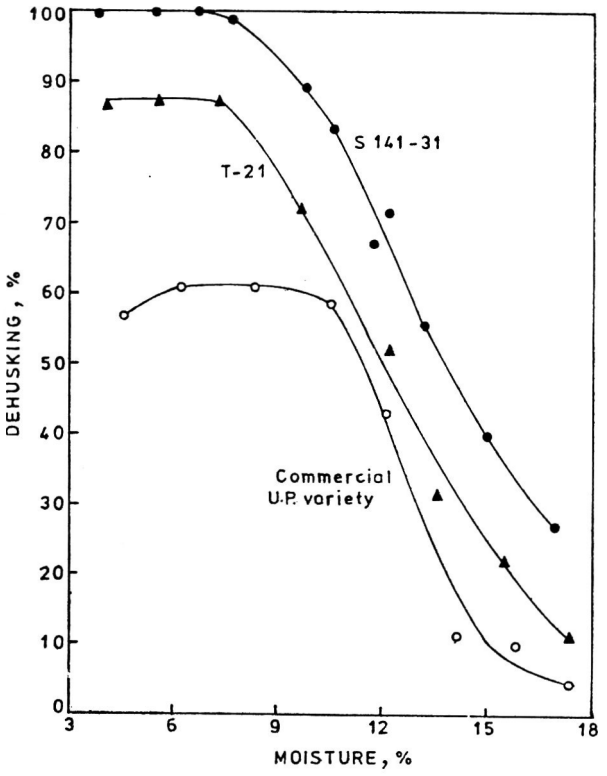

Fig.1. Influence of moisture on degree of dehusking.

(True yield is only a calculated value and a theoretical possibility. In actual milling, the yield on complete dehusking can be less due to further scouring).

Effect of moisture on degree of dehusking: This was evaluated for three cultivars of high, medium and low degrees of dehusking. Varieties were dried at 50°C or treated with calculated quantities of moisture to get moisture contents of approximately 4.0, 5.0, 6.0, etc. upto 18.0 per cent and equilibrated for 3 weeks. The samples were dehusked after estimating the moisture. Degree of dehusking was calculated and plotted against moisture content (Fig. 1).

Effect of temperature of premilling heat treatment on the degree of dehusking: This was determined with the (same) three cultivars. The grains were heated at air temperature of 50, 60, 70 and upto 150°C to a moisture level of 7.5 to 8.0 per cent and dehulled after equilibration. Degree of dehusking was calculated after dehulling and hand-separating the split *dhal* and unsplit pulse. Degree of dehusking was plotted against temperature of premilling treatment. (Fig. 2).

Conditioning: This was done by heating 230 g of each cultivar at 120°C air temperature for 2 min. when the grains attain a temperature of 80-85°C. The grains were tempered in insulated wire baskets for 2.5 hr. This treatment was repeated four times after which the moisture was determined by oven method prior to dehusking.

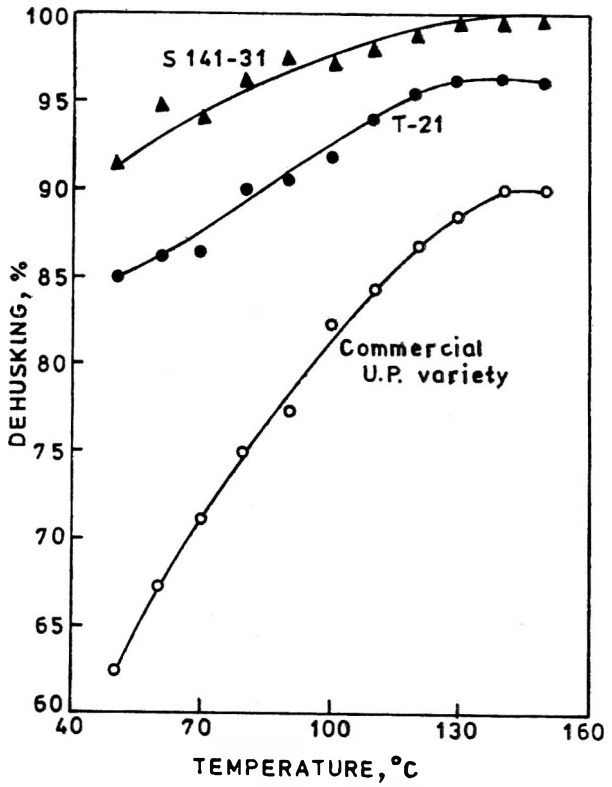

Fig. 2. Influence of Temperature of Premilling heat-treatment on degree of dehusking.

Conditioning unit: used for experiment was a laboratory model assembled for this study and consisted of a portable electric blower attached to an electrical air heater (3 Kw) from which heated air can be blown into a heating glass chamber. The blower and heater were provided with dimmerstats for controlling air-flow and heat. Thermometers were provided at the entry and exit points of the chamber for measuring air temperatures. The grains in the chamber were kept fluidised by adequate air-flow.

Dehusking: of the conditioned grains was done in a '*Karkoran*' barley de-braning machine (Karkoran, England) by milling 200 g of the grains for 45 sec. The husk and powder (coming from the machine) were discarded. Split *dhal* and unsplit pulses were collected, aspirated and weighed.

Results and Discussions

Thousand grain weight of the cultivars which is indicative of the mass (size) of the grains, given in Table 1, varied from 70.9 to 191.6 g. The grains were nearly spherical in shape. In traditional *dhal* milling practice, bold-grained varieties are preferred as they are easier to dehusk and considered to give a better yield of *dhal*. In a sample containing mixture of big and small grains, the bigger grains get dehusked first due to mechanical advantages, while smaller grains escape abrasion, leaving large amounts of unhusked grains.

TABLE 1. THOUSAND GRAIN WEIGHT, HUSK, COTYLEDON AND GERM CONTENT OF THE VARIETIES

Variety/cultivar	1000 grain wt (g)	Husk (%)	Cotyledon (%)	Germ (%)
Commercial var.	70.9	12.5	86.4	1.1
B.S. 1	76.8	14.5	84.2	1.3
T. 21	79.6	14.8	83.8	1.4
S-8	84.2	14.1	85.0	0.9
S-5	85.2	15.5	83.4	1.1
ICP-722 1	90.9	12.3	86.7	1.0
ICP-7118	95.6	14.0	85.0	1.0
ICP-1	97.3	13.9	85.3	0.8
G. Local	97.9	13.2	85.8	1.0
ICP-7120	98.9	14.1	85.1	0.8
Hy-1	103.1	12.7	86.3	1.0
ICP-7182	104.7	13.5	85.5	1.0
Hy-A	107.2	14.1	85.0	0.9
Hyd-2	119.9	12.7	86.6	0.7
Hy-2	132.9	11.6	87.5	0.9
ICP-7119	177.3	13.0	85.9	1.1
Hy-3C	188.1	11.0	88.2	0.8
Hy-3A	190.9	10.5	88.9	0.6
S-141-31	191.6	14.8	84.0	1.2

The husk content of the cultivars varied from 10.5 to 15.5 per cent (Table 1). Cotyledon content of grains vary from 83.4 to 88.9 per cent and germ content from 0.6 to 1.3 per cent (Table 1). Husk content is not influenced by the size of grain. However, smaller grains have relatively higher husk and germ contents.

Milling behaviour of cultivars vary as seen from data presented in Table 2. Initial moisture content of samples varied from 10.6 to 13.0 per cent (not reported here); while after conditioning the moisture content varied between 6.2 and 7.2 per cent indicating a loss of more than 4 per cent during conditioning. It is reported that the husk is attached to the cotyledons through a layer of gum, the chemical nature, quantity and level of hydration of which determine its tackiness and influence the milling behaviour of the grains.[4,5] These gums are reported to contain pentosans, hexosans, other polysaccharides and uronic acids.[8,10] Variations in the degree of dehusking under uniform conditions of processing indicate a varying influence of cultivar characteristics. The size of grain do not appear to influence dehusking. Grains with a low degree of dehusking are not necessarily those with a higher husk content. Abrasion efficiency of the dehusking machine, no doubt, has a significant

TABLE 2. DEGREE OF DEHUSKING OF THE VARIETIES

Variety/Cultivar	Moisture at milling (%)	Per cent dehusking		
		Split (%)	Unsplit (%)	Total (%)
Commercial var.	6.3	54.1	77.9	67.1
BS-1	6.9	92.1	85.4	88.6
T-21	6.9	92.3	90.5	91.4
S-8	7.2	88.3	86.9	88.0
S-5	6.4	72.9	78.0	75.2
ICP-7221	7.1	75.6	87.1	81.3
ICP-7118	6.8	94.4	94.0	94.2
ICP-1	6.6	68.0	72.6	70.3
G-Local	6.2	90.5	72.4	84.6
ICP-7120	6.5	76.4	86.4	82.3
Hy-1	7.0	67.7	78.4	72.1
ICP-7182	6.8	89.5	92.3	90.8
Hy-4	7.2	81.8	89.5	85.8
Hyd-2	6.5	87.6	74.4	85.0
Hy-2	7.2	79.7	85.5	84.1
ICP-7119	6.3	82.8	92.5	85.2
Hy-3C	6.8	83.6	91.9	85.0
Hy-3A	7.0	86.8	90.7	87.6
S-141-31	7.2	100.0	100.0	100.0

TABLE 3. APPARENT YIELD OF SPLITS AND UNSPLITS FROM DIFFERENT CULTIVARS AND CALCULATED (TRUE) YIELD ON COMPLETE DEHUSKING

Variety/Cultivar	Apparent yield (%)			True calculated yield (%)
	Splits	Unsplits	Total	
Commercial var.	35.9	44.3	80.2	76.8
B.S. 1	50.3	27.6	77.9	76.6
T-21	33.1	46.1	79.2	78.2
S-8	48.6	29.5	78.1	76.8
S-5	44.4	35.0	79.4	76.3
ICP-7221	25.8	54.9	80.7	78.8
ICP-7118	43.6	35.1	78.8	78.1
ICP-1	44.5	33.8	78.3	75.0
G-Local	66.6	7.1	73.7	72.3
ICP-7120	20.5	58.9	79.4	77.4
Hy-1	67.8	11.6	79.4	73.2
ICP-7182	55.5	23.1	78.6	77.6
Hy-4	36.6	41.9	78.5	76.9
Hyd-2	72.8	2.3	75.1	73.6
Hy-2	69.0	10.9	79.9	78.4
ICP-7119	71.1	7.0	78.1	76.6
Hy-3C	75.6	3.6	79.2	77.9
H7-3A	75.0	4.8	79.8	78.8
S-141-31	20.8	61.2	82.0	82.0

influence on dehusking, but identical conditions have been used to eliminate this influence. Variations in the degree of dehusking obtained with different cultivars are possibly the result of varying extent of loosening of husk from the cotyledons after pre-milling treatments reducing the influence of gums. It may, therefore be inferred that under optimum conditions of premilling treatments, maximum loosening of husk from the cotyledons can be obtained.

Apparent yield and extent (percentage) of splitting of grains are given in Table 3. The yield of *dhal* depends on the cotyledon content and the extent of peripheral scouring. Peripheral scouring is influenced by the hardness of the grain (to resist scouring) abrasiveness of the scouring machine and the duration of scouring. Addition of moisture softens the grains and make them susceptable to scouring; while drying hardens the grains and increase their resistance to scouring. The cultivars in the present trials have lost about 4 per cent moisture to reach 6.2-7.2 per cent moisture levels which gives them increased resistance to peripheral scouring. However, as Table 3 shows the yields of dehusked split and unsplit grains with similar cotyledon contents vary indicating that even at similar moisture levels, resistance to scouring vary among the cultivars. Similarly, extent of splitting of the grain also appear to vary with variety, in addition to the influence of moisture.

Splitting during milling is normally disadvantageous in milling operation as the splits get scoured at the edges causing powder loss (Hy-2' and 'G-local' in contrast to 'ICP-7120' and 'S-141-31'). However, in varieties like 'Hy-3C' and 'Hy-3A' with high cotyledon contents, the splitting was high (75-76 per cent) and the yields were also high due to less scouring losses possibly the grains were hard and resist abrasion. The yields are generally higher when splitting of cotyledons is less, and where cotyledons are hard enough to resist abrasion.

Effect of moisture on degree of dehusking in three selected varieties of pigeon pea ('S-141-31', 'T-21' and Commercial variety) is given in Fig. 1. When the moisture levels were progressively reduced, the degree of dehusking increased until it reached a maximum and it varied with variety. Dehusking is complete in variety 'S-141-31' while in variety 'T-21', it was 87 per cent. In commercial variety, it was only 62 per cent. Further reduction in moisture did not help in increasing the degree of dehusking. These moisture levels may be called 'critical' for that variety, as the grains showed maximum dehusking at or below that moisture level.

The critical moisture level may be reached by conditioning the grain at low temperatures for prolonged periods or at high temperatures for short time. The influence of the temperature of conditioning on the degree of dehusking is shown in Fig. 2. At higher temperatures of conditioning, the degree of dehusking improved and even the poor hulling the commercial variety showed, improved by dehusking (90 per cent). However, the temperature and duration of heating should be carefully selected as prolonged heating at high temperatures may adversely affect the flavour, taste, colour and quality of the product.

Different cultivars of pigeon pea display varying dehulling characteristics independent of their size and husk contents, but influenced by other varietal characteristics (like quality and quantity of gums, etc.) and the moisture level of the grain. Splitting appears to be a varietal character which is also influenced by moisture. Factors that generally reduce the yield are moisture which softens the grain and splitting which exposes more areas of the kernal to the abrasive action of machines. Eventhough, pigeon pea cultivars show maximum dehusking at moisture levels specific for that variety, the extent of dehusking can be improved by proper pre-milling heat treatments. Varieties with poor milling behaviour may give low yields of *dhal* on normal processing and offset the advantages of increased agricultural production.

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Studies on Linseed *(Linum usitatissimum)* as a Protein Source. 2. Evidence of Toxicity and Treatments to Improve Quality

V. M. MANDOKHOT* AND N. SINGH
Central Food Technological Research Institute, Mysore, India

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Deoiled, demucilaged and dehulled linseed meals were tested for various reported toxic factors. Vitamin B_6 antagonism was found to be the most prominent antinutritional factor. Among different methods, autoclaving was found promising in improving the quality of linseed meals.

Chicks fed with linseed meal develop toxic manifestations[1] which can be attributed to various factors[2,3]. Linseed has been reported to cause hydrocyanic acid poisoning and goitre in ewes and rats[4-6]. A pyridoxine antagonist, linatine, has been isolated from linseed[7], following the reports of vitamin B_6 deficiency symptoms in animals and their vital glands[8,9]. Allergens have also been reported to occur in linseed[10].

Investigations were carried out to test the presence of various toxic and antinutritional factors in deoiled

**Present address:* College of Animal Sciences, Haryana Agricultural University, Hissar, India.

linseed meals of natural. demucilaged and dehulled linseed and various methods were tried to improve the quality of linseed meals for use in chick rations. The results of these studies are presented in this paper.

Materials and Methods

Deoiled meals of whole linseed (L), demucilaged linseed (Lm) and dehulled linseed (Lp), were prepared as described earlier[1]. For allergens. the proteinaceous fraction was prepared from the LP material[11] by peptization at pH 6.8. Wheat gluten and salt mixture for goitrogenic activity were prepared according to Halverson *et. al*[12]; and the vitamin free casein by the method of Elsadr[13] for the basal vitamin B_6 deficient diet[14]. Isopropanol extraction of linseed was carried out according to the method of Klosterman *et al*[7].

Deoiled meal was autoclaved dry at 15 lb. pressure for 15 min. For wet autoclaving, the meals were slurried in hot water, allowed to settle, the supernant fluid was drained and the residue was autoclaved as before. The autoclaved materials were dried in a cross-flow drier at 60°C.

Analyses and tests for toxicity: Presence of hydrocyanic acid (HCN) was tested by Guignard's method[15] and quantitative estimation by standard A.O.A.C. method[16]. Selenium was estimated by the method of Cumins *et al.*[17] and thiocyanate by Johnson and Jones[18] method. Male guinea pigs were used[10],[11] for *allergen* test and male weanling albino rats for goitrogenic activity. The animals were kept on control diet (ground corn 68, wheat gluten 30, and salt mixture 2, parts), or on test diets with 50 or 75 per cent of gluten protein being replaced by linseed materials. Chicks of comparable weight from the test as well as control group from previous study[1] were used for obtaining vital glands. Procedure adopted for chick-growth assay for vitamin B_6 will be described later.

Chick experiments: One-day old vaccinated chicks of Arbor Acres broiler strain were wing-banded, weighed, and randomly sorted into different groups, and then reared on different diets as per the schedule. Linseed materials at different levels replaced protein in the control diet and made isocaloric and isonitrogeneous by adjusting the starch and/or other ingredients and served as test diet. Feed and water to the brooders were given *ad lib.* Weights of individual birds and group-wise feed intakes were recorded weekly.

Chick growth assay: Pyridoxin antagonism was conducted in 16 groups of each with 16 chicks. In the test diets, 10 or 25 per cent of the casein protein was replaced by Lp and Lm materials as shown in Table 1.

Lp meal, after extraction with isopropanol was used to study its effect on chicks, (5 groups of 16 each). One-fourth of the groundnut protein out of control diet was replaced by untreated or isopropanol extracted Lp meals, with or without vitamin B_6 supplementation.[1]

TABLE 1. CHICK GROWTH ASSAY FOR VITAMIN B_6 ANTAGONISM

Diet+	Vitamin B_6 (ppm)	3-wk expt feeding* Av. wt (g)	Av feed intake (g)
Basal	nil	—	—
Basal	8	135	184
Basal	16	165	242
Basal	24	134	188
Lp10	—	—	—
Lp10	8	145	218
Lp10	16	236	315
Lp10	24	143	185
Lp25	—	—	—
Lp25	8	133	163
Lp25	16	134	189
Lp25	24	137	185
Lm10	—	—	—
Lm10	16	172	216
Lm25	—	—	—
Lm25	16	133	189

*One-day old chick; initial wt 31-33 g.

+ Basal dietary protin replaced by Lp or Lm at 10 or 25 per cent levels as indicatec.

Autoclaved materials were used in determining the quality in two experiments, as shown in Table 2. In the 2 week study, there were 8 groups each with 23 chicks which were fed with test diet replacing 10 per cent of the groundnut protein by untreated or autoclaved materials. In the 3 week study, 6 groups of 16 chicks each were used, which were fed with test diet replacing 25 per cent of the control protein by the autoclaved materials.

Metabolic studies were carried out on 25 chicks each with 8 treatments having 5 replicates in each. One day old chicks kept on standard rations for one week were randomly sorted into different groups as given in Table 3. Nitrogen-free diet of Summer and Fisher[19] was used, and protein and fiber of the other diets were adjusted at 13 and 6 per cent respectively[20]. FAO reference protein formed the basis for amino acid supplementation[21]. The experiment lasted for 10 days. Individual birds were weighed and replicate-wise feed consumption recorded. Feed was stopped 12 hr before the termination experiment. The 12 hr

TABLE 2. EFFECT OF AUTOCLAVING ON QUALITY OF CHICK RATIONS

Diet	Average wt (g)	Average feed intake (g)
2-wk period		
Control	216	258
L10	197	236
Lm10	212	248
Lm10 (DA)	21[illegible]	251
Lm10 (WA)	226	258
Lp10	209	247
Lp10 (DA)	222	262
Lp10 (WA)	229	267
3-wk period		
Control	269^a	363
L25 (DA)	190^b	190
Lm25 (DA)	243^a	336
Lm25 (WA)	263^a	344
Lp25 (DA)	241^a	362
Lp25 (WA)	269^a	353

Test diets had various linseed materials, untreated or dry or wet autoclaved (DA or WA), replacing the control diet groundnut protein at 10 or 25 per cent levels.

Means with different superscript letters differ significantly ($P<0.05$).

The initial mean wt range of 2-wk and 3-wk period chicks was 41-43 and 55-59 g. respectively.

