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Studies on Packaging and Storage of North Indian Spiced Papads Made from Blends of Different *Dhals* (Split Pulses)

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Packaging and storage studies were conducted on North Indian spiced papads prepared from different blends of blackgram (*Phaseolus mungo* L.), Bengalgram (*Cicer arietinum* L.), lentils (*Lens culinaris* Medicu), red gram (*Cajanus cajan* L.), and greengram (*Phaseolus aureus* Roxb). Equilibrium relative humidity (ERH) of these papads (initial moisture 13.1-13.6%) ranged from 58.2 to 58.6%. Moisture, pH and alkalinity decreased gradually during 4 months storage under ambient conditions. Sensory quality of fried papads remained acceptable upto 4 months storage. The quality of papads made from blends containing Bengalgram or redgram was as good as that prepared from blackgram. However, the colour deteriorated slightly in papads prepared from blends containing lentils.

Papads are important savoury foods widely consumed in India. They are usually made from blends of pulse and cereal flour, with common salt, spices, edible oil, alkaline and mucilagenous additives.

Literature as well as market survey[1] revealed that the spiced papads are usually prepared from a blend of blackgram, greengram, corn flour and gelatinised starch[2] with salt and spices. The recipe varies from region to region. Mould growth was observed in the commercial papads at about 18 per cent moisture level[3]. Moisture sorption studies of blackgram papads and packaging requirements were reported by Balasubrahmanyam, *et al*[4]. Balasubrahmanyam *et al.*,[5] studied the factors responsible for changes in colour and pH of papads during storage under accelerated conditions. Use of fumigants in the prevention of insect infestation and mould growth in South Indian papads was reported by Narasimhan *et al.*[6] Saxena, *et al.*,[7] have explored the feasibility of using different dhal flours instead of blackgram flour in the preparation of North Indian spiced papads. This paper discusses the sorption isotherms, effect of storage, packaging requirement and quality evaluation of papads prepared from various blends of Bengal gram, blackgram, greengram, redgram and lentils.

Materials and Methods

Preparation of papads: Papads were prepared from blends of blackgram: Bengalgram (70:30); blackgram:lentil (80:20); blackgram:redgram (80:20); blackgram:greengram:lentil (60:25:15) and blackgram alone (control) with added spices and common salt. The papad doughs were made according to the standard procedure[8]. Papads were rolled into thin circular discs of about 0.6-0.85 mm thickness and 20-30 cm diameter using a wooden rolling pin. The adhering flour on the papads was brushed off. They were sun-dried to 13-14 per cent moisture content. About 250 g of papads were packed in (i) 100 gauge low density polyethylene (LDPE), (ii) 200 gauge low density polyethylene (LDPE), and (iii) 120 gauge polypropylene (PP) (flexible film) bags of size 30 × 25 cm. Six replicates of each sample were packed, heat sealed and exposed to the ambient conditions (13-42°C temperature and relative humidity of 42-86 per cent) for subsequent evaluation during storage.

Packaging: In order to ascertain the packaging requirement of papads, equilibrium RH studies were conducted at room temperature (30±5°C)[9]. Known weights of the samples were exposed to different relative humidities ranging from 11 to 92 per cent. Equilibrium moisture contents (EMC) of these samples were estimated when there was no further loss or gain in weight. Adverse effects such as loss of colour, texture, acceptability and mould growth appearance were recorded for different blends (Table 1). The critical moisture content for each of these blends is indicated in the graphical relationship of EMC and per cent RH (Fig.1). ERH of the sample was also determined by plotting graph of percentage loss or gain in weights against number of days stored.

Changes in proximate composition of papads during storage: Composite samples of freshly prepared papads as well as those stored were analysed for moisture, pH and alkalinity of ash using standard ISI

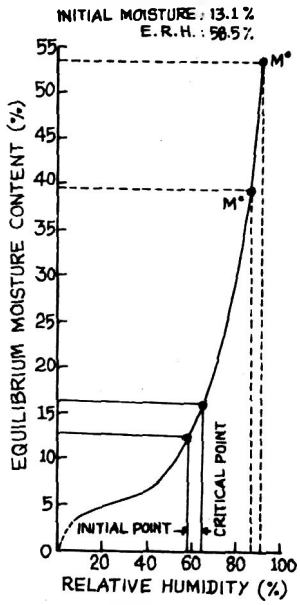

Fig. 1.. Humidity moisture equilibrium curve for spiced 'Papads' prepared from the blends of blackgram and Bengalgram flours (70:30)

methods[10] (Table 2). Samples were drawn periodically at 0, 1, 2, 3 and 4 months of storage.