TABLE 3. METABOLIC STUDIES OF CHICKS

Diet treatments	Mean N intake (g)	Mean wt (final) (g)	Mean apparent digest (%)	NPU (%)	LPU (%)
N-free diet	0.349^a	66.0^a	—	—	—
Casein control	9.559^b	101.2^b	98.1	32.89^a	1.79^{ab}
Lm (DA)	9.663^b	97.6^b	96.3	31.93^a	0.45^d
Lm (DA) + Lys	15.381^c	132.6^c	96.5	47.55^b	1.73^{ab}
Lm (DA) + Lys + Meth	18.671^d	154.8^d	96.8	61.92^c	1.77^a
Lm (WA)	18.034^d	145.7^e	97.4	44.42^b	1.23^a
Lm (WA) + Lys	22.678^f	172.0^f	96.5	65.82^c	1.82^{ab}
Lm (WA) + Lys + Meth	24.277^f	193.8^g	97.2	62.78^c	1.80^a

Means with different superscript letters differ significantly ($P<0.05$)

NPU: net protein utilization; LPU: Liver protein utilization

starved birds were anaesthised and the carcass cut open for taking out liver. Net Protein Utilization (NPU) and apparent digestibility were determined by conventional methods, and the Liver Protein Utilization (LPU) calculated according to Mokady *et al*[22].

Results and Discussions

Toxicity tests: Analyses for HCN showed values of 47-49 mg/100 g in different linseed materials, in agreement with the reported[15] literature values. Heat during expeller pressing reduces the HCN content due to inactivation of the cyanogenetic glucosidases[23]. Autoclaving reduced the HCN to negligible amount.

Selenium values were 1-2 ppm, falling within the reported values[24], and therefore not considered deleterious. Thiocyanates were absent.

None of the guinea pigs experienced shock in tests with the preparations made from linseed materials, indicating the absence of allergens. The thyroid glands of rats on the test diets for 7 weeks also gave no evidence of goitrogenic activity. From the weights of liver, kidney, thyroid, pancreas and adrenal glands of chicks surviving on linseed diets, the following generalisation was possible. With the exception of adrenals, all other glands showed some enlargement due to linseed in diets, more pronounced with higher levels than lower ones. Earlier reports[8,9,25,26,27]. have attributed such adverse effects to vitamin B_6 deficiency in diets.

Chick growth assay for vitamin B_6 antagonism gave the following results (Table 1). On basel diet and the linseed diets, not supplemented with vitamin B_6, no chicks survived the 3-week period. The chicks began to die on 6th and 9th day onwards on the 10 and 25 percent test diets respectively. These results were comparable to other findings[28,29]. Postmortem showed exudative infiltration in the abdominal region, more pronounced at higher levels. Liver and spleen were pale yellow with rounded borders, the enlarged gall bladder full of bile, the intestines practically empty and the mucous membrane showed petechial haemorrhage.

Based on 20 ppm vitamin B_6 supplementation for checking the mortality in chicks on linseed diets[30], 3 levels of supplementation, 8, 16 and 24 ppm were used. There was a progressive increase in feed intake and growth with increase in vitamin B_6 supplementation with maximum was observed at 16 ppm. The decrease noticed at 24 ppm supplementation may be due to vitamin imbalance[31,32]. The vitamin B_6 supplemented linseed diets showed better response than the vitamin supplemented basal diets, but only in 10 per cent test diets and not in the 25 per cent ones. Apparently the linseed-casein mixture protein was better than casein alone, but at higher linseed levels, the vitamin B_6 antagonist proved harmful. In this

experiment, among the supplemented diets Lp was found to be better than the Lm, and the best combination being vitamin B_6 at 16 ppm in the Lp 10 test diets.

Methods to improve quality: The isopropanol extracted linseed meal was definitely better than untreated one, but not superior to the latter when supplemented with vitamin B_6. However, in neither case the response was comparable to the control.

Incorporation of untreated linseed materials replacing the control protein at 10 per cent level (Table 2) led to deterioration with respect to both growth response and feed intake. However, Lm and Lp were better than the L meals. Dry autoclaving of the Lp did improve the quality over the untreated material, but not in case of Lm. Wet autoclaving of the materials led to further improvement in Lp, but very much greater in Lm. Autoclaving definitely improved the quality, the wet one being better than dry.

In the second experiment with 25 per cent replacement of groundnut protein by the autoclaved materials, Lm and Lp were better than the L, (Table 2) and again the wet autoclaving was found better than dry one, specially with Lm material. Between the control, wet autoclaved Lm, and dry and wet autoclaved Lp materials, there was no significant difference in the weight of chicks. These results suggest that the demucilaged linseed (Lm) is better starting material, as the removal of hulls would not be advantageous appreciable enough to justify the costs of further processing, specially for use in poultry feeds.

The results of metabolic studies are presented in Table 3. Between the casein control and dry autoclaved Lm diet, there was no difference in respect of the N intake, final weight, apparent digestibility and NPU values, with the LPU value being much lower in the latter case. There was not much difference in the apparent digestibility values among different treatments. Wet autoclaving was always better than dry autoclaving. Lysine supplementation of either dry or wet autoclaved materials led to improvements. Combined supplementation with lysine and methionine improved in the case of dry autoclaved material for all parameters, but not in the case of wet autoclaved material. Liver protein utilisation values suggested that wet autoclaved material, supplemented with lysine alone was best.

From these studies, the vitamin B_6 antagonism was found to be the most pronounced antinutritional factor. In terms of starting material, demucilaged material was the best. Wet autoclaving of the demucilaged material was indicated to be adequate for amending the vitamin B_6 antagonism. The metabolic studies suggested that the wet autoclaved demucilaged material could be greatly improved for chick nutrition by mere supplementation of lysine.

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Studies on Linseed *(Linum usitatissimum)* as a Protein Source. 3. Comparison with Other Vegetable Protein Sources and a Trial in Broiler Production

V. M. MANDOKHOT* AND NARENDRA SINGH
Central Food Technological Research Institute Mysore-570 013, India

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Autoclaved, demucilaged linseed meals were found to be inferior to other vegetable protein sources in comparative chick growth studies. However, the wet autoclaved meals (Lm.WA) did not show any adverse effects in broiler chicken when incorporated at 50% protein replacement of groundnut meal in rations.

The results of earlier short-duration chick growth experiments[1] showed the promise of wet autoclaving of demucilaged linseed meals as a detoxification treatment, with no mortality and vitamin B_6 deficiency symptoms in the chicks. In the present study, a 6-week chick-growth study was carried out initially to compose the autoclaved demucilaged linseed meals with other vegetable proteins used in poultry rations. Thereafter, wet autoclaved linseed meals were evaluated in broiler production, replacing half or all of the groundnut protein in the rations.

Materials and Methods

Autoclaved linseed meals were prepared as described earlier[1]. Cottonseed meal was obtained from the Regional Research Laboratory, Hyderabad, and other vegetable protein materials were collected from within the Institute.

Comparison with other vegetable protein sources was done in a 6-week chick growth study. The standard diet had 25 per cent groundnut meal (GN). In the test diets, the groundnut meal was totally replaced by deoiled cottonseed, sesame, soybean or wet autoclaved demucilaged linseed (Lm. WA) meals or leaf protein. For isonitrogenic diets, adjustments were made in the corn starch and/or maize contents of the standard diet. All diets had 24 per cent crude protein ($N \times 6.25$) content.

The plan of chick experiments was generally as done earlier[1]. One-day old chicks, fed commercial feed for 4 days, were randomly sorted into different groups of 25 each, the latter in 5 replicates, and fed different diets for 6 weeks.

Broiler production study included the starter and finisher rations, as described in Table 1. The starter ration had 24-25 per cent protein and the finisher ration 21 per cent protein, in either case half of the protein was derived from the vegetable protein sources.

Present address: College of Animal Sciences, Haryana Agricultural University, Hissar (Haryana), India.

TABLE 1. BROILER DIETS COMPOSITION*

Ingredients	Starter**					Finisher**				
	Control C	L.WA+GN A	Lm.WA+GN B	L.WA D	Lm.WA E	Control C	L.WA+GN A	Lm.WA+GN B	L.WA D	Lm.WA E
Groundnut meal (GN)	24.60	12.30	12.30	—	—	19.20	9.60	9.60	—	—
Lysine monohydrochloride	0.7	0.70	0.70	0.80	0.80	0.55	0.55	0.65	0.65	0.65
DL-Methionine	0.10	0.10	0.10	0.10	0.10	0.05	0.05	0.05	0.05	0.05
Ground yellow corn	57.00	53.00	53.00	49.00	49.00	62.60	59.50	59.50	56.30	56.30
Linseed meal, wet autoclaved (L.WA)	—	16.30	—	32.50	—	—	12.70	—	25.4	—
Demucilaged linseed meal, wet autoclaved (Lm. WA)	—	—	16.30	—	32.50	—	—	12.70	—	25.40

*Common ingredients (%): lard 4.0, corn gluten meal 5.0, fish meal 5.0, lucerne meal 1.5, dicalcium phosphate 0.95, calcium carbonate 1.05; salt mixture 0.35, and the recommended vitamin and feed supplements.

**A and B diets had 50% ground nut protein replaced by linseed meals, whereas D and E 100%.

In the test diets either half or total protein of groundnut meal was replaced by treated linseed meals. One day old chicks were fed poultry mash for four days, and then weighed and randomly distributed into five treatments, each with 48 birds divided into four replicates, and reared on different diets in standard brooders. At the end of fifth week, the chicks were transferred to floor pen and put on finisher rations. The digestibility study was conducted in the second week of experimental period.

At the end of the experiment, the processing of birds for ready-to-cook chicken was done by standard procedures. The individual carcasses were graded into I, II, and III on the basis of confirmation, fleshing, breast and back finish and then processed further. The back fat was estimated by the method of Moran *et al*[2].

Results and Discussion

Comparative evaluation of various materials: The results of growth studies are given in Table 2. All diets showed variable food intakes, weight gains, FER, PER and digestibility quotients, with the highest values in leaf protein and the lowest in linseed meal diets. As there were no toxicity symptoms in chicks on diets containing treated linseed meal, the nutritional inferiority might be due to other factors, specially when total vegetable protein was derived from linseed materials.

Broiler production studies: The 10-week cumulative data of chicks on the control (C) and test diets (A, B, D, E) containing wet autoclaved linseed materials at two levels are given in Table 3, which shows that between the two levels of linseed, the replacement of groundnut protein at 50 per cent (A, B) was better than at 100 per cent (D, E). Demucilaging led to definite improvements over the use of mucilage containing meals. At 50 per cent groundnut protein replacement, the wet autoclaved demucilaged linseed (Lm. WA) was comparable to control in respect of feed intake, FER and PER, but slighly inferior in respect

TABLE 2. COMPARATIVE EVALUATION OF CHICK GROWTH RESPONSE ON DIFFERENT PROTEIN SOURCES

Group	Diets	Feed intake (g)	Gain in wt. (g)	FER	PER	Digestibility quotient
A	Groundnut meal-control	6956^{a}	3888^{b}	0.56^{a}	2.36^{a}	60.33
B	Cottonseed meal	5840^{a}	3327^{a}	0.57^{a}	2.39^{a}	58.54
C	Sesame meal	4934^{b}	2739^{c}	0.55^{ab}	2.37^{a}	55.28
D	Leaf protein	7839^{c}	4654^{d}	0.60^{ab}	2.43^{a}	65.53
E	Soybean meal	6955^{a}	3795^{b}	0.54^{ab}	2.28^{a}	54.01
F	Demucilaged linseed meal WA	3355^{d}	1421^{f}	0.42^{c}	1.79^{b}	41.35

Means with different superscripts differed significantly ($p<0.05$). All values are averages of 5 replicates of 5 chicks each.

TABLE 3. CUMULATIVE FEED INTAKE AND WEIGHT GAINS AND OTHER GROWTH RESPONSE VALUES OF CHICKS FROM 10-WEEK FEEDING EXPERIMENT

Group	Diet	Cumulative feed intake (mg)**	Cumulative wt gain (g)**	Mean wt gain (g)	FER	PER	Digestibility (%)
C	Control-GN	47360^a	22920^a	1910^a	0.483^a	2.127^a	60.81
A	L.WA+GN	43830^a	20388^b	1699^b	0.465^a	2.065^a	66.06
B	Lm.WA+GN	46115^a	21840^b	1820^b	0.474^a	2.119^a	64.20
D	L.WA	19268^b	17440^c	620^c	0.386^c	1.670^b	54.00
E	Lm.WA	27792^b	11820^d	985^d	0.425^b	1.896^c	66.18

*For diet symbols see Table 1. A and B had 50% groundnut protein replaced by linseed meals, whereas D and E total groundnut protein replacement.

**Means different superscripts differ significantly ($P<0.05$) values are average of 4 replicates of 12 chicks each.

of weight gain. Because of significant inferior growth response of the chicks on linseed diets with 100 per cent protein replacement of groundnut meal, these birds were excluded from subsequent quality assessment and gradation.

The losses and gains during processing are given in Table 4. The weights with respect to all the processing stages were significantly more in C group followed by B, which was superior to A in all characters. There was no significant difference in the weight of head and legs among the chick on different diets. There was no variation due to sex.

The weights of different commercial cuts and the back fat values are given in Table 5. In general, linseed incorporation led to broilers with commercial cuts of lower weight than the control. However, demucilaged material (Lm. WA-50) was better than mucilage-containing one (L. WA-50). Males showed a heavier weight trend than females in the commercial cuts, except in case of wings. The judgement of the carcass quality by back fat showed the incorporation of wet autoclaved demucilaged linseed (Lm. WA) at 50 per cent protein replacement of groundnut to be as good as the control, but not the incorporation of the wet autoclaved linseed (L. WA).

There was no significant difference in the connective tissue contents of the dark and light muscles in chicks on different diets with the range being 3.477-4.341 and 1.953-2.810 per cent respectively. This indicated that the incorporation of wet autoclaved linseed at 50 per cent protein replacement had no effect in respect of tenderness of poultry meat.

On the basis of health, feathering, confirmation, fleshing and fat, all of the live birds and carcasses were graded for quality assessment. In grade I, there were 89 per cent of birds for control group, 68 per cent for A group (L. WA+GN) and 84 for B group (Lm. WA+GN). The rest of the birds in group A and B, classified as Grade II were also up to the standard in all the characters except the unusual enlargement of the crop. Birds in groups D and E were not graded, because of unsatisfactory growth response as mentioned earlier.

Summary and Conclusions: Comparative growth response study in chicks on various vegetable protein sources showed leaf protein giving best results followed

TABLE 4. WEIGHTS AT DIFFERENT STAGES OF PROCESSING OF CHICKS AND LOSSES OCCURRING THERE OF DURING BROILER PRODUCTION

Group	Diets	wt on starving (g)	Plucked carcass wt (g)	wt of warm eviscerated carcass (g)	wt after chilling (g)	wt of giblet (b)	wt of head and legs (g)
C	Groundnut (GN)	1386^a	1673^a	1293^a	1383^a	83^a	135^a
		(4.54)^a	(15.38)^a	(22.79)^a	(7.11)^a	(6.46)^a	
A	L.WA+GN	1671^b	1478^b	1104^b	1190^b	76^b	129^a
		(5.32)^{ab}	(16.26)^{ab}	(25.63)^b	(7.95)^{ab}	(6.93)^b	
B	Lm.WA+GN	1771^c	1564^b	1219^c	1298^c	77^b	134^a
		(6.05)^b	(17.17)^b	(21.69)^a	(6.60)^b	(6.33)^a	

Means with different supercripts differ significantly ($P<0.5$); Figures in parenthesis are per cent losses at different stages of processing

TABLE 5. WEIGHTS OF DIFFERENT COMMERCIAL CUTS AND ANALYSIS OF BACK SKIN FAT (% OF DRY WEIGHT)

Groups	Diets	Wings (g)	Thighs (g)	Drumstick (g)	Breast (g)	Back (g)	Neck (g)	Back skin fat (g)
C	Control	174.0^a	226.5^a	214.8^a	399.5^a	290.9^a	82.1^a	86.53^a
A	L.WA+GN	169.7^a	196.8^b	190.4^b	321.5^b	244.7^b	71.7^b	82.74^b
B	Lm.WA+GN	164.2^a	213.6^a	198.8^b	357.9^c	272.3^c	71.4^b	85.04^c

Means with different superscripts differ significantly ($P<0.05$)

in successive order by groundnut, soybean, cottonseed, and the wet autoclaved demucilaged linseed meals. The inferiority of linseed meals can be attributed to the presence of factors, other than toxicity. Broiler production studies have revealed that in the commercial rations, wet autoclaved demucilaged linseed meal can replace half of the main vegetable protein without any adverse effect. Complete replacement of vegetable protein by such treated linseed is not possible.

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Studies on Extending the Shelf-life of Soybean Curd

USHA GROVER, SURJAN SINGH AND B. K. MITAL
Department of Food Science and Technology, G. B. Pant University of Agriculture and Technology, Pantnagar, Nainital, U.P., India

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The effect of different treatments like immersion in water or aqueous solutions of 2 per cent citric acid, 5 per cent sodium chloride or a combination of both for 24 hr and daily replacement of the solutions with fresh water and deep fat frying at 180°C for 4 min on the shelf-life of soybean curd stored at 5 and 30°C was studied. The treated samples were analysed for changes in chemical composition and microbiological status and were also evaluated organoleptically. It was found that the curd kept immersed in water or 5 per cent sodium chloride solution at 30°C was acceptable for a single day. However, the curd preserved in 2 per cent citric acid or citric acid and sodium chloride remained wholesome for 6 days at 30°C and for 15 and 18 days when stored at 5°C. Curd, deep fried in fat and stored at 5°C was found acceptable upto 12 days.

Soybean curd or *tofu*, is a popular food of East Asia. In Japan, nearly 42 per cent of the total consumption of soybeans is in the form of curd[1]. It is an excellent and cheap source of quality proteins[2]. Soybean curd resembles *paneer* in colour, appearance and consistency. It could, therefore, serve as an excellent and cheap substitute for *paneer* prepared from cow milk.

Soybean curd contains 80-88 per cent water[3,4] and is subject to rapid microbial spoilage. Under refrigerated conditions, its shelf-life is 1-2 days[5] only which would be much less under the atmospheric conditions prevailing in India. The present investigation was directed towards developing a process to enhance the shelf-life of soybean curd kept at room temperature.

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Materials and Methods

Preparation of Soybean milk: Soybeans (variety 'Bragg') was cleaned, washed and soaked overnight in water, (quantity used being three times, by weight to that of seed) rinsed 2-3 times with water and ground in a colloid mill with hot water (85-90°C). The ratio of dry beans to water (W/V) was 1:10. The resulting suspension was boiled for 10 min with continuous stirring and filtered through two layers of cheese cloth. The soy milk thus obtained contained 6-7 per cent solids.

Preparation of soybean curd: Scybean milk heated to 85°C, and calcium sulphate (0.4 per cent W/V) was added while stirring vigorously. The contents were left undisturbed for 10-15 min in a water bath maintained at 85°C. Later, the vessel was kept at room temperature till the setting of the curd, and the whey was siphoned off and the curd was pressed by applying pressure equivalent to 10 times the weight of soybeans.

Preservation: Freshly prepared curd was cut into blocks of 5 cm×5 cm×3 cm and divided into lots. One lot each was immersed in water or solutions of 5 per cent NaCl, 2 per cent citric acid, and a combination of 2 per cent citric acid and 5 per cent salt. After 24 hr, the curd was stored at 5 or 30°C. Thereafter, the solutions were replaced with tap water daily throughout the storage period.

Pieces of 2.5 cm×1.0 cm×1.0 cm were cut from fresh curd blocks and were deep fried in hydrogenated vegetable oil at about 180°C for 4 min. The surface of curd pieces toughened slightly and became brownish during frying. The fried curd pieces were cooled to room temperature, packed in 50-60 g quantity in polyethylene bags and were stored at 5 and 30°C.

Sampling: Approximately 50 g of the sample was removed using sterilized spatula by cutting the curd without breakage. Moisture on the surface of the cut portion was removed with filter paper. The sample was then macerated aseptically in a clean, dry mortar and pestle and used for analysis. Fat fried curd pieces were crushed and mixed well to obtain a representative sample for analysis.

Chemical analysis: Protein, moisture, fat, ash and calcium were determined by AOAC procedures[6]. Carbohydrate content was calculated by difference.

Non-protein nitrogen was determined by Bhatty and Finalaysen[7] procedure with a slight modification using 10 per cent trichloroacetic acid to precipitate the proteins. Free fatty acids were determined according to the procedure of David[8]. One gram fat was dissolved in 50 ml of 95 per cent heated and neutralized alcohol and titrated hot against 0.05N sodium hydroxide. Free fatty acids were calculated as per cent oleic acid.

For titratable acidity determination, 2 g of the sample was macerated with 20 ml of distilled water and titrated against 0.025 N sodium hydroxide to pH 8.4[9]. Acidity was calculated by applying the equation:

$$\text{Meq acid/g} = \frac{\text{Normality of NaOH} \times \text{Volume of NaOH}}{\text{Weight of curd sample}} \times 10^{-3}$$

Microbiological status: For microbial count, 11g of the sample was macerated aseptically using pestle and mortar. Serial dilutions were prepared using sterilized 2 per cent sodium citrate solution. Appropriate dilutions were used in plating. Tryptone Dextrose Agar was used for determining standard plate count (SPC) and psychrotrophic counts. Plates were incubated at 37°C for 24-48 hr for the former and at 7°C for 10 days for the latter. Coliform count was determined using violet red bile agar. The plates were incubated at 32°C for 18-24 hr. Potato dextrose agar was used for the determination of yeast and mold counts. The plates were incubated at 22°C for 4-5 days.[10]

Organoleptic evaluation: For organoleptic evaluation, the taste panel consisted of 11 members of the departmental staff. The samples were rated on hedonic scale using numerical values from 1 to 9 where value of 1 on this scale denoted extreme dislike and 9 denoted extreme liking[11].

Results and Discussion.

Composition of fresh and fat fried soybean curds are presented in Table 1. Similar values have also been reported by Schroder and Jackson[12]. Deep fat fried curd contained moisture 25.87, protein 21.29, fat 33.91, ash 1.99 and carbohydrates (by difference) 16.94 per cent. Omura *et al*[13]. reported a higher protein content of 26.60 per cent and a lower carbohydrate content of 11.50 per cent for *Aburage*-a deep fat fried soybean curd preparation.

Preservation in water: The moisture content of

TABLE 1. COMPOSITION OF FRESH AND FRIED SOYBEAN CURDS

Constituent	Fresh Curd	Fried Curd
Moisture%	79.80	25.87
Protein%	11.33	21.29
Fat%	5.16	33.91
Ash%	1.28	1.99
Carbohydrates%	2.41	16.94
Crude Fiber%	0.00	0.00
Calcium (mg/100g)	197.5	450

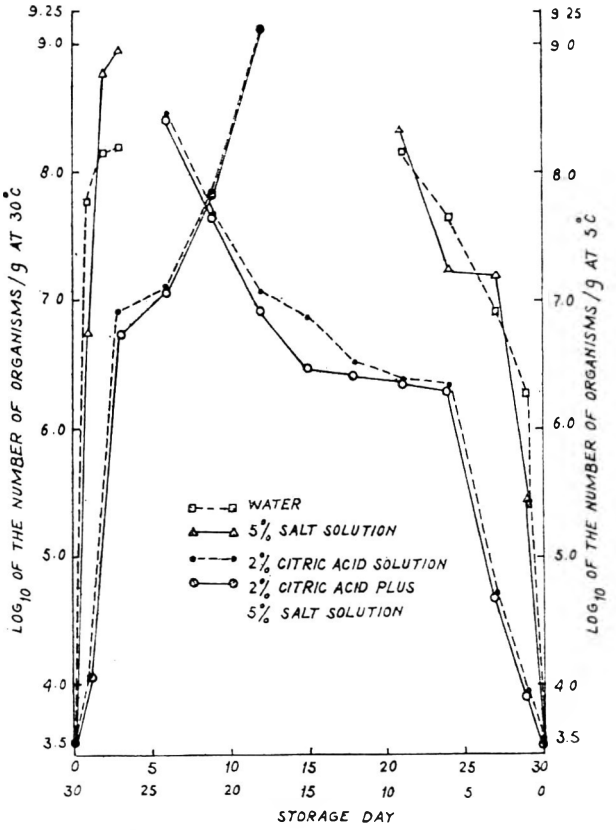

Fig. 1. Effect of various preserving solutions on standard plate count of curd during storage.

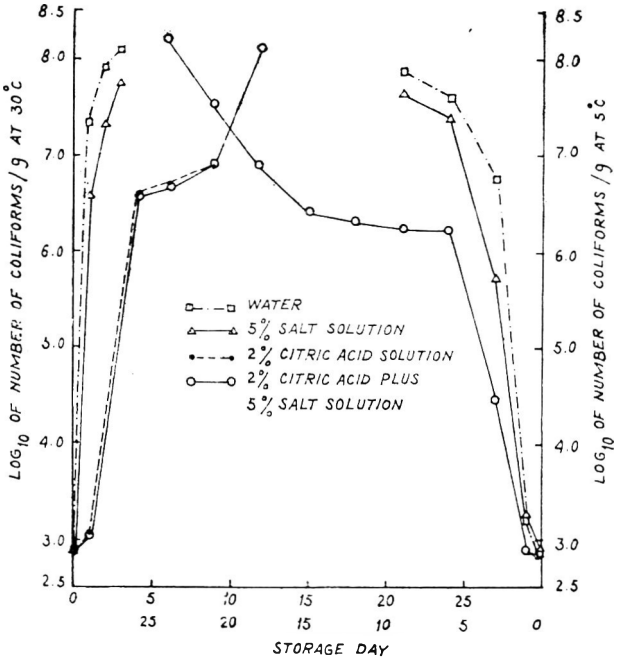

Fig. 2. Effect of various preserving solutions on coliform count of curd during storage.

soybean curd increased from 78.07 per cent to 84.21 per cent and 82.47 per cent at 30 and 5°C storage, respectively, over a period of 3 days. Similar observations have been made by Pontecorvo and Bourne[4]. The protein content of curd (on dry weight basis) decreased from 54.45 to 48.48 per cent at 30°C and to 53.03 per cent at 5°C for the same period. At the end of 3 days storage, the titratable acidity of curd increased from 15×10^{-6} to 83×10^{-6} meg/g at 5°C and to 92×10^{-6} meg/g at 30°C.

The standard plate count and coliform count increased significantly at both storage temperatures (Fig. 1 and 2). The psychrotrophic count of curd stored at 5°C increased from 7.31×10^{2} to 1.65×10^{7}/g at the end of 3 days. Yeasts and molds were not detected in freshly prepared curd, but appeared after 1 day at 30°C and after 3 days at 5°C. Their number increased to 8.85×10^{7}/g at the end of 3 days at 30°C and 1.30×10^{7}/g at the end of 9 days at 5°C storage. Earlier reports[5,14] have also indicated similar changes in microbial population of liquid surrounding soybean curd.

Soybean curd remains wholesome for 10-12 hr under tropical storage conditions. Curd sample preserved in water was found acceptable upto 24 hr stored at 30°C as against 72 hr of sample stored at 5°C. Wu and Salunkhe[13] reported that in-packaged soybean curd had shelf-life of 7 days stored at 4.5°C and it could be extended upto 21 days by in-packaged micro wave heat treatment.

Preservation in NaCl solution: The increase in microbial population of curd preserved in 5 per cent NaCl solution was comparitively less than that observed for in-water preservation (Fig. 1 and 2). Similarly acid production (53×10^{-3} meg/g) in these samples was less than that observed for samples preserved in water (95×10^{-6} meg/g) and stored over 9 days at 5°C.

The soybean curd preserved in 5 per cent NaCl solution remained acceptable upto 6 days as compared to 3 days stored in water at 5°C. However, the curd stored at 30°C was not acceptable after 1 day.

Preservation in citric acid solution: The absorption of moisture by soybean curd preserved in 2 per cent citric acid solution was in the same range. However, after 12 days storage, the protein content of curd preserved in 2 per cent citric acid was 46.78 and 52.52 per cent at 30 and 5°C, respectively. Citric acid depressed the growth of microorganisms (Fig. 1 and 2), and extended the shelf-life of curd to 15 days at 5°C. However, the shelf-life of curd stored at 30°C was found to be only 6 days.

Preservation in a combination of citric acid and NaCl: Lower microbial load was observed when a combination of citric acid and salt was used (Fig.1 and 2).

The shelf-life of soybean curd preserved in such a

mixture was extended upto 18 days as compared to the one preserved in 2 per cent citric acid at 5°C. However. shelf-life of the curd was 6 days stored at 30°C. Pontecorvo and Bourne[3] have claimed that the shelf-life of curd could be extended to 10 days by using 10 per cent lime juice and 4 per cent NaCl solution under tropical conditions.

Deep fat frying: Non Protein Nitrogen (NPN) and Free Fatty Acid (FFA) contents of deep fried product increased upon storage (Fig 3) less at 5°C than at 30°C.

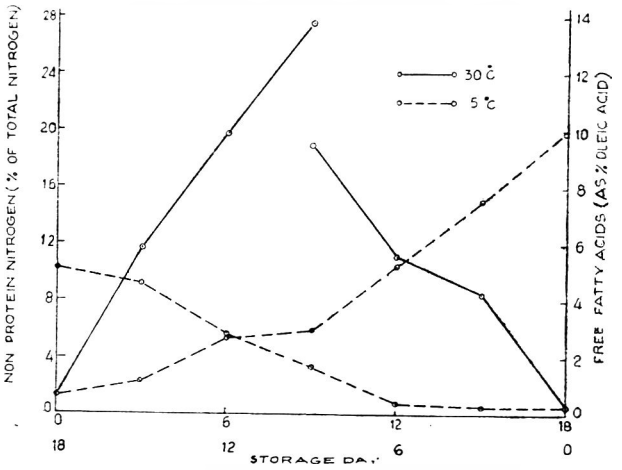

Fig. 3. Effect of storage temperature on non protein nitrogen & free fatty acid contents of fried curd.

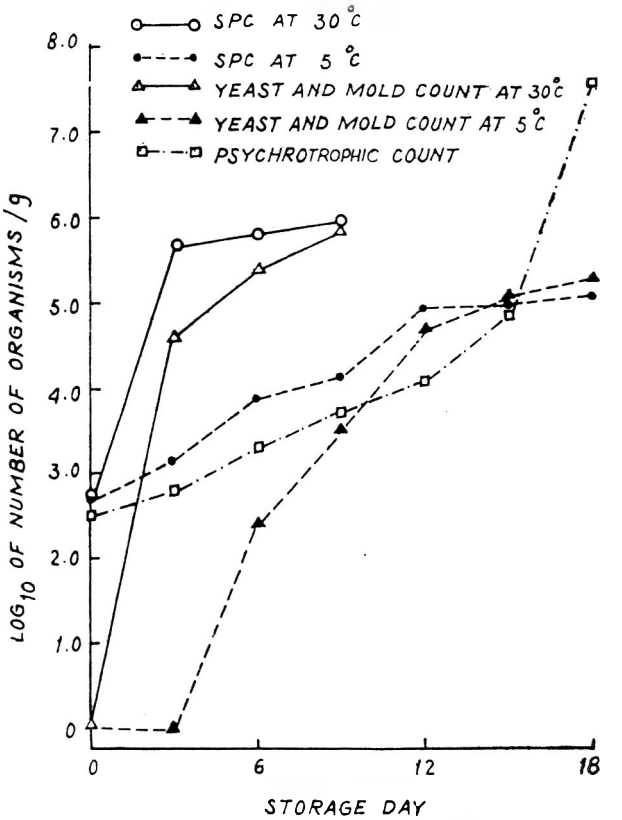

Fig. 4. Microbial count of fried curd during storage.

The microbial counts at different intervals of storage were lower than that observed for other treatments (Fig. 4). The deep fat fried curd was found acceptable upto 12 days at 5°C. Development of mold growth on the surface thereafter decreased its acceptability.

The results of this investigation indicate that preservation of soybean curd in 2 per cent citric acid or 2 per cent citric acid plus 5 per cent salt extended the shelf-life of curd at 30°C. The shelf-life could be further enhanced upon storage at 5°C.

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Effect of Some Physical and Chemical Treatments on Cassava Flour Quality

S. N. MOORTHY
Division of Technology, Central Tuber Crops Research Institute, Trivandrum-695 017, India

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Cassava flour was subjected to treatments like oxidation, acid and base treatments, low levels of derivatisation, mild fermentation, and autoclaving to reduce stickiness and improve the organoleptic quality. It was found that orthophosphoric acid treatment and conversion to succinic ester derivative improved the quality, but the latter imparted slight bitter taste to the product and hence only orthophosphoric acid treatment was found suitable.

Out of a total production of 5.2 million tonnes of Cassava (*Manihot esculenta* Crantz), around 50 per cent is consumed as a staple food in Kerala, India. It is cooked and consumed along with some side-dish. Because of inherent stickiness while cooking, cassava flour is not used in different food preparations. Stickiness is considered to be due to the particle size[1-3], higher amylopection content and weakness of the starch granules[4].

Efforts to reduce stickiness by cross-linking of starch molecules have been attempted in rice, potato and waxy maize[5-7]. Bridges so formed result in strengthening of the granular form of the starch and reduction of cohesiveness[8]. The most common cross-linking agents used are epichlorhydrin, sodium metaphosphate and phosphorus oxychloride with appropriate conditions of pH and temperature to reduce stickiness[9]. The physical properties of cassava starch have been modified by derivatisation[10,11] as well as by physical treatment, without affecting the basic structure of starch and the consumption of the modified starch has no harmful effects.[12,13].

Effect of oxidation, acid and base treatments, low levels of derivatisation, mild fermentation and autoclaving on the quality of cassava flour and possible effects on the stickiness and organoleptic quality were studied.

Materials and Methods

Sun-dried chips of the cassava variety 'H-1687' was powdered and used without sieving. Analytical grade reagents were employed for the experiments.

Steam treatment: The flour was autoclaved at different pressures for varying lengths of time in a pressure cooker (0.35 kg/cm^2 for 15 and 30 min., 0.71 kg/cm^2 for 15 and 30 min and 1.06 kg/cm^2 for 15 and 30 min.) and after cooling, was stored in polythene bags.

Chemical treatment: Cassava flour was added to an aqoues solution of oxidising agent, acid or base, shaken continuously for varying lenths of time, filtered, washed and dried at 50°C. The details of acid and bases added are given in Table 1.

Preparation of derivatives: The ester derivatives of acetic acid, propionic acid, maleic acid and succinic acids were used. Fifty grams of flour was added to 200 ml water in a beaker and pH adjusted to 8.0 by adding 3 per cent sodium hydroxide. Then 0.05 molar acid anhydride was added periodically with vigorous stirring maintaining the pH around 8.0. The partially esterified flour was filtered, washed and dried at 50°C. The degree of substitution (D. S.) was obtained by standard procedure[14].

Fermentation: After removing the rind, the tubers were kept immersed under running water for 40 hr to allow partial fermentation and then crushed, dried and powdered.

Phosphorous content in samples treated with orthophosphoric acid was determined by phosphomolybdate method. Reducing values of the treated samples were determined by Schochs procedure[15].

Treated samples were assessed for stickiness and other organoleptic qualities by steaming the flour with minimum water for 10 min in a pressure cooker. *Puttu* a traditional preparation from rice flour served as control. The cooked product was evaluated for stickiness, flavour, taste and overall acceptability, based on visible and organoleptic evaluation by 5 judges. Rice flour *Puttu* was given maximum score of 10 and untreated tapioca *Puttu* a score of 5 for measurement of stickiness. Taste and flavour were

TABLE 1. EFFECT OF TREATMENT OF CASSAVA FLOUR WITH OXIDISING AGENTS, ACIDS AND BASES FOR DIFFERENT LENGTHS OF TIME ON THE ACCEPTABILITY OF *PUTTU* PREPARED FROM IT

Quantity of reagent used (%)*	Treatment time (hr)	Stickiness	Taste and flavour	Overall acceptability	Quantity of reagent used (%)*	Treatment time (hr)	Stickiness	Taste and flavour	Overall acceptability
		Bromine					**Perchloric acid**		
0.1	2	5	A	A	0.05	2	5	JA	JA
	6	5	A	A		6	5	JA	JA
	16	5	A	A		16	5	JA	JA
0.2	2	6	JA	NA	0.10	2	4	NA	NA
	6	7	JA	NA		6	4	NA	NA
	16	7	NA	NA		16	2	NA	NA
0.5	2	8	NA	NA	1.0	2	2	NA	NA
	6	8	NA	NA		6	2	NA	NA
	16	8	NA	NA		16	2	NA	NA
		Hydrogen peroxide					**Ammonia**		
0.25	2	5	A	A	0.5	2	5	A	A
	6	5	A	A		6	5	A	A
	16	5	A	A		16	5	A	A
0.5	2	5	A	A	2.0	2	5	A	A
	6	5	A	A		6	5	A	A
	16	5	A	A		16	5	A	A
1.0	2	5	A	A	5.0	2	5	A	A
	6	5	A	A		6	5	A	A
	16	5	A	A		16	5	A	A
		Orthophosphoric acid					**Trimethylamine**		
1.0	2	6	A	A	0.5	2	5	A	A
	6	8	A	A		6	5	A	A
	16	8	A	A		16	5	A	A
0.5	2	8	A	A	2.0	2	5	A	A
	6	9	A	A		6	5	A	A
	16	9	A	A		16	5	A	A
1.0	2	9	A	A	5.0	2	5	A	A
	6	9	A	A		6	5	A	A
	16	9	A	A		16	5	A	A

*Based on starch used.

A: Acceptable; JA: Just acceptable; NA: Not acceptable

graded as Acceptable (A); Just acceptable (JA) and Not acceptable (NA).

Results and Discussion

The stickiness and organoleptic quality of *Puttu* obtained by various treatments are given in Tables 1-4. It is seen that an acceptable *Puttu* with reduced stickiness could be obtained when treated with orthophosphoric acid.

Effect of steam treatment: Steam pressure treatment of cassava starch has been found to rearrange the associative forces without causing any degradation of the starch molecules[17]. Similar changes might be expected in the flour also. The results indicate that

TABLE 2. EFFECT OF STEAM PRESSURE TREATMENTS ON THE QUALITY OF *PUTTU* PREPARED

Steam pressure (kg/cm^2)	Heating time (min)	Stickiness score	Taste and flavour	overall acceptability
0.35	15	5	A	A
0.35	30	5	A	A
0.71	15	5	A	A
0.71	30	4	A	A
1.06	15	4	JA	JA
1.06	30	3	JA	NA

Fermented flour had a stickiness score of 3 and just acceptable with regard to taste and flavour, but not acceptable in overall quality.

at higher pressures and longer duration of treatment, stickiness gets increased. It may be due to the reduction in granular strength and probably due to weakening of associative forces; hence, steam presure treatment is ineffective in improving the texture.

Effect of oxidising agents: Oxidation of starch can either cleave the 2, 3-glycol bond to give a dialdehyde starch or under milder conditions convert the primary hydroxyl groups to aldehyde or acid. The conditions used cannot bring about the cleavage, but only the latter type of conversion. The effect of substitution of CH_2OH groups by aldehyde groups may be to strengthen the hydrogen bonds, but how far it will help in strengthening the granule is not clear. No improvement was obtained on treatment with H_2O_2 solution. It may be due to mild oxidation or hydrogen bond might not have exerted enough strength to the granule. Bromine did not bring about any change at lower concentrations, but at higher levels stickiness of the flour was slightly reduced. However, there was slight reduction in yield of flour and taste was adversely affected and hence bromine oxidation cannot be considered as a beneficial treatment.

Effect of acids: Since starch undergoes degradation at higher temperatures with higher concentration of acids, the treatments were carried out at low concentrations at room temperature. Perchloric acid decomposes starch to a large extent even under these conditions, as indicated by the increase in reducing values. However, most of the treatments with orthophosphoric acid gave flour of reduced stickiness.. Correlation was obtained between phosphorus content and quality. (Table 3). The improvement in texture may be attributed to the formation of phosphate ester linkages in the starch. Cross linking can also occur, since phosphate group can act as trifunctional agent, Thus, the starch granules may be strengthened by inter-as well as intra-molecular linkages which leads to improved flour character. The treatment is also simple.