Quality characteristics of raw/fried papads: Raw/fried papad samples were evaluated for quality parameters like visual colour, appearance, texture and taste/flavour by a panel of judges using the method described by Govindarajan *et al.*[11] (Tables 3 and 4).

Results and Discussion

Sorption behaviour of different blends of spiced papads: The sorption behaviour of control and experimental spiced papads is presented in Table 1. The ERH of spiced papads prepared from blackgram (control) and from blend of blackgram:Bengalgram (initial moisture content of 13.1 per cent in either) were 58.0 and 58.5 per cent respectively. The product remained good with respect to colour, texture and overall acceptability upto 63 per cent RH but became brittle below 52 per cent RH. When exposed to 75 per cent RH, the product attained EMC value of 24.8

TABLE 1. RELATIONSHIP BETWEEN RELATIVE HUMIDITY (RH), EQUILIBRIUM MOISTURE CONTENT (EMC) AND NUMBER OF DAYS TO ATTAIN EQUILIBRIUM FOR SPICED PAPADS

RH %	EMC	Days to attain equilibrium	Quality characteristics		
			Colour	Texture	Acceptability
		Blackgram:Bengalgram (70:30), (Initial moisture 13.1%)			
52	9.0	7	Pale yellow	Very brittle	Not acceptable
63	15.7	7	"	Pliable	Acceptable
75	24.8	11	Light brown	Very soft	Not acceptable
86	39.1*	10	—	—	—
		Blackgram:Lentil (80:20), (Initial moisture 13.5%)			
52	9.0	9	Pale yellow with brown tinge	Very brittle	Not acceptable
63	15.6	7	"	Pliable	Acceptable
75	23.9	12	Light brown	Very soft	Not acceptable
86	40.1*	9	—	—	—
		Blackgram:Redgram (80:20), (Initial moisture 13.6%)			
52	9.1	10	Creamish yellow	Very brittle	Not acceptable
63	15.8	6	"	Pliable	Acceptable
75	22.9	11	Light brown	Very soft	Not acceptable
86	40.2*	9	—	—	—
		Blackgram:Greengram:Lentil (65:25:15), (Initial moisture 13.2%)			
52	8.9	4	Pale yellow	Very brittle	Not acceptable
63	15.9	9	"	Pliable	Acceptable
75	22.6	12	Light brown	Very soft	Not acceptable
86	39.1*	9	—	—	—
		Blackgram Alone (Control), (Initial moisture 13.1%)			
52	9.0	10	Pale yellow	Very brittle	Not acceptable
63	16.1	7	"	Pliable	Acceptable
75	23.1	10	Turned light brown	Very soft	Not acceptable
86	40.1*	9	—	—	—

*Mould appearance

per cent, became very soft and hence unacceptable. Papads of control sample and that from blend of blackgram:Bengalgram developed mould at moisture levels between 39.1 and 53.6 per cent (RH 86-92 per cent). The critical moisture contents for control and experimental spiced papads were found to be 16.1 and 15.7 per cent respectively.

However, there was no significant change in ERH of the papads prepared from the blends of blackgram: lentil; blackgram:redgram and blackgram:greengram: lentil. All papads were brittle and not acceptable when stored below 52 per cent RH. The products remained good at 63 per cent RH and moisture levels of 15.6, 15.8 and 15.9 per cent respectively. The critical moisture contents for these products were 15.6, 15.8 and 15.9 per cent.

Changes in proximate chemical composition of papads during storage: Data presented in Table 2 reveal that the moisture content of papads packed in 100 and 200 gauge LDPE and 120 gauge PP bags increased for the first two months of storage and then decreased. This may be due to variation in atmospheric relative humidity which ranged from 42 to 86 per cent during the storage period. Greater fluctuations in moisture loss or gain were noticed in 100 gauge LDPE bags probably because of higher water vapour transmission rate compared to other packaging materials used. There was a gradual decrease in alkalinity and pH of the papads in all the packaging materials during four months storage at room temperature (13°-42°C). The corresponding values of moisture, pH and alkalinity in papads for the storage periods of 1 and 3 months did not differ much from values for those papads stored for 2 and 4 months respectively. All the packages were free from insect and mould infestation throughout the storage period.