TABLE 3. EFFECT OF ORTHOPHOSPHORIC ACID TREATMENT ON THE QUALITY OF *PUTTU* PREPARED

Acid concn (%)	Treatment time (hr)	Phosphorus content (%)	Stickiness score	Taste and flavour	Overall acceptability
0.1	2	0.019	5	A	A
0.1	6	0.025	7	A	A
0.1	16	0.032	9	A	A
0.5	2	0.026	6	A	A
0.5	6	0.030	9	A	A
0.5	16	0.030	8	A	A
1.0	2	0.033	9	A	A
1.0	6	0.032	9	A	A
1.0	16	0.034	9	A	A

TABLE 4. EFFECT OF DERIVATISATION ON THE QUALITY OF *PUTTU*

Anhydride used	Degree of substitution	Stickiness score	Taste and flavour	overall acceptability
Acetic	0.045	5	JA	JA
Propionic	0.042	5	JA	JA
Maleic	0.033	5	JA	JA
Succinic	0.037	8	NA	NA

Effect of bases: Ammonia or trimethylamine treatment did not improve the quality. This may be due to the fact that no strong linkage can be formed between starch molecules and the weak bases.

Effect of fermentation: Mild fermentation of cassava starch has been found to breakdown starch molecules internally through enzymatic action. This is confirmed by the result that fermented flour exhibited higher stickiness (Table 2).

Effect of chemical derivatisation: Four anhydrides namely acetic, propionic, maleic and succinic anhydrides were employed. The latter two being bifunctional agents, can cross link the starch, whereas the other two can form only ester derivatives. The results (Table 4) with succinic ester confirms the assumption, as the flour stickiness was very much reduced. However, the product developed slight bitter taste and was unacceptable. Lack of improvement when maleic anhydride was used may be attributed to structural rigidity of maleic anhydride.

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Some Studies on the Suitability of Kinnow Mandarin and Blood-red Orange for Comminuted Squashes and their Nutritional Quality and Storage Behaviour

URMIL MEHTA AND SATINDER BAJAJ

Department of Foods and Nutrition, College of Home Science. Punjab Agricultural University, Ludhiana, India

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'Kinnow' and 'Blood-red' varieties of citrus fruits can successfully be utilized in the form of comminuted squahses. Nearly 50% of the peel and pulp can be incorporated into comminuted squashes without sacrificing the quality. Organoleptic evaluation has shown that there is favourable consumer acceptance. Squashes were stored for 9 weeks without any deterioration in quality. There is nearly 50% saving in the cost of raw material compared to squashes prepaed from juice alone.

Citrus fruits are commonly processed into products like juices and squashes. Recently much attention is paid to produce comminuted squashes. Investigations carried out with two varieties of oranges namely 'Kinnow' and 'Blood-red' are presented in this paper.

Braverman and Levi[1] described a process of preparing comminuted paste from whole oranges after cooking them under pressure. According to Charley[3] these novel drinks made from the whole fruit have a richer, more enduring and stable flavour, denser and stable cloud and a heavy body due to the pectin content. The advantages claimed for the comminuted beverages are: (1) less labour in preparing the base; (2) maximum utilization of the fruit; (3) reduction in the quantity of disposable waste, better colour, flavour, cloud stability and keeping quality.

Casimir and Chandler[2] have recommended formulations for canned comminuted orange and orange-lemon drinks. Lime and Cruse[4] prepared drinks of satisfactory quality and cloud stability from whole fruit/puree. Nichols *et al.*[5] reported that whole fruit drinks received better ranking than fruit juices when evaluated by 600 families in Texas and Ohio.

Rao *et al*[6]. have prepared comminuted squashes from Indian 'Sathgudi' sweet orange and loose jacketed oranges. Verma and Sastry[7] have made a comparative study of the quality of squashes and comminuted beverages prepared from Indian Coorg mandarin, 'Sathgudi' sweet orange and lime. These comminuted squashes were preferred to conventional squashes organoleptically and had better keeping quality during storage.

Materials and Methods

Fruits were dipped in one per cent HCL for one minute to remove spray residues. They were then washed in running tap water and utilized for the preparation of comminuted squashes by the following methods:

Comminuted squashes using different parts of fruit: Fruits were peeled, the segments separated and juice was expressed in a screw type extractor. Peel was cooked for 10 min in a pressure cooker, cooled, minced in a blendor with an equal quantity by wt. of sugar syrup (70° Brix) and sieved through 30 mesh sieve. Juice was pasteurized by holding at 85°C for 2 min and then for 10 min in boiling water. Pulp and rags were processed for 4 min in a pressure cooker, cooled, minced in blender and sieved through 30 mesh sieve. In order to prepare the comminuted squashes, 30 parts of such processed juice, 4 parts of processed peel and 6 parts of pulp were mixed thoroughly. More sugar syrup was added to raise the Brix to 45°. Acidity was maintained at 1.25 per cent.

Comminuted squashes using whole fruit: The 'Kinnow' fruits were pressure cooked for 6 min and 'Blood red' fruits for 10 min. Fruits were cut into bits, pulped and sieved through 18 mesh sieve. One Kg of this pulp was mixed with 1 kg juice and 4 kg of sugar syrup so that Brix and acidity of the product were 45° and 1.25 per cent respectively. (Control squash had 30 per cent juice, 45° Brix and 1.26 per cent acidity).

Squashes were filled in sterilized bottles and stored at 37°C for 9 weeks. Samples were analysed for chemical constituents and tested for their organoleptic quality initially and after storage.

Colour of the products was determined by comparison with the Horticultural Colour Charts I, II, III and IV[8]. Acidity was estimated by titrating the sample against 0.1 N NaOH using phenolpthalein as indicator. pH was determined with philips pH meter. Ascorbic acid was estimated titrimetrically by 2, 6- dichlorophenol-indophenol method. Soluble solids were recorded with a hand refractometer and corrected to 20°C. Carotenoids were estimated by the method described by Ranganna[9] and total flavonoids by the method of Balbaa *et al.*[10] cloud stability was observed visually. Statistical analysis of sensory evaluation was made by Renksum method.

TABLE 1. EFFECT OF STORAGE ON THE CHEMICAL CONSTITUENTS OF COMMINUTED SQUASHES PREPARED FROM 'KINNOW' MANDARIN AND 'BLOOD RED' ORANGES

Constituents	'Kinnow'/comminuted squash						'Blood-Red'/Comminuted squash					
	Juice alone		30% juice+6% pulp+4% peel		Whole fruit squash (pressure processed)		Juice alone		30% juice+6% pulp+4% peel		Whole fruit squash (pressure processed)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Colour	Yellow orange 22A	Greyed orange 165B	Greyed orange 163B	Greyed orange 164B	Greyed orange 163A	Greyed orange 164B	Greyed yellow 160B	Greyed Brown 199C	Greyed Yellow 160A	Greyed orange 164B	Yellow 13B	Greyed orange 163B
Ascorbic acid (mg)	10.20	4.61	15.16	11.84	14.28	11.23	27.99	14.73	31.33	21.34	43.09	25.43
Acidity (as anhydrous citric acid (g)	1.25	1.20	1.25	1.22	1.25	1.23	1.25	1.21	1.25	1.19	1.25	1.20
°Brix	46.4	47.4	46.4	47.4	46.4	46.9	46.4	47.4	46.4	46.9	46.4	47.4
°Brix/acid ratio	37.1	39.5	37.1	38.9	37.1	38.1	37.1	39.2	37.1	39.4	37.1	39.5
pH	2.5	2.6	2.9	3.0	2.5	2.6	2.5	2.5	3.0	3.0	2.5	2.6
Total cartotenoids (as β-carotene) (mg)	0.40	0.22	0.63	0.47	0.74	0.55	0.05	0.02	0.06	0.04	0.06	0.05
Total flavonoids (as rutin) (mg)	0.33	0.27	0.67	0.54	0.80	0.62	0.45	0.22	2.56	1.71	3.08	2.47
Cloud	Little Uniformaly spread	settled	Moderate fairly heavy surface deposit	Moderate uniformaly spread	Moderate stable	Moderate stable	Moderate surface deposit	Moderate uniform	Dense more surface deposit	Dense no surface deposit	Heavy very slight surface deposit	Heavy no surface deposit

*Values reported are for 100 g. Final values determined 9 weeks after storage at 37°C.

Results and Discussion

Comminuted squashes contained higher amounts of ascorbic acid, carotenoid pigments and flavonoids as compared to control samples (Table 1). This might be due to the inclusion of peel and pulp in comminuted squashes.

Effect of Storage: Change in colour was noticed in the control as well as communited squashes of both the varieties (Table 1). Retention of ascorbic acid (78 per cent) was significantly higher in 'Kinnow' comminuted squashes as compared to its control (45 per cent). Comminuted squashes are reported to be better protected against oxidative spoilage[3]. Verma and Sastry[7] observed no difference in the retention of normal ascorbic acid content of comminuted squashes of Coorg mandarin oranges stored at 37°C for 24 weeks. There was negligible difference in the retention of ascorbic acid in the control and comminuted squashes of 'Blood-red' variety (53 per cent retention in control and 54-59 per cent in comminuted) during storage. Similar trend has been observed by Verma and Sastry[7] for 'Sathgudi' orange squashes.

In both the varieties decrease in acidity and increase in pH and total soluble solids were observed. This has resulted in the change of Brix-acid ratio. Even after 9 weeks storage, there was better retention of carotenoid pigments in comminuted squashes of both the varieties.

These findings are in agreement with the findings of Verma and Sastry[7] for Coorg 'mandarin' and 'Sathgudi' oranges.

Extent of flavonoid retention was practically the same both in the control and in comminuted 'Kinnow' squashes. However, 'Blood-red' comminuted squashes had better retention (66-80 per cent) than control (49 per cent). Verma and Sastry[7] have reported similar results for Coorg and 'Sathgudi' comminuted squashes.

Cloud stability of comminuted oranges were also better than those of controls.

Effect of storage on sensory characterstics: Keeping quality of the squashes were good till 9 weeks (Table 2). The comminuted squashes prepared by two different methods were rated superior to their respective controls. However, the comminuted squashes were slightly bitter which was acceptable to consumers. Flavour, colour and cloud stability of comminuted squashes were better because of the utilization of the whole or most parts of fruits. Control samples developed terpenoid flavour after storage.

Since 49-54 per cent of pulp and peel are utilized in the squashes, it brings down the the cost of raw materials. This also offers protection against deterioration of the product.

TABLE 2. EFFECT OF STORAGE ON ORGANOLEPTIC EVALUATION OF COMMINUTED SQUASHES

Type of squash	Flavour		Colour		Bitterness		Consistency		Overall acceptability		Remarks	
	I	II	I	II	I	II	I	II	I	II	I	II
					'Kinnow'							
Juice	2.6	2.3	2.1	2.0	3.3*	3.1*	2.4	2.4	2.6	2.5	Terpeny	Terpeny
30% juice*, 4% peel and 6% pulp	3.4*	3.0*	3.3*	3.1*	3.0*	2.6	3.1*	3.0*	3.1*	2.9*	Acceptable	Acceptacle
Pressure processed whole fruit	3.5*	3.4*	3.6*	3.5*	3.2*	3.0*	3.6*	3.4*	3.5*	3.3*	Highly acceptable	Highly acceptable
					'Blood-Red'							
Juice	2.6	2.1	2.0	1.7	3.5*	3.2*	2.7*	2.2*	2.7*	2.3	Terpeny	Terpeny
30% juice, 4% peel and 6% pulp	3.1*	2.9*	2.7*	2.7*	3.4*	3.1*	3.1*	2.9*	3.1*	2.9*	Acceptable	Acceptable
Pressure Processed whole fruit	3.1*	2.9*	3.7*	3.5*	2.7*	2.4	3.1*	3.0*	3.1*	3.0*	Highly acceptable	Highly acceptable

Rank sums are average of 8 judges; Dilution rate is 1:3;
I=Initial; II=Stored for 9 weeks at 37°C.
Maximum marks 4 for each parameter.
*Significant at 5% level.

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Some Chemical, Pasting, Rheological and Textural Characteristics of Composite Flours Based on Wheat and Tubers

S. CHANDRA SHEKARA AND S. R. SHURPALEKAR

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

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Pasting and dough characteristics of blended wheat and tuber flours (5-15%) were evaluated with the help of visco-amylograph, farinograph, extensograph and texturometer. There was wide variation in the damaged starch content of the constituent flours (potato: 31.3%; cassava: 4.8% and wheat: 11.4%) and hence, significant differences were observed in the rheological, textural and handling properties of the doughs. Because of greater difference in rheological characteristics of wheat-potato flour blends, utility of blend containing higher proportions of potato flour was limited.

Though rice and wheat form the staple food of majority of the world's population, in many developing countries, agro-climatic conditions favour the cultivation of maize, sorghum, cassava (tapioca), potato, yam, etc. However, wheat based bakery products have gained popularity and efforts are being made for partial substitution of wheat with the locally available grains or tubers.

The use of non-wheat cereals and tubers in wheat based bakery products has been comprehensively reviewed[1-4]. Relatively higher levels of non-wheat flour could be incorporated in bread by adopting, highly capital intensive processes, such as mechanical dough development in place of conventional processes of straight-dough or sponge and dough where, incorporation can be to a maximum of 10 per cent. Since processes like mechanical dough development involves use of imported machinery and high energy, they are not of immediate application to developing countries. Hence, utilization of locally available non-wheat flours at levels higher than 10 per cent by appropriate modifications in the conventional methods would be of importance.

It is however, necessary to assess the chemical, pasting, rheological and textural characteristics of flours processed from tubers used namely, cassava and potato, blended with wheat flour at different levels, before undertaking baking studies. The results of such a study are presented in this paper.

Materials and Methods

Wheat flour: Wheat flour of variety '*WG*-357' milled to an extraction rate of 71 per cent in the Buhler laboratory mill (MLU- 202) was used.

Cassava flour: A sweet commercial variety of cassava 'H-2304' collected from the Central Tuber Crops Research Institute, Trivandrum was processed into flour according to Kaputo[5]. The tubers were washed, deskinned, sliced, dried and the chips were milled and sieved through 10XX sieve (136 μ).

Potato flour: A high yielding potato variety 'Kufri Chandramukhi', obtained from Potato Research

Station, Pune, was processed into dried chips and ground to flour according to Bhullar[6].

Blending of flours: The flours processed from cassava and potato were blended separately at levels ranging from 5 to 25 per cent with wheat flour in a Hobart planetary mixer (C-300).

Chemical characteristics of wheat-tuber flour blends: AOAC[7] methods were followed for determining the proximate composition of flours. Starch and sugars-both reducing and non-reducing, were determined according to the methods described by Kent-Jones and Amos[8]. Damaged starch and diastatic activity were determined according to AACC[9].

Pasting characteristics of slurries of wheat-tuber flour blends: Visco-amylograph characteristics, like peak viscosity, stability and set-back for a slurry concentration at 13.33 per cent were recorded for wheat flour/blends using 700 cmg sensitivity catridge.

Rheological characteristics of doughs of wheat-tuber flour blends: Farinograph characteristics at the desired dough consistencies were studied with reference to water absorption, dough development time, dough stability, mixing tolerance index and farinogram band width using Brabender farinograph, while resistance to extension, extensibility, ratio figure and area were determined using Brabender extensograph according to standard methods of AACC[9].

Textural characteristics of doughs based on wheat-tuber flour blends: The texture profiles[10] of doughs of desired consistencies were recorded on the General Foods Texturometer (Model GTX) with the following conditions arrived at on the basis of the preliminary trials.

Voltage: 0.5 V; Clearance between plunger and platform: 2mm,

Plunger: Chrome-20 mm; platform: meat cup-40 mm diameter and 13 mm depth. Attenuator: 1, Bites: 12/min, Chart speed: 750 mm/min.

Results and Discussion

Chemical characteristics of wheat-tuber flour blends: Cassava flour is a poor source of protein unlike potato flour which had a protein content comparable to that of wheat (Table 1). Cassava flour had the highest starch content followed by potato flour. Non-reducing sugars accounted for about 75, 66 and 50 per cent of total sugars in cassava, wheat and potato flours respectively.

For bread preparation, 7 to 8 per cent of damaged starch is desirable in the panary fermentation. Because of the low damaged starch content of 4.8 per cent in cassava flours (as compared to 11.4 per cent of wheat flour) there was a reduction of about 0.25 per cent for every 5 g cassava flour added to the wheat flour

TABLE 1. CHEMICAL COMPOSITION OF WHEAT AND TUBER FLOURS*

Constituent (%)	Wheat flour	Cassava flour**	Potato flour
Crude protein‡	10.8	1.7	8.1
Total ash	0.7	2.0	2.5
Ether extractives	1.3	0.7	0.8
Crude fibre	0.6	0.9	1.3
Carbohydrates (by diff.)	86.6	94.7	87.3
Starch	79.1	85.2	81.2
Sugars (reducing)	0.8	1.6	2.2
Sugars (non-reducing)	1.9	5.5	2.3

*Values expressed on moisture free basis; moisture in wheat flour; 10.5%, cassava flour 9.6%, potato flour; 9.7%

**Cassava flour sieved through 10 XX sieve (136 μ)

‡Calculated as N×5.7 for wheat and N×6.25 for cassava and potato

(Fig. 1). This agrees with the observations made by Hudson and Ogunsua[11]. The reduced percentage of starch damage in cassava flour may be due to the easy-to-grind and crumbly nature of the dried chips.

Use of potato flour, with its high damaged starch content of 31.3 per cent, results in an increase of about 1.5 per cent damaged starch with every 5 per cent increase of potato flour in the blends (Fig. 1). Higher damage to potato starch may be due to (a) the partial

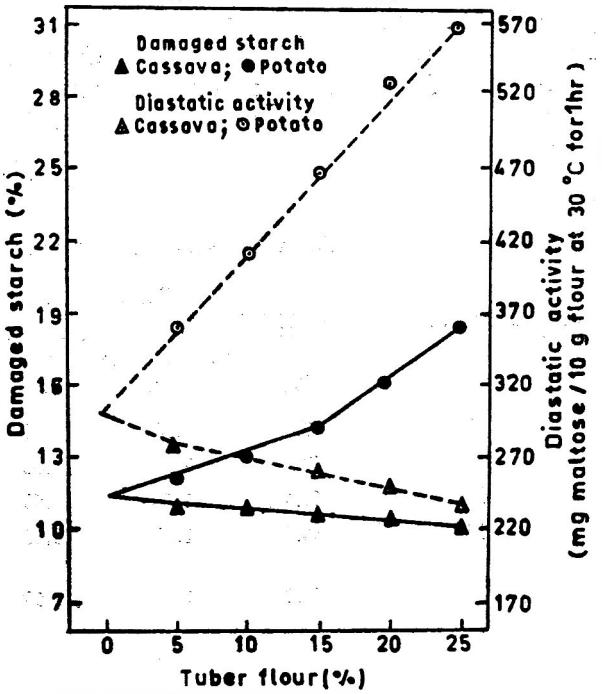

Fig. 1. Damaged starch and diastatic activity in wheat-tuber flour blends.

TABLE 2. VISCO-AMYLOGRAPH CHARACTERISTICS OF WHEAT, FLOUR AND WHEAT-TUBER FLOUR BLENDS

Flour/blend	Slurry concn. (%)	Viscosity (B.U.)			Breakdown a–b (B.U.)	Set-back c–a (B.U.)
		Peak a	Hot paste b	Cold paste c		
Wheat	13.33	870	565	1030	305	160
Wheat-cassava (85:15)	13.33	940	540	870	400	- 70
Wheat-potato (85:15)	13.33	700	510	870	190	170

gelatinization occurring during blanching rendering the dried chips to become hard and hence more susceptible to starch damage during grinding, (b) larger size of starch granules (mean diameter about 100μ) and (c) higher degree of swelling during gelatinization.

Because of the low diastatic activity of cassava flour (130 mg maltose/10 g flour) as compared to wheat flour (299 mg/10 g) the diastatic activity decreased correspondingly with the quantity used (Fig. 1). This necessitates use of malt in the formulation for optimum fermentation as reported by Hudson and Ogunsua[11] and Rasper *et al*[12]. On the other hand, the values obtained in the experiment showed that there was significant increase in the diastatic activities of the blend with the increase in the proportion of potato flour inspite of its low diastatic activity of 180 mg. This can be attributed to the high damaged starch content of potato flour. Similar observation has been reported by Mangels[13] and Jones[14].

Pasting characteristics of slurries of wheat-tuber flour blends: Cnaracteristics of blended wheat-tuber flour mixtures, at 15 per cent level as recorded by visco-amylograph are presented in Table 2. The results indicate the relative susceptibilities of the different starches to gelatinization, breakdown and retrogradation. Even low levels of cassava flour caused a steep rise in viscosity, steep breakdown and a negative set-back which indicates the 'in-tact' nature of its starch granules with a low amylose content. Inherent two-stage swelling of wheat and other cereal starches, as reported by Whistler and Paschall[15], also gets totally masked by the inclusion of cassava flour. Low level of amylose in the cassava flour, may be helpful in retarding the staling of bread as suggested by Rasper[16].

Potato starch has been reported to result in viscous paste, on heating because of large number of phosphate groups present. Low peak viscosity (700 B. U.) observed in wheat-potato flour blend could be attributed to the passive role played by the ruptured potato starch granules in increasing the viscosity by swelling.

Rheological characteristics of doughs of wheat-tuber flour blends: Farinograph characteristics presented in Table 3 indicate that, a dough consistency of 500 B. U. was optimum for wheat-cassava flour blends at all levels. In contrast, wheat-potato flour blends yielded a sticky dough at a consitency of 500 B. U.

TABLE 3. FARINOGRAPH CHARACTERISTICS* OF WHEAT-TUBER FLOUR BLENDS

Level of tuber flour (%)	Cassava blends				Potato blends			
	Water absorb (%)	Dough develop. time (min)	Dough stability (min)	Mixing toler. index (B.U.)	Water absorp (%)	Dough develop. time (min)	Dough stability (min)	Mixing toler. index (B.U.)
0	67.4	3.50	7.50	30	67.4	3.50	7.50	30
5	67.0	2.00	6.00	40	72.0	1.00	3.50	70
10	66.4	1.75	5.50	40	77.4	1.50	2.25	90
15	66.2	1.50	5.00	40	82.0 (77.6)	2.00 (2.00)	1.75 (1.50)	120 (170)
20	66.0	1.25	4.50	50	87.6 (83.2)	2.50 (2.75)	1.75 (1.50)	140 (185)
25	65.6	1.25	4.00	60	92.0	2.75	1.75	180

*Values refer to 500 B.U. consistency of the dough, except those in parentheses, which are for doughs of 600 B.U. consistency.

When potato flour was used at 15 to 20 per cent level, a consistency of 600 B. U. was required for proper handling of the dough, while at 25 per cent, even a high consistency of 700 B. U. resulted in a sticky dough with poor handling property.

At the same level of blending, the changes in farinograph characteristics were greater with potato flour as compared with those of cassava flour. Because of the variation in the extent of starch damage, a gradual decrease in water absorption was observed with increasing levels of cassava flour in the blends, while in the wheat-potato flour blends there was a steep increase.

Increases in dough development time noted with doughs based on wheat-potato flour blends can be attributed to the preferential absorption of water by the damaged starch component as compared to the gluten component. This corroborates the results of Ciacco and D'Appolonia[17]. At similar levels of blending, potato flour exhibited a greater degree of weakening than cassava flour. The band width of the farinogram, an index of mobility, was not affected in the case of doughs based on wheat-cassava flour blends (50 B. U.), while the band width narrowed down from 50 to 15 B. U. as the level of potato flour increased from 0 to 25 per cent.

The result of extensograph studies followed a similar pattern as with farinograph studies, with potato flour exhibiting more significant changes than cassava flour.

Even at 5 per cent addition, potato flour brought

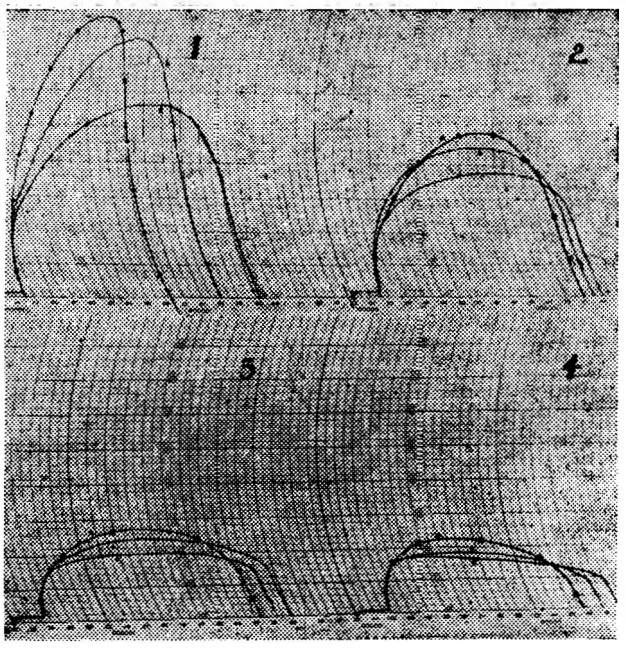

Fig. 2. Extensograms for wheat-potato flour blends

1—100:0; 2—95:5; 3—90:10; 4—85:15

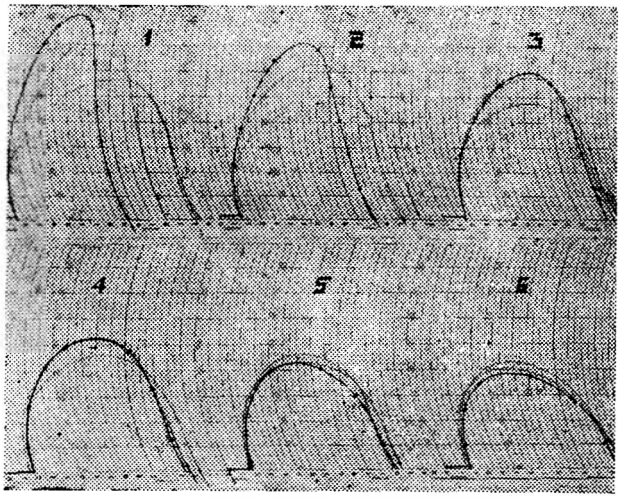

Fig. 3. Extensograms for wheat-cassava flour blends

1–100:0; 2–95:5; 3–90:10; 4–85:15; 5–80:20; 6–75:25

about highly significant changes in extensograms by lowering the resistance to extension (R), area (A)-indicating the strength of the flour and ratio figure (R/E) and increasing the extensibility (E) (Fig. 2). These values are comparable to those obtained with 20 per cent wheat-cassava flour blend (Fig. 3). The high level of damaged starch in potato flour necessitates requirement for more gluten to strengthen the dough.

Textural characteristics of doughs based on wheat-tuber flour blends: The effect of blending wheat flour with varying quantities of tuber flours, showed that compressibility and cohesiveness of doughs remained almost unaltered. This may be attributed to variation in the quantity of water added to obtain doughs of

TABLE 4. SOME TEXTURAL CHARACTERISTICS* OF DOUGHS BASED ON WHEAT-TUBER FLOUR BLENDS

Level of tuber flour (%)	Adhesivenes (cm^2)	
	Cassava blends	Potato blends
0	3.8	3.8
5	8.3	8.0
10	7.1	9.3
15	4.0	10.0
20	3.4	11.0
25	2.0	11.5

*Differences among values both for compressibility (kg/V) and cohesiveness were negligible; average values for cassava blends: 8.0 and 0.87 respectively and for potato blends: 6.4 and 0.95 respectively.

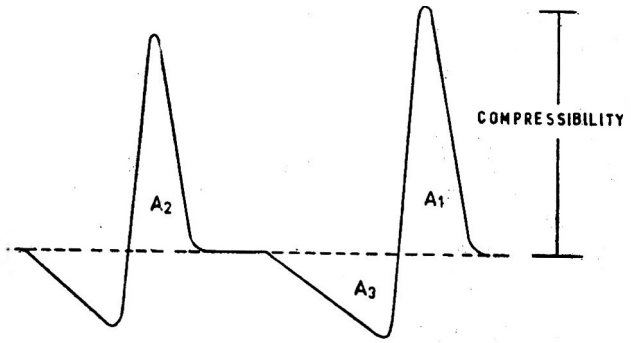

Fig. 4. A typical texturometer curve for interpretation of dough characteristics

(A: Area) A_2/A_1: Cohesiveness; A_3: Adhesiveness

similar consistency. Increase in adhesiveness with potato flour can be attributed to the partial gelatinization of starch in potato flour, which limits its use for blending at higher levels with wheat flour (Table 4). The norms followed for evaluating the texture profiles of dough are presented in a typical texturometer curve (Fig. 4).

It is evident from the results that damaged starch contents of constituents flours of the blend govern (i) the level of blending, and (ii) the pasting, rheological and textural characteristics which are important factors influencing the quality of breads based on composite flours.

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BLENDING OF GRAPE MUSTS FOR PRODUCTION OF RED WINES

Grapes from 'Gulabi' variety was blended with grapes of deep coloured varieties such as 'Baily Alicante' and 'Rubired' in different proportions to improve the colour of wines. Blending at 2:1 and 3:1 level gave wines with desirable colour. White varieties, 'Thompson Seedless' and 'Anab-e-Shahi' were blended with 'Rubired' in different proportions to produce red table wines. The blending at 1:1, 2:1, 3:1 and 4:1 levels were found suitable for making dry table wines. A blend of 1:1 or 2:1 could be used for making dessert wines, since these blends had sufficient intensity of colour.

Blending of wines is an important cellar operation, carried out to develop specific types of wines and to maintain uniformity in quality and character of these wines[1]. Ough and Amerine[2] studied the effect of antefermentation blending of 'Cabernet Sauvignon' musts with white juice to improve colour and wine quality and found some practical advantages. Recently, Akopyan[3] reported that the quality of red wines can be improved by proper blending of grapes. The present paper, reports the studies carried out to produce acceptable quality red table wines by blending deep coloured varieties such as, 'Baily Alicante' and 'Rubired' with commercial varieties like 'Gulabi', 'Thompson Seedless' and 'Anab-e-Shahi'. The composition and qualities of the resulting wines are reported in this communication.

Two white varieties namely, 'Thompson Seedless' and 'Anab-e-Shahi' and three red varieties 'Gulabi', 'Rubired' and 'Baily Ali cante' were used in this investigation. They were grown at the Experimental Farm of the Indian Institute of Horticultural Research, Bangalore and harvested in Feb.-April, 1980. Blends were prepared by mixing grapes in different proportions (wt/wt). About 2 kg of the blends as well as individual varieties were crushed in the presence of 100 ppm of SO_2 and subjected to fermentation at $20 \pm 1°C$ after adjusting the sugar content to 20-22° Brix with cane sugar. The white wines from 'Thompson Seedless' and 'Anab-e-Shahi' were prepared from pressed juice. The procedure for preparation of wine has been described in an earlier communication[4]. The must and wine samples were analysed according to the methods of Amerine and Ough[5]. The wine samples were organoleptically evaluated by a panel of three judges on the basis of score card described by Ough and Baker[6].

The variety 'Gulabi' is a light coloured grape with muscat flavour, but gives wine with light colour. In order to improve the colour, it was blended with deep coloured varieties such as 'Baily Alicante' and 'Rubired'. The composition of musts and wines from different blends are presented in Table 1. It is evident that there is a marked improvement in the colour of wines in all the blends. Based on the chemical composition and organoleptic evaluation, the wines obtained by blending 'Gulabi' with 'Baily Alicante' or 'Rubired' in the proportions of 2:1 and 3:1 were proved better than other samples.

The colour of wines from 'Rubired' was deep, but wine was highly acidic and astringent. To overcome this defect, 'Rubired' was blended with 'Thompson Seedless' and' Anab-e-Shahi' grapes, while making wine. The composition of musts and wines are shown in Table 2. As a result of blending, the high acidity and tannin content of 'Rubired' wine was reduced considerably. Chemical composition and organoleptic evaluation suggested that the combinations of 'Rubired' with 'Thompson Seedless' and 'Anab-e-Shahi' in proportions ranging from 1:1 to 1:4 were found suitable for making red dry table wine. However, wines from blends of 1:1 and 1:2 had sufficient intensity in colour for dessert wines. The wine colour in the blends of colourless varieties was more than that of 'Rubired-Gulabi' blends. This trend was not observed in wines from 'Baily Alicante–Gulabi' blends, probably due to some oxidation. The results obtained suggest that blending can be adopted to improve the quality of wines from the varieties deficient in colour and possessing chemical composition not suitable for wine making.

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Indian Institute of Horticultural Research,
255, Upper Palace Orchards,
Bangalore-560 080, India.

E. R. SURESH
S. ETHIRAJ
H. ONKARAYYA

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TABLE I. COMPOSITION OF MUSTS AND WINES FROM BLENDS OF 'GULABI' WITH 'BAILY ALICANTE' OR 'RUBIRED'

Varieties used	Blending ratio	MUST COMPOSITION				WINE COMPOSITION							
		T.S.S. (°Brix)		Titratable acidity (g. tartaric acid/100 ml)	pH	Alcohol %	Titratable acidity (g. tartaric acid/100 ml)	pH	Volatile acidity (g. acetic acid/100 ml)	Tannins (mg/l)	Reducing sugars (%)	Colour[c] (1 : 10 dilution)	Average score
		Initial	Final[a]										
Gulabi	—	20.0	21.2	0.76	3.50	11.7	0.76	3.65	0.036	2280	0.188	0.163	13.08
Baily Alicante	—	17.0	21.8	1.01	3.30	11.7	1.03	3.35	0.048	3760	0.294	1.174	11.75
Rubired[b]	—	19.0	21.6	1.41	3.20	11.5	1.26	3.30	0.036	6400	0.372	2.44	11.33
Gulabi + Baily Alicante	2:1	19.2	21.6	0.90	3.35	11.2	0.88	3.50	0.039	2560	0.188	0.647	14.58
Gulabi + Baily Alicante	3:1	19.2	21.4	0.86	3.40	11.5	0.87	3.55	0.048	2440	0.244	0.452	13.38
Gulabi + Baily Alicante	4:1	19.0	21.0	0.86	3.40	11.2	0.84	3.50	0.039	2250	0.188	0.343	12.42
Gulabi + Rubired	2:1	19.4	21.2	1.01	3.35	11.5	0.91	3.50	0.048	4000	0.200	0.368	14.58
Gulabi + Rubired	3:1	19.4	21.4	0.97	3.35	11.2	0.89	3.55	0.036	3000	0.166	0.348	13.25
Gulabi + Rubired	4:1	19.2	21.4	0.89	3.45	11.5	0.84	3.55	0.036	2560	0.156	0.274	11.50

(*a*) Adjusted with cane sugar; (*b*) Harvested on 5.3. '80 and used for blending; (c) Sum of absorbance at 420 and 520 nm.

TABLE 2. COMPOSITION OF MUSTS AND WINES FROM BLENDS OF 'RUBIRED' WITH 'ANAB-E-SHAHI' OR 'THOMPSON SEEDLESS'

Varieties used	Blending ratio	MUST COMPOSITION				WINE COMPOSITION							
		T.S.S. (°Brix)		Titratable acidity (g. tartaric acid/100 ml)	pH	Alcohol %	Titratable acidity (g. tartaric acid/100 ml)	pH	Volatile acidity (g. acetic acid/100 ml)	Tannins (mg/l)	Reducing sugars (%)	Colour[c] (1 : 10 dilution)	Average score
		Initial	Final[a]										
Rubired[b]	—	21.0	21.0	1.03	3.30	11.2	1.09	3.45	0.036	5720	0.412	1.84	11.33
Thompson Seedless	—	22.2	22.2	0.56	3.75	12.6	0.65	3.90	0.042	640	0.124	—	14.58
Anab-e-Shahi	—	16.8	20.8	0.44	3.75	11.7	0.61	3.70	0.033	572	0.124	—	12.66
Rubired + Thompson Seedless	1:1	21.0	21.0	0.78	3.55	12.1	0.84	3.65	0.057	3040	0.234	0.818	13.83
Rubired + Thompson Seedless	1:2	21.2	21.2	0.71	3.65	12.1	0.71	3.65	0.060	2160	0.176	0.496	14.42
Rubired + Thompson Seedless	1:3	21.0	21.0	0.65	3.70	11.5	0.67	3.65	0.069	1860	0.116	0.396	13.92
Rubired + Thompson Seedless	1:4	21.0	21.0	0.62	3.70	11.7	0.67	3.60	0.057	1560	0.124	0.262	13.75
Rubired + Anab-e-Shahi	1:1	19.0	21.2	0.74	3.45	11.5	0.80	3.65	0.033	3200	0.220	0.734	14.25
Rubired + Anab-e-Shahi	1:2	18.2	21.4	0.63	3.55	12.1	0.71	3.65	0.042	2500	0.166	0.497	14.30
Rubired + Ananb-e-Shahi	1:3	17.8	21.4	0.56	3.60	11.5	0.66	3.65	0.048	2100	0.144	0.233	13.67
Rubired + Anab-e-Shahi	1:4	17.4	21.6	0.54	3.65	11.2	0.64	3.65	0.036	1680	0.144	0.257	14.46

(*a*) Adjusted with cane sugar; (*b*) Harvested on 13.3. '80 and used; (*c*) Sum of absorbance at 420 and 520 nm.

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ANTIBIOTIC RESISTANT COLIFORMS IN *KHOA*

One hundred and eighty seven samples of *Khoa* were tested for the presence of drug resistant coliforms and enteropathogenic *E. coli*. Ten different serotypes of *E. coli* were isolated, of which 91.7% were resistant to one antibiotic or other used in the study. Most of the members of coliform group were resistant to penicillin and erythromycin, but sensitive to streptomycin and chloramphenicol. Many were also resistant to a mixture of antibiotics.

Presence of coliforms, specifically *E. coli* are generally used as an index of sanitary quality of food. Some of the serotypes of *E. coli* are also enteropathogenic in nature.

Antibiotic resistant coliforms are being isolated in increasing numbers from different foods[1,2]; which are of ecological and epidemiological significance[3]. However, there is no report on the presence of antibiotic resistant coliforms from *Khoa*, a processed milk product used as such or in the preparation of sweetmeats and other food products. This note pertains to the investigation with the resistotypes and serotypes of coliforms obtained from market samples of *khoa* in Calcutta.

One hundred and eighty seven samples of *khoa* were collected in sterile containers from vendors in and around Calcutta over a period of three years. The samples were immediately brought to the laboratory, homogenised in sterile buffer of pH 7.2, inoculated into MacConkey broth and incubated at 37°C for 48 hr. From the tubes showing bacterial growth, loopful of broth was streaked on MacConkey agar plates and incubated at 37°C for 16 hr. Coliform colonies were isolated and identified on the basis of cultural and biochemical characteristics[4,5]. *E. coli* strains were sent to National Salmonella and Escherichia Centre, Kasauli, India, for serotyping. Antibiotic resistance of the isolates of coliform was studied against penicillin, ampicillin, streptomycin, erythromycin and tetracycline by disc diffusion method as described in an earlier report[6]. Reference grade antibiotics used in the study were obtained from Central Drugs Laboratory, Calcutta.

Coliform was detected in 85.6 per cent of samples and in 12.8 per cent of samples confirmed as *E. coli* on the basis of biochemical and serological reactions. Coliforms are heat labile and their isolation from large number of samples indicate postprocessing contamination from food handlers, equipment and presumbly unhygeinic environment. Presence of *E. coli* is undesirable in *khoa* and is of considerable public health significance.

Out of 350 isolates, 33 were identified as *E. coli*, 38 as *Klebsiella*, 12 as *Enterobacter* and 11 as *Citrobacter*. Ten different serotypes of *E. coli* namely 01, 017, 022, 025, 029, 059, 060, 084, 0119 and 0125 were isolated. Eight isolates could not be typed serologically. More than one serotype was detected in three samples. Among the different serotypes isolated, four namely 022, 025, 0119, and 0125 are reported to be enteropathogenic[7,8].

Antibiotic resistance patterns of coliforms are presented in Table 1. Basically, most of the strains of all the genera are resistant to penicillin and erythromycin, but sensitive to streptomycin and chloramphenicol. More isolates were resistant to tetracycline than to streptomycin and chloramphenicol. It was noted that isolates of *E. coli* were more resistant to antibiotics than isolates belonging to other genera. The data presented in Table 2 exhibited by most of the strains belonging to all the genera tested, indicate the pattern of resistance to multiple antibiotics. Out of 12 enteropathogenic strains of *E. coli*. isolated, 3 were resistant to a combination of 5 antibiotics, 3 isolates to 4 antibiotics and 5 to 2 or 3 antibiotics. Multiple resistance to penicillin, ampicillin and erythromycin constitituted more than 54 per cent of the total number of coliforms studied.

TABLE 1. RESISTANCE OF COLIFORMS TO ANTIBIOTICS (EXPRESSED AS % OF THE TOTAL NUMBERS)

Organisams	*Pen.*	*Amp.*	*Strep.*	*Ery.*	*Chloro*	*Tetra*
E. coli	91.1	60.6	24.2	81.8	21.2	60.6
Klebsiella	89.5	68.4	13.2	78.9	13.2	31.6
Enterobacter	66.7	25.0	8.3	75.0	8.3	8.3
Citrobacter	81.8	63.6	9.1	63.6	9.1	36.3

TABLE 2. MULTIPLE RESISTANCE OF COLIFORM STRAINS TO ANTIBIOTICS

Antibiotic combination	No. of strain resistant			
	E. coli	*Klebsiella*	*Enterobacter*	*Citrobacter*
PA	7	4	1	0
PE	3	3	4	2
PT, PS, PCT	1	0	0	0
PAS	0	1	0	0
PET	4	1	0	0
PAT	0	0	0	2
PAE	3	8	1	2
PSE, PAEC	0	2	0	0
PAET	5	7	0	1
PASE	1	1	1	0
PACT	1	1	0	0
PECT	0	0	0	0
PAST	0	0	0	1
PSEC	2	0	0	0
PASET	4	1	0	0
PAECT	4	2	0	7

P=Penicillin, A=Ampicillin, E=Erythromycin, T=Tetracycline, S=Streptomycin, C=Chloramphenicol.

The genera included in the study were normal benevolent intestinal flora, but *E. coli* and *Klebsiella* can cause infection under certain conditions. But they may constitute an important source of R-factors and may transfer the resistance factors to more dangerous organisms like *Shigella*[9-12]. It has been reported that multiple drug resistance can be transferred to almost any genus of the family Enterobacteriaceae[13-15]. *E. coli* or other intestinal microflora can be induced to drug resistance by injesting R-factors containing *E. coli*. In such an individual, intestinal, pathogenic organism may be converted to drug resistant types due to transfer of R-factors. However, the transfer of R-factors normally occurs less readily in the intestine than *in vitro*[16]. The results obtained in this study indicate the presence of a large number of antibiotic resistant bacteria in *khoa*.

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Central Food Laboratory,
3, Kyd Street,
Calcutta-700 016, India.

A. K. GUHA*
H. N. DAS
R. ROY
B. R. ROY

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Present address: Macro molecules Department, I.A.C.S. Calcutta—32, India.

ACID PRODUCTION BY YOGHURT STARTERS AND THEIR EFFECT ON PROTEOLYSIS AND LACTOSE UTILIZATION IN BUFFALO SKIM MILK

The rate of acid production, increase in cell number, lactose utilization and proteolysis by yoghurt starter bacteria when grown in buffalo skim milk individually and in combination at 40 and 45°C were investigated. The results indicate that the highest rate of acid development, lactose utilization, increase in viable count and proteolytic degradation and uptake of T.C.A. soluble protein fractions were at 45°C and with the mixed cultures when they were in combination.

Yoghurt is a fermented milk derived using *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Quality of the product depends on the rate of acid development and controlled proteolysis. Buffalo milk has been used in India for yoghurt preparation, but information on the rate of acid production, proteolysis, lactose utilization and changes in viable cell numbers during the product manufacture is scanty. Work carried out to elucidate the information are reported here.

Streptococcus thermophilus isolated in the department and *Lactobacillus bulgaricus* LBW procured from National Dairy Research Institute, Karnal, were used in these experiments. The cultures were maintained on sterilized buffalo skim by weekly tranfers and stored in refrigerator. Inoculum was prepared after activating the cultures by three successive transfers on sterilized buffalo skim milk and incubation at 40 and 45°C for 16 hr. The inoculum from each bacterium was utilised as such or mixed in the ratio 3:1 (v/v) for mixed culture cultivation. Sterile buffalo skim milk was inoculated at 5 per cent level, mixed well and distributed in 18×150 mm sterile test tubes in 10 ml quantities. Each set of tubes were incubated at 40°C and 45°C. Duplicate samples were drawn from each set of tubes at random at hourly intervals and analysed for acid development and viable count. Lactose utilization was determined after 4 hr and proteolytic degradation after 8 hr. The titratable acidity expressed as lactic acid was estimated by titration with 0.1 N NaOH using phenolpthalein as indicator. Viable count was assessed by utilizing lactic agar and lactose utilized was estimated by Fiona Frais[1] method using 1 g sample for each estimation. Five gram samples were used for estimation of 4 per cent T. C. A. and 12.5 per cent T. C. A. soluble protein fractions which are expressed as tyrosine[2].

Rate of acid development and viable counts of yoghurt cultures incubated at 40 and 45°C are given in Fig 1 and 2. It is observed that acid development and viable count were better in cultures incubated

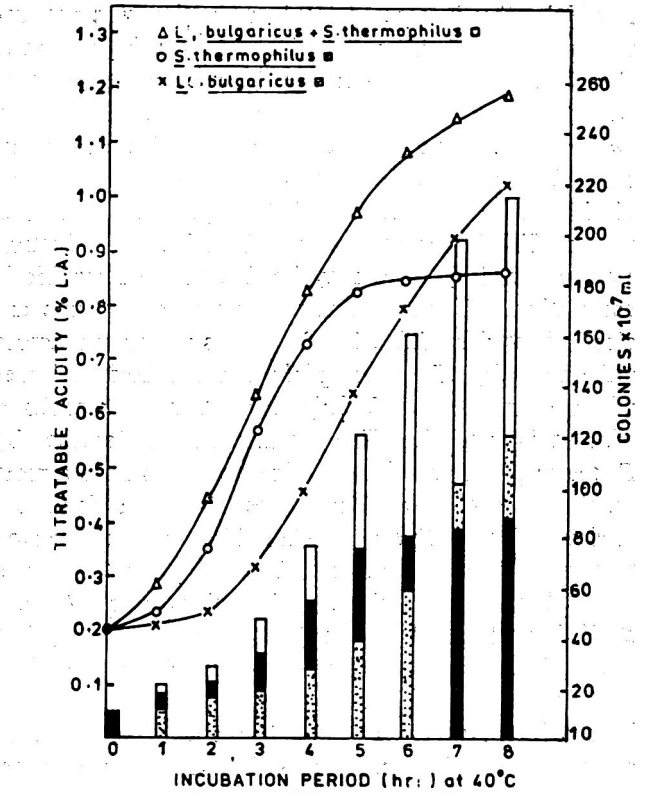

Fig. 1. Rate of acid development and increase in viable count of Yoghurt cultures in buffalo skim milk at 40°C.

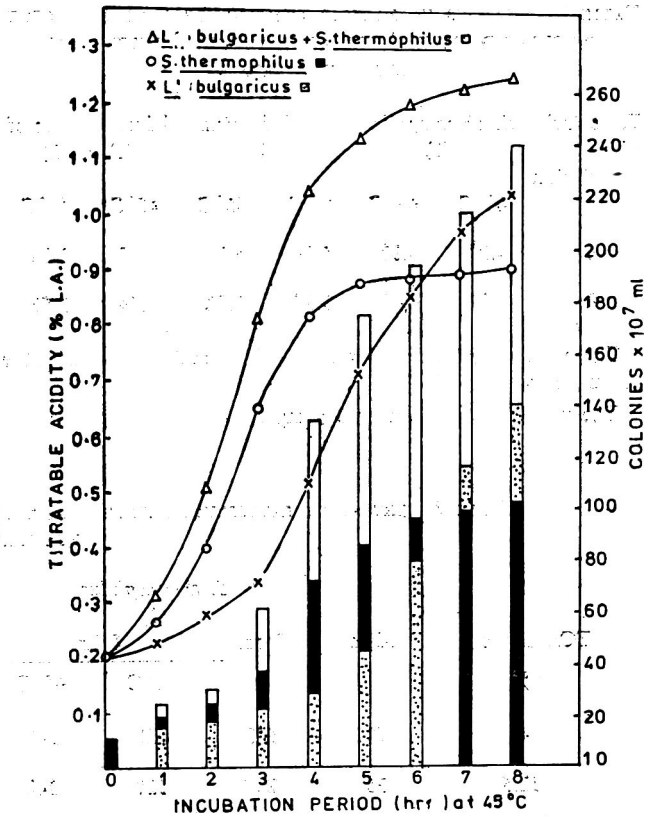

Fig. 2. Rate of acid development and increase in viable count of Yoghurt cultures in buffalo skim milk at 45°C.

at 45°C. The increase in acid production was proportionate to the increase in viable counts in all cases. The fastest increase was noted when mixed culture was used at both the temperatures studied. Among the two cultures at both the temperatures, *S. thermophilus* showed a faster rate of growth and acid production, till 6 hr of growth, after which *L. bulgaricus* gave higher values. The higher rate of growth and acid production by the mixed cultures may be due to their symbiotic association. Lactose utilization by these cultures are also indicated in Table 1. There was maximum lactose utilization by *S. thermophilus* and mixed cultures within 4 hr, while in *L. bulgaricus* utilization was faster after this period. The utilization of lactose by *S. thermophilus* and in the mixed cultures runs almost parallel upto 4 hr which indicates that lactose is consumed initially by *S. thermophilus*. Kroger[3] has observed that acid development is a basic requirement for a good flavour in yoghurt. Several workers[4-7] have studied acid development by yoghurt cultures, but the mode of production has not received any attention. The data clearly indicate that 0.8 per cent acidity can be obtained by using mixed cultures at 5 per cent inoculum level by incubating at 45°C within three hours.

TABLE 1. CHANGES IN LACTOSE CONTENT OF MILK (PER CENT) INOCULATED WITH 5 PER CENT YOGHURT CULTURE AT DIFFERENT INTERVALS

Time (hr) after inoculation	*S. thermophilus* (1)		*L. bulgaricus* (2)		Mixed culture of (1) & (2)	
	40°C	45°C	40°C	45°C	40°C	45°C
4	4.20	4.12	5.34	5.20	3.98	3.85
8	4.07	4.00	3.97	3.82	3.64	3.58

The lactose content of milk was 5.55% and at the time of addition of culture was 5.6%.

The values are average of 5 samples.

Table 2 shows, degree of proteolysis determined as tyrosine in the filtrates after 4 and 12.5 per cent T. C. A. treatment of the curd. Soluble protein content was markedly lower in all samples after the growth of lactic cultures. This decrease was maximum with *S. thermopilus* followed by the mixed culture. This may be due to lower proteinase activity and slower utilization of soluble proteins by *S. thermophilus*[8]. Asperger[9] has also reported similar results wherein tyrosine content of yoghurt was used as one of the criteria quality tor its evaluation. Miller and Kandler[10] observed higher release of free amino acids in milk by different strains of *L. bulgaricus* and utilization of tyrosine and a few other amino acids by all the strains tested when they were actively growing.

The increased cellular activities like acidity rise, rise in viable count, and drop in T. C. A. soluble protein components observed with the mixed cultures in the present study indicate that buffalo milk is equally suitable as cow's milk for the preparation of yoghurt within 3 hr at 45°C using the inoculum at the rate of 5 per cent. It is interesting to note that at 45°C with the mixed culture, 12.5 per cent T. C. A. soluble protein fractions were higher than when *L. bulgaricus* was used alone. This is attributed most probably to the higher peptidase activity of *S. thermophilus*[8].

Department of Dairy Microbiology,
Gujarat Agricultural University,
Dairy Science College,
Anand Campus, Anand-388 110, India.

A. M. PATEL
J. M. DAVE
S. S. SANNABHADTI

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TABLE 2. EXTENT OF PROTEOLYSIS REPORTED AS TYROSINE (μG/ML IN FILTRATE) IN MILK INOCULATED WITH YOGHURT CULTURES

TCA levels (%)	Milk	*S. thermophilus* (1)			*L. bulgaricus* (2)			Mixed culture of (1) and (2)		
		0 hr.	8 hr.		0 hr.	8 hr.		0 hr.	8 hr.	
			40°C	45°C		40°C	45°C		40°C	45°C
4	720	550	500	240	1000	780	820	1040	500	660
12.5	250	200	120	340	550	550	550	550	360	500

Values are average of 5 samples

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BIOLOGICAL EVALUATION OF WHEAT VARIETIES

Four varieties of wheat, viz., WH-147, WH-157, WH-283 and WH-291 were evaluated on the basis of proximate composition, chapati making quality and protein efficiency ratio. Moisture, ash, crude protein, ether extract, crude fibre contents and nitrogen free extract ranged from 7.88-8.96; 1.43-1.79; 10.11-11.77; 1.74-2.93; 0.98-1.49 and 74.03-78.24 per cent respectively. Calcium and phosphorous contents ranged from 88.78-119.10 and 224.35-349.41 mg/100g respectively. Chapati making quality of these varieties were also studied. PER ranged from 1.99 to 2.46. The variety 'WH-283' had higher PER and good chapati making qualities. The quality characteristics of 'WH-147', 'WH-157' and 'WH-291' were also satisfactory.

Protein efficiency ratio[1-2] is the most practical procedure for monitoring protein quality on a routine basis. Having overcome the problems of quantity, as a result of high yielding varieties of wheat[3], there has been an increasing awareness about the nutritional quality, both among growers and consumers. Recently, two new varieties 'WH-283' and 'WH-291' have been developed and identified. The quality characters of these two wheats are evaluated and compared with those of 'WH-147' and WH-157'.

Grain samples of 'WH-147', 'WH-157', 'WH-283' and 'WH-291' varieties of wheat grown at Haryana Agricultural University, Hissar were collected.

AOAC[4] methods were followed for determining proximate composition. Phosphorous was estimated by the method of Fiske and Subba Row[5]. PER was determined according to AOAC[6] method. Chapati making qualities were studied as described by the method of Austin and Ram[7].

The data on the proximate composition are presented in Table I. Moisture content of the varieties ranged from 7.88 to 8.96; total ash from 1.43 to 1.88; crude protein from 10.11 to 11.77; ether extract from 1.74 to 2.93; crude fibre from 0.98 to 1.49; NFE from 74.03 to 78.24 per cent. Calcium and phosphorous contents (mg/100g) ranged from 88.78 to 119.10 mg and 224.34 to 349.41 mg respectively. The variety 'WH-283' seems to be superior because of its higher calcium and phosphorous contents and lower ash content. Protein content of the varieties was found to vary depending upon the place. Such differences may be attributed to the locational effect and to various agronomic practices[8].

Table 2 presents the gain in weight, food intake, protein intake and PER. PER ranged between 1.99 and 2.46 as compared to 2.84 for casein. 'WH-283' was found to have maximum PER (2.46) followed by 'WH-291', 'WH-157' and 'WH-147'. PER value of 1.35 to 2.00 in some pre-released high yielding wheat varieties has been reported by Sangeetha[9]. While Narayana-Swamy *et al.*[10] have reported a PER range of 1.89 to 2.38.

Data on chapati-making qualities presented in Table 3, show that the varieties had water absorption ranging between 64 and 72 per cent. Rattan Singh and Bailey[11] observed that Indian wheats have relatively high water absorption which may be due to their high

TABLE 1. PROXIMATE COMPOSITION OF WHEAT VARIETIES

Variety	Moisture (%)	Ash (%)	Crude protein (%)	Ether extract (%)	Crude fibre (%)	NFE (%)	Calcium (mg/100 g)	Phosphorous (mg/100 g)
WH-147	7.88	1.65	10.11	1.74	0.98	78.2	114.7	304.0
WH-157	7.98	1.79	11.29	2.74	1.36	75.1	91.2	224.35
WH-283	8.61	1.43	10.77	2.93	1.49	74.0	119.1	349.4
WH-291	8.96	1.88	11.77	1.95	1.27	74.2	88.8	298.1

TABLE 2. EFFECT OF FEEDING WHEAT VARIETIES ON RAT GROWTH

Vairety	Body wt. Intial (g)	Body wt. Final (g)	Gain in wt. (g)	Food intake (g)	Protein consumed (g)	PER
Casein (conrol)	31.9	84.9	45.9	179.50	16.15	2.84
WH-283	32.6	70.5	36.9	166.0	14.93	2.46
WH-291	33.0	69.6	35.6	170.8	15.37	2.31
WH-157	30.8	63.5	31.9	169.2	15.21	2.12
WH-147	34.4	64.6	29.5	164.5	14.80	1.99
S.E.	—	—	2.8	3.4	0.30	—
C.D. at 5%	—	—	11.1	13.5	1.21	—

TABLE 3. CHAPATI-MAKING QUALITY O THE WHEAT VARIETIES AND THE SCORES FOR CHAPATI

Variety	Water absorption capacity (%)	Puffing/score	Aroma score	Texture of chapati		Taste score
				Initial	After 4 hr	
WH-147	64	Full/7	6	8	Soft-7	7
WH-157	65	Full/7	7	8	Soft and pliable-8	6
WH-283	72	Full and rapid/8	6	8	Soft and pliable-8	4
WH-291	70	Full and rapid/8	6	8	Soft and pliable-8	7

All the varieties had cream colour dough which gave cream coloured chapati. The handling property of the dough of all varieties was medium strong, non-sticky and the texture of chapati obtained was soft and pliable in all the varieties.

starch damage during milling. Higher water absorption is generally indicative of good chapati making quality. All these varieties are suitable for chapati making. Austin and Hanslas[12] reported the bread making properties of 'WH-283'.

It may be concluded that, 'WH-283' is the outstanding variety possessing all the qualities required for both bread as well as chapati making.

Dept. of Chemistry and Biochemistry,
Haryana Agricultural University,
Hissar-125 004, India.

SHASHI TANEJA
KAUSHAL GUPTA
D. S. WAGLE
K. S. DHINDSA

Received 18 *October* 1982
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A STUDY ON DISTRIBUTION AND QUALITY OF DIFFERENT SIZED 'KINNOW' MANDARIN ORANGES HARVESTED AT OPTIMUM MATURITY

'Kinnow' mandarin oranges were harvested at optimum maturity, during 1980 from a 11-year old tree and graded according to the size and quality. Out of 1844 fruits, maximum number (44.75 percent) belonged to B grade. Peel thickness and vitamin C content were found to increase with fruit size. There was no appreciable difference in rag content among fruits of different grades. The highest solid/acid ratio was recorded in B grade fruits. Higher percentage of juice and total soluble solids were obtained in medium sized fruits belonging to grade B.

'Kinnow' mandarin orange (hybrid between 'King' and 'Willow-leaf' mandarin) is becoming popular with citrus growers in North India due to its performance and good eating quality. Large acreages are being brought under this fruit in Punjab, Haryana, Rajasthan and Himachal Pradesh. It has been recommended that the best time for picking fruits is from the middle of January to the middle of February in different regions. However, variations in the size and quality of the fruits are noticed as the fruits of outer periphery are of better quality than those borne in the interior of the canopy which are still green[3].

Harvesting of citrus fruits at proper stage and their grading is of atmost importance for marketing the produce and obtaining maximum returns. Present investigation was carried out to grade the fruits according to their size and to assess the quality of fruits falling in each grade.

One thousand eight hundred and fortyfour fruits from a 11-year old healthy 'Kinnow' tree (grafted on Rough lemon (*Jatti Khatti*) rootstock) growing in the Regional Fruit Research station, Abohar, were harvested on 17 January, 1980 and sorted into five grades, A through E, on the basis of skin colour and size as given in Table 1.

Twenty fruits were taken at random from each group and their characteristics worked out. The diameter of the fruits, peel thickness and the weight of the fruit, peel and rag (waste material after extracting juice) were recorded. Fruits from each grade were evaluated for organoleptic qualities by a panel consisting of four judges, based on general appearance, taste and flavour (10 points each). Fruits were rated as fair (5.5 - 6.4 points), good (6.5-7.4 points), very good (7.5-8.4 points) and excellent (8.5-10 points). Total soluble solids (TSS) were measured with hand refractometer. Acidity was determined by titrating a known volume of fruit juice against 0.1N NaOH using phenolphthalein as indicator. Ascorbic acid was measured titrimetrically by using 2, 6-dichlorophenol indophenol dye.

Maximum number of fruits were found in grade B (44.75 per cent), while in grade C, there were 401 fruits and in grade A 352 fruits. The fruits belonging to A and B which accounted for 64 per cent, had deep orange colour and the most acceptable by consumers. Grade D consisted of 11.7 per cent fruits while 2.66 per cent belonged to grade E.

There was a lot of variation in physical characteristics between the fruits in each grade.

Peel thickness was higher in larger fruits. Maximum quantity of peel was found in grade A fruits, whereas

TABLE 1. DISTRIBUTION AND PHYSICAL CHARACTERISTICS OF GRADED 'KINNOW' FRUITS

Grade	Fruit size	Colour	No. of fruits	Distribution* (%)	Fruit dia (cm)	Fruit wt (g)	Peel thickness (cm)	Peel (%)	Rag (%)
A	Big	Deep orange	352	19.08	8.46	254.66	0.44	31.53	20.11
B	Medium	Deep orange	825	44.75	7.24	200.80	0.36	24.98	22.25
C	Small	Deep Orange	401	21.74	6.17	148.33	0.35	25.80	22.30
D	Medium small	Light green Orange tinge	217	11.77	6.93	174.67	0.34	26.56	20.86
E	Very small	Light green Orange tinge	49	2.66	5.86	129.53	0.31	28.88	19.93
	C.D. at 5%				0.18	10.60	0.04	2.35	NS

*As % of 1844 fruits taken for observations

TABLE 2. CHARACTERISTICS OF 'KINNOW' FRUITS BELONGING TO DIFFERENT GRADES

Grade	Palatability rating	Juice (%)	TSS (%)	Acidity (%)	TSS/acid ratio	Vitamin C (mg/100g)
A	8.00	48.35	10.12	0.840	12.05	27.84
B	8.62	52.77	11.24	0.871	12.90	27.18
C	8.25	51.89	11.08	0.880	12.59	26.67
D	7.00	51.88	9.86	0.905	10.90	24.24
E	5.62	51.19	9.07	0.982	9.24	23.07
C.D. at 5%		2.66	0.45	0.018	2.47	2.29

minimum quantity was found in grade A fruits. Similar observations have been recorded by Jawanda *et al.*[2] with 'Kinnow' fruits. There was no significant difference in the quantity of rags obtained from fruits of different grades. The data on the characteristics of '*Kinnow*' fruits of different grades are presented in Table 2.

Fruits belonging to B grade was adjudged 'excellent' after organoleptic evaluation, while those belonging to A and C grades were rated as very good. Fruits D grade was evaluated as good and those of E grade as 'fair'.

Maximum yield of juice was obtained from fruits of B grade. Jawanda *et al.*[2] have also reported that 'Kinnow' fruits of 8-9 cm size yielded maximum juice. The yield of juice from C, D and E grades was moderate while the fruits of A grade gave lowest yield.

TSS was the highest in grade B fruits (11.24 per cent) closely followed by C grade (11.08 per cent). TSS content of juices from other grades were significantly lower. Josan *et al.*[3] reported that green fruits of 'Kinnow' located inside the canopy of the tree had lower TSS content. Chundawat *et al*[1]. also have made similar observations.

The acidity of fruits progressively increased with the decrease in size of the fruit. Findings of Jawand *et al*[2]. are also similar.

TSS/acid ratio is considered as good index for judging the maturity of citrus fruits. It has been reported that 'Kinnow' fruits should be picked when they attain a solid/acid ratio of 12:1 for fruits borne on the open side of the tree and 14:1 for fruits found in the inner side of the tree[2]. The highest TSS/acid ratio was recorded in grade B fruits closely followed by fruits of grade C and grade A. The TSS/acid ratio decreased progressively with fruits of lower grade, minimum being recorded with fruits of E grade.

It was also found that larger fruits contained more vitamin C. Chundawat *et al.*[1] also have reported similar findings. The vitamin C content decreased with decrease in size of the fruit. Fruits belonging to grade B were the best based on peel thickness, juice, yield, palatability, TSS and TSS/acid ratio. Number of harvested fruits falling in grade B was also the highest (44.75 per cent).

Regional Fruit Research Station,
Punjab Agricultural University,
Abohar-152 116, India.
J. S. BAL
G. S. CHOHAN
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EFFECT OF DIETARY INTAKE OF HEXACHLOROCYCLOHEXANE ISOMERS ON SOME HAEMATOLOGICAL PARAMETERS

Dietary intake of β—and γ—isomers of hexachlorocyclohexane in rats (at 800 ppm level) over two weeks period increased the blood clotting time considerably. The blood cell counts and other parameters like erythrocytes sedimentation rate, osmotic fragility of erythrocytes were not affected, while blood cell volumes were sligthly elevated only by γ—hexachlorocyclohexane treatment.

Although technical hexachlorocyclohexane (HCH) and lindane (γ-HCH) have been employed in pest

control for a number of years, their effect on the blood cell parameters are not reported. These may be affected by changes either in the blood forming organs or in other tissues of the body. Dietary intake of β- or γ-HCH at 800 ppm level for more than two weeks is known to result in severe toxicity symptoms culminating in death of majority of animals[1]. α-and δ-isomers of HCH at similar concentrations in the diet have been demonstrated to be ineffective[2]. In the present study, the effect of dietary β- and γ-isomers of HCH on some haematological parameters was investigated in albino rats.

Young male albino rats (Wistar strain) weighing 65-70g were fed with diets containing 800 ppm β-HCH or γ-HCH for two weeks. The basal diet contained (g/100g): casein, 18; cane sugar, 10; corn starch, 55.1; groundnut oil, 10; mineral salts[3], 4; vitamin mixture[4], 2.5; choline chloride, 0.4 and fat soluble vitamins as solution in groundnut oil. The animals were sacrificed at the end of two weeks. For heamatological studies, blood was collected in tubes coated with EDTA. For erythrocytes preparation, blood was centrifuged at 500×*g* for 10 min and plasma was separated by aspiration. The erythrocytes were washed with 0.9 per cent saline, centrifuged and finally suspended in 0.9 per cent saline. Serum samples for calcium were obtained by leaving the blood to clot for one hour and then centrifuging it.

The mean corpuscular volume, packed cell volume, erythrocyte sedimentation rate and total counts of the erythrocytes, leucocytes and differential counts were determined by the methods described by Kolmer and Boerner[5]. Blood clotting time was measured by capillary tube method[6]. The osmotic fragility of erythrocytes was determined by the method described by Karkert[7]. Serum calcium was estimated according to Baron and Bell[8].

Data on the blood cell concentrations, viz., RBC, WBC and differential counts in HCH treated and control rats are presented in Table 1. The RBC counts were in the range of normal values in HCH treated rats. WBC counts were decreased, but not significantly. Differential counts on leucocytes did not show any marked differences. Data on blood cell volume, erythrocyte sedimentation rate, hemoglobin content and blood clotting time are given in Table 2. Mean corpuscular volume and packed cell volume were significantly higher only with γ-HCH treatment. The hemoglobin content of blood as well as the hemoglobin content per erythrocyte viz., mean corpuscular hemoglobin were in the range of normal values in the HCH treated rats. But the mean corpuscular hemoglobin concentration which expresses the hemoglobin per unit volume of erythrocytes was lower in γ-HCH treated animals. Blood clotting time was significantly higher in both the groups of treated animals. Dietary HCH isomers showed no effect on serum calcium level (Table 2).

TABLE 1. BLOOD CELL COUNTS IN β— AND γ—HCH TREATED RATS

Parameter	*Control*	*β—HCH*	*γ—HCH*
RBC (million/cu.mm)	8.37±0.12	8.14±0.38	7.93±0.47
WBC (thousands/cu.mm)	10. 1±2.13	8.37±0.71	8.50±1.49
Differential counts (% total)			
Neutrophil	16. 5±2.6	14. 1±1.8	15. 1±1.3
Lymphocyte	79. 0±2.4	80. 9±2.7	79. 8±2.5
Monocyte	3. 7∓0.3	4. 0±0.4	4. 3±0.4
Eosinophil	0. 8±0.2	1. 0±0.3	0. 8±0.2
Basophil	0	0	0

Values are mean±SEM of 6 animals in the control group and of 9 animals in the experimental groups.

The effect of ingestion of HCH isomers on the osmotic fragility of erythrocytes is shown in Fig. 1. In all the three groups, hemolysis began in hypotonic saline at 0.5 per cent concentration and completed at 0.3 per cent concentration with mean cell fragility (50 per cent hemolysis) being evident at 0.4 per cent sodium chloride concentration. There was no difference in the pattern of hemolysis between control and HCH treated groups.

TABLE 2. HAEMATOLOGICAL PARAMETERS AND SERUM CALCIUM IN β— AND γ—HCH TREATED RATS

Parameter	*Control*	*β—HCH*	*γ—HCH*
Mean corpuscular vol. (cu. microns)	64.5±2.4	72.4±3.2	78.8±2.1[a]
Packed cell vol. (%)	54.0±2.0	52.7±3.0	63.1±3.0[c]
Erythrocyte sedimentation rate (mm/1 hr)	1.7±0.2	2.0±0.2	2.0±0.2
Hemoglobin (g/100 ml)	16.1±0.2	15.5±0.4	15.3±0.6
Mean corpuscular hemoglobin (nanogram)	19.1±0.3	19.2±0.4	19.4±0.3
Mean corpuscular hemoglobin concn (%)	30.0±1.2	26.9±1.0	24.3±0.5[a]
Blood clotting time (sec)	27.0±1.6	47.1±2.8[a]	44.3±4.4[b]
Serum calcium (mg/100 ml)	13.9±0.1	12.5±0.4	13.0±0.1

P values: a:<0.001; b:<0.005; c:<0.05

Values are mean±SEM of 6 animals in the control group and of 9 animals in the experimental groups

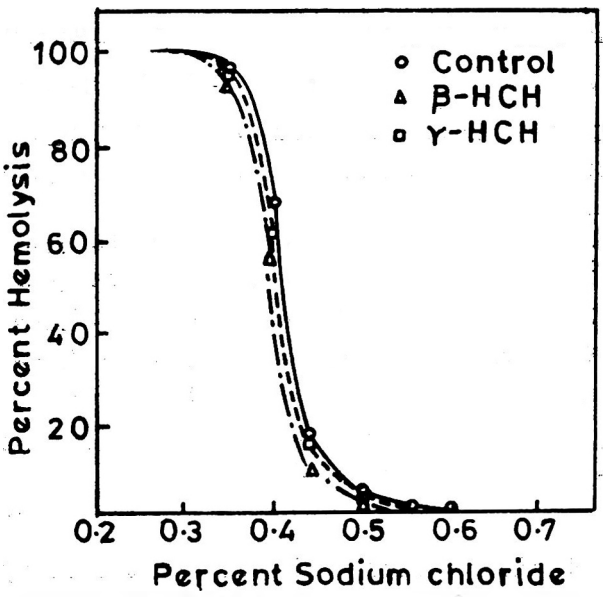

Fig. 1. Erythrocyte fragility response in sodium chloride solution (Each point represents average of 6 values.)

The haematological parameters are studied here as they constitute certain health indices. Erythrocyte sedimentation rate is a specific parameter for general health. Other measurements, viz., hemoglobin, RBC, WBC, mean and packed cell volume disclose the possible reactions of blood and blood forming tissues to the pesticide treatment.

The present study has demonstrated that the animals are able to maintain the concentration of erythrocytes and leucocytes in normal range during HCH isomers treatment. Further, the relatively insensitive parameters on erythrocytes like mean corpuscular volume and packed cell volume did not respond to β-HCH treatment although these parameters were slightly elevated in γ-HCH treatment. The lowered mean corpuscular hemoglobin concentration observed here is due to the increased mean corpuscular volume by γ-HCH treatment. The hemolytic study has revealed that fragility of erythrocytes is comparable to that of normal rats, which is an indication that membrane composition of erythrocytes is not much altered. In conditions of hemolytic anemia, erythrocytes are known to be deficient in the activity of the enzyme glucose-6-phosphate dehydrogenase[9]. It has been shown that during β-and γ-HCH treatment, glucose-6-phosphate dehydrogenase activity of the erythrocytes is either normal or even higher[1]. This is in consistence with the result of hemolytic study presented here.

Increased blood clotting time observed in HCH isomers treated animals could be associated with the liver damage caused by these compounds[1] resulting in low blood prothrombin levels. The fact that blood calcium levels are normal in HCH treated rats eliminates the possibility of increased blood clotting time resulting from low calcium levels. Barsel'yants[10] has reported prolonged blood clotting time in humans subjected to long term exposure to HCH and other pesticides. It has been claimed that abnormalities in blood coagulation and fibrinolysis may lead to or play a part in the development of renal damage[11]. It is to be noted here that prolonged dietary intake of β-or γ-HCH results in kidney malfunction[1].

The authors wish to express their gratitude to Mr. C. P. Ramasundaram for his help in haematological studies.

Discipline of Biochemistry & Applied Nutrition, Central Food Technological Research Institute, Mysore-570 013, India.
K. SRINIVASAN
R. RADHAKRISHNAMURTHY

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BOOK REVIEWS

Food Proteins: by Box P. F. and Condon. J. J. (Eds) Applied Science Publishers, New York, 1981; pp: 361, Price: £ 38.00.

The proceedings of the International Symposium on Food Proteins sponsored by Kellogg foundation and held in 1981 at Cork, Ireland has been brought out in a book form. Ninteen papers have been included in the book and they are on world food supply, basic aspects of proteins, chemistry and technology of three important food proteins namely milk, cereal and meat proteins and some unconventional proteins. The papers have not been arranged in any logical order which is a distraction.

Irish people consume more than three times the recommended level of proteins, and food proteins *per se* have an important place in Irish economy. There is good scope to start major protein-based industries since raw materials and expertise are available in Ireland. The increase in protein/food supply has been rather marginal and there is a need for world community to have economic integration foı rational distribution of proteins. However, this idea may remain only ideal.

Food proteins have their own unique functional properties. The possibilities of texturising some seed proteins to obtain meat-like or cheese-like products have been well recognised. The need for more basic studies into the physical properties of proteins has been stressed. A comprehensive list of references has been given on this topic.

Establishing the optimal level of protein-calorie requirements has been a topic of endless debate; it is a complex problem and is as controversial as the protein gap. The issues have been elaborated once again without offereing any solution.

Inspite of the reliable correlation between chemical score and biological value, researchers prefer only PER, NPR etc. as the best method of nutritive evaluation. The need for a change to other methods has been convincingly brought out.

Milk proteins have been dealt in three chapters which include chemistry and physics, manufacture of milk protein and textured food from milk proteins. The details on these are very comprehensive with extensive citations from published literature. Extrusion is unsuitable for milk protein texturation; dry spinning of casein appears to be a better alternative. Processing of skim milk powder into textured protein and preparation of whey syrups have been suggested as profitable propositions.

Meat proteins as a food component have not received as much attention as milk or vegetable proteins. Muscle proteins and their impoıtance in meat technology and proteolysis of meat have been discussed in three chapters. Muscle proteins are not merely important in rigor, but also for the tenderness of meats and in cooking. The discussion on the properties of myofibrillar proteins and collagen has been limited. References cited in these chapters are few.

Leaf protein preparation on domestic scale to augment proteins in protein-deficient diet has been particularly discussed. Too much optimism for practical application has been expressed without taking into consideration the reality of the situation. The chapter on biological nitrogen fixation and its importance to food industry briefly catalogues the soil organisms that can symbiotically or otherwise fix nitrogen in agricultural plants. The possibility of genetic engineering as a tool to fix free N_2 is also detailed.

Recovery of proteins from food factory wastes is discussed in the last two chapters. One chapter details the potential from meat industry discharges using lignosulphanates and ion exchange resin for separation of proteins. The economic gains by such a recovery are apparently impressive and makes one wonder why these projections have not been realised in practice. Obviously, such a diverse array of topics cannot be discussed in detail in the brief reviews presented at the symposium. However, researchers in the area of food proteins will find the book a good addition, since recent information on the status on the varied topics of food proteins is provided by the book.

L. V. VENKATARAMAN
M. S. NARASINGA RAO
C.F.T.R.I., MYSORE

Refrigeration of perishable products for distant markets: by International Institute of Refrigeration, 177., Boulevard, Malesherbes-F 75017, Paris, France, 1982(1), Pp 461; Price: 160 F. F.

This book is proceedings of the meeting of the Commissions C_2, D_1, D_2 and D_3 of the IIR which met at Hamilton from January 26-29, 1982.

It comprises 56 research papers, together with the essentials of discussions constitute the 52 volume of the IIR series on refrigeration science and technology.

The proceedings comprises of 9 sections: Section I

has 3 articles—(*i*) The food/energy dilemma, what can refrigeration technology do? by G. Lorentzen, (Norway) (*ii*) Energy consumption in the cold chain by L. Mattarolo (Italy) and (*iii*) Refrigeration in New Zealand by R. L. Earle (Newzealand) were presented. The rest of the sections were devoted to individual commodity/aspect.

Section 2, on Meat, comprises 12 research papers on (1) electrical stimulation on meat quality, (2) practical considerations in the freezing of electrically stimulated lamb carcases, (3) The intricacies of freezer chain, (4) Misconceptions of meat and fish chilling, (5) effect of processing variables on the storage stability of frozen lamb, (6) on the ice growth mechanisms during beef freezing, (7) Microbiological considerations in cold storage of meat, (8) frozen and refrigerated comminuted meat (beef & pork] microbial and physicochemical properties evolution during storage (in French), (9) a large capacity plate freezer for meat cartons, (10) survey of weight loss from lamb in cold storage, (11) weight losses of Newzealand lamb carcases from slaughter to market and (12) vacuum packed telescoped lamb: an innovative technique in whole carcass packaging.

Section 3 has 5 research papers on fish: (*i*) the importance of appropriate refrigeration to the Newzealand fishing industry, (*ii*) A model for fish freezing and storage on board small Newzealand fishing vessels, (*iii*) New prospects for refrigeration applied to fishery products in developing countries of the Asian and Pacific regions, (*iv*) The importance of chilling in producing top quality snapper (chrysophsys aurantus) for the Japanese market and (*v*) the chilling of Southern blue tin tuna.

Section 4 is devoted to quality and packaging, comprises 4 research papers (*i*) influence of chilling and freezing on seed quality and viability (french), (*ii*) Human anxieties about persishable food processing and distribution, (*iii*) Evaluation test in packaging of perishable products to be transported in refrigerated media and (*iv*) prediction of shelf life of stored foods.

Section 5 on storage consits of 6 research papers on (*i*) Developments of reflective insulation in refrigerated warehouse construction, (*ii*) The application of mechanical refrigeration to cave storage of fruit, (*iii*) controlled atmosphere storage using plastic tents, (*iv*) the refrigerated storage conditions necessary for the export of cheese, (*v*) alternatives for meeting peak cold storage demand in Newzealand seasonal industry and (*vi*) The time temperature relation on chilling injury to cucumber in refrigerated storage.

Section 6 on transport covering 11 research papers 3 on commodity basis-factors affecting the carriage of meat in containers, transport of oranges in refrigerated containers under high ambient temperatures and container transport of bananas in the South Pacific, the rest are of general nature like heat transmission through ship structures, export of woody ornamentals under refrigeration, dockside refrigeration, shipping conditions for butter cup squash exports from Newzealand, consumption and predetermination of dry ice quantity depending on the boundary values for cooling perishable goods in air-transport, refrigerated transport of perishables from Isreal to Europe, problems and solutions and evalutating the thermal property of refrigerated transport medium through Q/K method.

Section 7 is on 'container testing' with 4 reserach papers-Australian container test facility: description and test-A numerical method for determining the overall heat transfer co-efficient for containers, measurement of gas-tightness with an automatic pressure decay timer and measurement of air circulation rate in integral refrigerated shipping containers.

Section 8, is on current interest topic 'Energy'-covering 6 papers, one on estimation of power cost for quick frozen minced steak, and the other on minimum chilling costs to maintain raw milk quality on the commodity basis, and the rest 4 are of general nature concerning energy (Two of the six are in French).

The last section is on 'calculation and modelling' comprising 4 research papers.

Some of the methods suggested like storage in plastic tents and cave storage are very useful for adopting in developing countries with immense benefits.

This book will be a good addition to any library and serve as a useful reference book for researchers in the field of refrigeration of perishable products.

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

Fire prevention and safety in cold stores: International Institute of Refrigeration, Paris, 1982; Pp., 187, Price: 100 FF

This book is a record of the 27 papers presented at a joint meeting of Commission D 1 of the International Institute of Refrigeration and specialists from the European Association of Refrigeration Enterprises held in Paris in 1982.

Although fires in cold stores are less frequent than in many other industries, in the present day safety conscious industrial world, cold stores cannot be excluded from the applicability of fire prevention and fire fighting measures. The stores by the very nature of

their design are sealed buildings which means that fire even in its initial stage can quickly spread. Even if the fire is contained in one place the smoke emanating from it can taint and make unfit for consumption the entire stock of meat.

The Foreword contains the major conclusions arrived at the Paris meeting.

Detailed papers are presented on the cause-wise analysis of major fires in cold stores in some of the countries in Europe and in Newzealand.

Regulations for constructions and fire test methods in cold stores in Norway and Newzealand are also given. A number of papers deal with newer construction materials and layout of modern cold stores which have already been put into service.

France, being the host country, has contributed the maximum number of papers dealing with all aspects of fire proof construction, fire prevention/warning systems. Among the latter are ionic detectors, carbon dioxide snuffing system, fire breaks equipped with fire proof doors, sprinkler systems and water curtains. Proper training of personnel manning the stores and the fire fighting staff have also been emphasized. A paper from Poland deals with the physiological effects of cold environment on the workers inside the cold stores.

Of the 27 papers presented, 18 are in French and 9 in English. However, the Editors have thoughtfully included a summary in English at the end of each paper in French and *vice versa*. Most of the papers, are printed in too small print. A few minor spelling mistakes have crept in, probably originating at the authors' end and being continued through reproduction of the papers.

The book should be useful to those who build and run cold stores and to all those who are interested in refrigeration.

L. A. RAMANATHAN
DEFENCE FOOD RESEARCH LABORATORY, MYSORE

Modern Techniques of Raising Field crops: by Chidda Singh, Oxford and IBH Publishing Company, New Delhi; 1983; Pp: 524; Price: Rs. 34.00

The improvements that are being witnessed in the production of crops is a continuous one, which are mainly based on our greater understanding of the basic aspects of crops coupled with the results of field experiments conducted all over the country. Our agricultural system influenced by soil and agro-climatic condition is so varied that such experiments are of utmost importance to draw definite conclusions applicable to each locality.

The book under review incorporates all the results of latest findings on various aspects of crop production covering 38 crops grown in our country. These include: cereals and millets (rice, wheat, maize, sorghum, bajra, barley, finger millet, proso millet, barnyard millet, khodo millet, and Italian millet), pulses (gram, lentil, peas, arhar, green gram, black gram, cowpea, rape and mustard seeds, linseed, safflower, sunflower) fiber crops, (cotton, jute and sann hemp), forage crops (oats, berseem, lucerne, guar, and napier grass), sugar crpps (sugarcane and sugarbeet) and miscellaneous crops (potato and tobacco). Each crop is discussed under well recognised sections coming under the study of crop production which include origin and history, area and distribution, botanical description, varieties, soil and climate, rotation, seed and sowing, cultivation methods, manures and fertilizers, water management, weed control, diseases and pests, harvesting and yield. The space devoted for each crop in the book is according to their importance in the Indian agricultural system. Information on high yielding varieties, crops suitable to different agro-climatic zones, identifying and control of pests and diseases are not exhaustively treated although attempts have been made to cover the entire country. Today the information on field crops has accumulated so much mainly due to variations in soil and climatic conditions of our country that a book of this magnitude will hardly meet the demands. In fact, book of this magnitude is needed on each crop, which I hope the author will be able to do in course of time.

Recently after the starting of Food Technology Departments in many of the agricultural universities, post-harvest technology has assumed a part of the study of agriculture and there are many interdisciplinary areas where technology has also got to play a very important role in agricultural programmes. It will be of great value, if the author, in the future editions devotes a section to discuss the post-harvest technology of crops.

In our country, where agriculture is the main profession, the books published on agriculture are very few and I am sure the present book will be of great use to both students of agriculture and extension workers. It is really unfortunate that the publication which will be a constant source as a reference book has been brought out using sub-standard paper with poor binding which will indicate that the book may not physically lost long.

K. A. RANGANATH
C.F.T.R.I., MYSORE

Blue-green Alga-Spirulina: by L. V. Venkataraman, Central Food Technological Research Institute, Mysore, 1982; Pp: 100; price: unpriced.

This booklet purports to be a monograph on the Bio-technology and Applications of the blue-green alga *Sprirulina platensis.* The author in his introduction states that the current knowledge on the subject is detailed therein with emphasis on the work carried out at the CFTRI. Howevei, the emphasis is almost wholly on the work done at CFTRI and hence it reads more like a project report than a monograph, down to the statement of experimental results in the past tense.

The author has summarized the results of nearly ten years of his research on this valuable alga, under the sections Cultivation, Outdoor cultivation, Processing, Algal Productivity Measurements, Yield, Chemical Composition, Contamination, Nutritional Studies, Metabolic Studies, Supplementation Studies, Toxicological Studies, Animal Feed and Therapeutical uses. He then lists, the Centres engaged in simailar work within and outside India—with brief comments on the nature of work—and to detail the potentialities and possible application of this alga.

The studies described are exhaustive and have yielded valuable information on various aspects of the biotechnology of mass production of this alga for better yield, while at the same time cutting down the cost of production by the use of cheap and locally available substitutes for raw materials. The author's efforts at cutting down on the energy input for the operations involved, are most commendable.

According to the author, the most important application of this work would be in environmental control-specifically, sewage and waste disposal, with the incidental production of a valuable animal feed supplement that is nutritious and without toxic effects.

On the whole, this would be a valuable reference work for those interested in environmental control and biomass production. As such, this book would merit wider reading if presented in a more readable form. The microscopic offset printing, though very well executed, is the main deterrent. The matter could have been presented under fewer headings, with a wider general coverage of each topic and suitable numbering. The references may also be cited in the text so as to enable readers to seek further information on specific topics, instead of merely being listed at the end. The animal studies could be supplemented with photographs for direct evidence.

The illustrations leave something to be desired. The taxonomic account under the head 'The alga' is inadequate, photographic reproduction is rather poor, the cartoons are out of place in a scientific work and the poem that follows 'Acknowledgements' is clumsy; in fact these detract from the merit of the work that is presented.

I. K.
UNIVERSITY OF MADRAS, MADRAS

Dietary Fibre: By G. G. Birch and K. J. Parker (Eds), Applied Science Publishers, London and New York, 1983; Pp: 304; Price: £ 28.00

This book comprises of sixteen papers presented at the 13th Annual International Symposium held at the University of Reading, National College of Food Technology, Weybridge, Surrey from 29th to 31st March 1982, under the auspices of a joint industry-University Organising Committee. Each paper is dealt with a comprehensive and up-to-date review of an aspect of dietary fibre by an expert in the field.

The role of dietary fibre is traced in this book, starting from the testing of the hypothesis that dietary fibre is a protective factor against many diseases prevalent amongst Western Communities. Recent advances on the chemical characterisation of fibre components of different foods-including heat treated products-and the performance and application of different analy tical procedures is presented in one chapter. The interactions between dietary fibre and nutrients influencing bioavailability has been discussed in depth with recent findings. The role of fibre in the aetiology and management of clinical disorders such as diabetes mellitus and intestinal diseases is a very useful chapter. Nutritional importance of gut micro flora, the influence of fibre on xenobiotic metabolism by gut bacteria and fibre and chronic intestinal diseases are dealt elaborately in three separate chapters. Each chapter carries a list of up-to-date and relevant references for further study, illustrations and tables with data. The book as a whole is directed to the food technologists, but will also be of value to nutritionists and physicians. This volume would be a welcome addition in all libraries.

S. PREAMKUMARI
SRI AVINASHILINGAM HOME SCIENCE COLLEGE,
COIMBATORE

Manganese: Environmental Health Criteria 17: published under the joint sponsorship of the United Nations Environment programme, the International Labour organization and the World Health Organization; World Health Organization, Geneva, 1981; Pp 110.

The document is one among the seventeen documents published so far in the Environmental Health Critena series and has been prepared by a WHO Task group. Manganese is an essential trace element for both animals and man. The daily requirement for adults is 2-3 mg/day and that of pre-adolescent children, atleast 1.25 mg/day. It is necessary for the formation of connective tissue and bone, and for growth, carbohydrates and lipid metabolisim, the embryonic development of the inner ear, and reproductive function.

Higher levels of manganese is toxic to man. It is characterized by psychological and neurological manifestations. Chronic manganese poisoning which is a hazard in the mining and processing of manganese ores, in the manganese alloy and dry-cell battery industries, and in welding, leads to lesions of the central nervous system. Organomanganese compounds which are used in fungicide in edible crops, and manganese tricarbonyl compounds which are used as additions to gasoline are also potential sources of manganese toxicity.

The document consists of ten chapters dealing with properties and analytical methods, sources of manganese in the environment, environmental levels and exposure, transport and distrubution in environmental media, metabolism of manganese and its deficiency, experimental studies on the effect of manganese, human epidemological and clinical studies, and evaluation of the health risks to man from exposure to manganese and its compounds. Each chapter gives a comprehensive account of the known knowledge on the particular aspects. The first chapter gives summary and recommendations for further studies since there are many "grey" areas in our knowledge especially on metabolism and epidemiology. The document contains 407 references, and is based on original publications listed in the reference section. In addition useful information has also been obtained from authoritative reviews.

In view of the diverse areas covered in the document research workers in a variety of disciplines will find it useful. It should specially interest those working in analytical chemistry, biochemistry, nutrition and toxicology, medicine, environmental pollution control etc.

M. S. NARASINGA RAO
C.F.T.R.I., MYSORE

Advances in Biochemical Engineering: Space and Terrestrial Biotechnology, Vol 22: by A Fiechter, Spring-Verlog, Berlin, Heidelberg, New York, 1982, Pp 230; Price: Unpriced

The twentysecond volume in this series is devoted to reviews of recent advances in space and terrestrial biotechnology. The present volume covers the following chapters: Biotechnology in space technology; basic concepts in microbial aerosols and characterization and performance of single and multistage tower reactors without loop for cell mass production.

The first chapter deals with biotechnology in space technology. With the advent of the space shuttle and of the space lab, we are at the begining of a new era in space technology. Material sciences and bioprocessing are becoming new importanct disciplines in space sciences. This review discusses mainly experiments and technology dedicated to the study of the behaviour in space of microorganisms and animal cells cultured *in vitro*. The effect of stress of space flight on the organisms and the effect of oxgen on the biological system is investigated. The survival and proliferation of mammliam cells in altered gravitational fields is a challenging aspect of space technology. Biostach, one of the first sophisticated experimental devices carried out in space, studied the effect of cosmic radiation on a variety of biological objects: microorganisms, eggs and plants. The effect of space-flight on human embryonic lung cells WI-38, studied during the 56-day Skylab-3 mission, indicated that cells growing in suspension, and the possibility to induce them to differentiate, are better objects for investigation in space.

One of the most important achievements of Soviet activities in space biotechnology was the finding that artificial gravity restores favourable conditions in space for the adoption at development and survival of higher plants and animals. However, artificial gravity has never been tested on humans. Specelab is a manned orbital laboratory providing a pressurized module and an unpressurized platform with a number of standard facilities. In contrast to its predecessors skylab and Salyut, it is a reusable system. The life sciences projects of Spacelab-I include physiological investigations on humans and biological experiments on plants, microorganisms and cells *in vitro*. Only a great multinational and multi-disciplinary effort can guarantee an optimum and fruitful exploitation of the space resources.

The second chapter contains information pertaining to bacterial aerosol viability and electrostatic surface charges of bacteria. The variables involved in the survival of bacterial aerosols include the method of droplet formation and intrinsic characteristics of gas

in which the bacteria containing droplets are suspended. The factors involved in microbial aerosol studies are bacterial strain, incubation medium, temperature cellular physiology, oxygen concentration, light and irradiation and ion transport disruption. The review also contains information on overall microbial aerosol assay techniques.

There are many stresses acting on the viability of aerosolized microbes which may act collectively or individually depending on the environmental conditions. A small part of the study deals with survival of mycoplasma, L-forms and fungal spores in aerosoles.

The third chapter reviews the bench-scale towerloop reactors under different operational conditions. Stirred tank reactors are the most important reactor types employed in biotechnology. But other types of reactors are also developed for special manufacturing processes, i.e., large-scale single-cell protein, plant and animal tissue culture production. Comparison of different systems have been carried out using the same biological sytem, i.e., strain, medium, growth conditions, etc. As biological system is complex in nature and numerous parameters effect the product formation, it is a difficult task to characterize and predict the performance of towerloop reactors. The performance of various types of reactors is supported with mathematical derivations and adequately illustrated with figures.

This is a very valuable compilation of review articles written by the renowned scientists in their respective fields. With the successful flights of space shuttle and spacelab, it has opened new vistas in space biotechnology. The exploitation of space resources and the establishment of space colonies is becoming a realistic goal of the next decades. This book will be a valuable addition to the libraries.

M. S. KALRA &
AJITH SINGH
PUNJAB AGRICULTURAL UNIVERSITY, LUDHIANA

Advances in Biochemical Engineering Vol. 25, Chromatography: Fiechter, A. (Ed.), Springer-Verlag, Berlin, Heidelberg, New York, 1982; Pp.145; Price: $ 27.20.

This volume has '*Chromatography*' as its subject of review, which is one of the most important analytical techniques used for analyses in almost all branches of science based on chemical and biochemical technology. The first one of the four articles, contributed by Yang and Tsao, deals with adsorption theorles and their application to affinity chromatography. They have reviewed the basic theories of chromatography and their application to affinity chromatography. The theories dealt are:—The plate theory first introduced by Martin and Synge, the rate theories of chromatography, the rate theories of frontal analysis and the rate theory of elution development. The authors conclude that very little theoritical work has been done on affinity chromatography which is attributed to the difficulty encountered in the mathematical treatment. The second chapter written by the same authors pertains to the general characteristics, principles and application aspects of affinity chromatography. Five desirable qualities of solid matrix supports are enumerated first. Then the authors discuss characteri stics of the commonly used solid matrix materials, namely, cellulose, agarose, dextran and polyacrylamide. Recent developments in the field of affinity chromatography like *high performance liquid affinity chromatography* (HPLAC) and *magnetic affinity chromatography*, are also discussed. Other areas covered in this articles are: chemistry of adsorbent preparation and its characteristics, the attached ligand, the spaces-arm-(leash) structure, interfaces in affinity chromatography, and significance of gel-capacity and equilibrium constants.

Third chapter of the book refers to 'Large scale chromatography of proteins', wherein the basic theory of chromatography and its implication in scaling up are discussed in detail After dealing with the advantages and disadvantages of different column designs, the authors compare the properties of various column materials and also describe the procedures for their maintenance. Engineering aspects of the construction of large-scale columns, the operational behaviour of bed packings and the design of column end pieces are treated exhaustively. The choice of equipment and fabrication of the column automation of chromatographic processes are also mentioned. Industrial applications of gel filtration, ion exchange chromatography and affinity chromatography with particular reference to some pharmaceutical products are reported.

The last chapter of this book reports the survey on Cibacron Blue F3G-A and related dyes used as ligends in affinity chromatography. After a brief introduction, the authors discuss the historical aspects of the application of Blue Dextran in affinity chromatography. Chemical structures of Cibacron-Blue F3G-A and related dyes, nature of the dye-protein interactions, procedures for dye immobilization and application of the technique in the purification of proteins are discussed in detail. A summary of the experimental conditions adopted by various workers for a successful purification of enzymes, application of Cibacron Blue F3G-A and related dyes for the purification of plasma

proteins are presented in tabular forms. The authors finally project the outlook for the use of dyes as affinity adsorbents for isolation and purification of enzymes and other proteins.

This volume contains useful reviews on affinity chromatography and therefore, will be a useful reference book for the researchers in the field.

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

Biotechnology: International Trends and Perspectives: Alan T. Bull, Geoffrey Holt Malcolm D. Lilly, Oxford & IBH Publishing Co, New Delhi-110 001, 1983; pp 84; Price: Rs. 30.

This booklet published originally by the organisation for Economic Co-operation and Development (OECD) deals with the current knowledge in the field of biotechnology emphasizing important trends and issues. Both in the forward and in the introduction, importance of the definition and the activities covered in the area of Biotechnology have been brought out. Conclusions and recommendations of the experts who met at the OECD meeting on 17th and 18th March 1982 are presented prior to presentation of actual papers. These recommendations reveal the present status of biotechnology and also suggest the priorities that have to be provided for its development. They are: 1. The need for common definition, 2. R and D priori ies, 3. Training, 4. Industry-University links, 5. Culture collection and data banks, 6. Economic conditions of biotechnology, 7. Economic impacts of biotechnology, 8. Patents and 9. Safety regulations.

The main report consists of three chapters, the first of which is titled, "Potential of Contributing Sciences and Technologies to Biotechnology". Topics covered in this chapter include Microbiology and Biochemistry, wherein the importance of organisms, physiology and chemical activities are discussed. The other aspects presented in this chapter are genetic manipulation and engineering.

In the second chapter, some major scientific and technological resource constraints in the development of biotechnology, have been enumerated. First, the authors have dealt with raw material-both energy and fermentation feed stocks. Importance of basing them on renewable sources is emphasized. Problems peculiar to developing countries have been identified and included in this chapter. Role of water and problems associated with its utility both during fermentation and in product recovery have been well outlined. Importance of product recovery, strain improvement and extending the use of biocatylists have also received attention. The last chapter is assigned to the issues concerning the developments in Biotechnology. The impact of governmental policies on R and D, education and manpower, finance and business aspects, safety regulations and patents on the development of biotechnology have been well presented. The book also contains a number of appendices which high light the present status of biotechnology in several countries and examples of applications of genetic manipulation in biotechnology. Important publications relevent to the subjects in the field are included in the bibliography.

This book is a very useful document for all the Institutions engaged in R and D activities and who contemplate planning research in Biotechnology.

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

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PUBLICATIONS

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Vol. 21 No. 1 **Contents of forthcoming issue** **Jan./Feb. 1984**

Research Papers

Research Notes

Printed and Published by Dr. S.C. Basappa, Secretary, AFST (India), CFTRI, Mysore-570013, at Sharada Press, Mangalore-575001.

INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (*in triplicate*) should be typewritten in double space on one side of bond paper. The manuscripts should be complete and in final form, since only minor corrections are allowed at the proof stage. The paper submitted should not have been published or data communicated for publication anywhere else. Review papers are also accepted.

2. Short communications in the nature of Research Notes should clearly indicate the scope of the investigation and the salient features of the results.

3. Names of chemical compounds and not their formulae should be used in the text. Superscripts and subscripts should be legibly and carefully placed. Foot notes especially for text should be avoided as far as possible.

4. **Abstract:** The abstract should indicate the principal findings of the paper. It should be about 200 words. It should be in such a form that abstracting periodicals can readily use it.

5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. They should be typed on separate paper. Nil results should be indicated and distinguished clearly from absence of data, which is indicated by '—' sign. Tables should not have more than nine columns.

6. **Illustrations:** Graphs and other line drawings should be drawn in *Indian ink* on tracing paper or white drawing paper preferably art paper. The lettering should be in double the size of printed letters. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size 16cms (ox axis) $\times$ 20cms (oy axis); photographs must be on glossy paper and must have good contrast; *three copies* should be sent.

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   (*e*) *Thesis:* Sathyanarayan, Y., Phytosociological Studies on the Calcicolous Plants of Bombay, 1953, Ph.D. Thesis, Bombay University.

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