The 200 gauge LDPE and 120 gauge PP bags were considered superior to 100 gauge LDPE bags with respect to the extent of loss of moisture from papads during storage. Loss of moisture from different varieties of papads packed in 120 gauge PP bags did not differ much.

Quality characteristics of raw and fried papads: The sensory quality for individual characteristics viz., colour, appearance, texture, flavour and overall quality of raw papads as well as those of deep-fat-fried papads are presented in Tables 3 and 4. The papads prepared from the different blends of blackgram and Bengalgram flour; blackgram and redgram flour; blackgram and lentil; blackgram, greengram and lentil

TABLE 2. CHANGES IN MOISTURE, pH AND ALKALINITY OF PAPADS DURING TWO AND FOUR MONTH STORAGE AT ROOM TEMPERATURE (13-42°C)

Package (gauge)	Moisture (%)		pH		Alkalinity (%)	
	2	4	2	4	2	4
Blackgram:Bengalgram (70:30)	(13.1)		(8.0)		(2.1)	
100 LDPE	14.0	11.6	7.6	7.3	1.9	1.7
200 LDPE	13.6	12.8	7.6	7.4	1.9	1.8
120 PP	13.5	12.6	7.7	7.4	2.0	1.9
Blackgram:Lentil (80:20)	(13.5)		(7.8)		(2.1)	
100 LDPE	14.1	11.9	7.7	7.2	1.9	1.8
200 LDPE	13.9	12.6	7.7	7.0	1.9	1.8
120 PP	13.7	12.4	7.7	7.3	2.0	1.8
Blackgram:Redgram (80:20)	(13.6)		(8.1)		(2.2)	
100 LDPE	13.9	11.0	7.7	7.5	1.9	1.9
200 LDPE	13.8	12.6	7.9	7.6	2.0	1.8
120 PP	13.7	12.9	8.0	7.8	2.0	1.9
Blackgram:Greengram (65:25:15)	(13.2)		(8.0)		(2.0)	
100 LDPE	13.8	11.5	7.6	7.2	1.8	1.7
200 LDPE	13.6	12.8	7.6	7.4	1.8	1.7
120 PP	13.4	12.8	7.6	7.5	1.9	1.9
Blackgram alone (control)	(13.1)		(8.0)		(2.1)	
100 LDPE	13.9	11.6	7.5	7.2	1.8	1.7
200 LDPE	13.5	12.8	7.9	7.3	1.8	1.8
120 PP	13.4	12.9	7.9	7.5	2.0	1.9

LDPE – Low density polyethylene; PP – Polypropylene; All values are means from two replicates
Figures in parenthesis are the initial values

TABLE 3. QUALITY CHARACTERISTICS OF RAW SPICED PAPADS DURING TWO AND FOUR MONTH STORAGE AT R.T. (13-42°C)

Package (gauge)	Colour (max. 40)		Appearance (max. 60)		Overall quality score (max.100)	
	2	4	2	4	2	4
Blackgram:Bengalgram (70:30)	(35.0)		(50.0)		(85.0)	
100 LDPE	28.0	20.0	32.0	21.0	60.0	41.0
200 LDPE	28.0	22.0	35.2	24.0	63.2	46.0
120 PP	28.0	22.0	34.8	23.9	62.8	45.9
Blackgram:Lentil (80:20)	(28.0)		(42.0)		(70.0)	
100 LDPE	22.0	16.0	30.0	20.0	52.0	36.0
200 LDPE	23.2	16.2	32.0	21.2	55.2	37.4
120 PP	22.8	16.3	33.2	24.3	56.0	40.6
Blackgram:Redgram (80:20)	(34.0)		(48.0)		(82.0)	
100 LDPE	30.0	18.0	36.1	22.0	66.1	40.0
200 LDPE	31.8	19.0	36.4	23.6	68.2	42.6
120 PP	32.0	18.0	36.0	24.5	68.0	42.5
Blackgram:Greengram (65:25:15)	(28.0)		(42.0)		(70.0)	
100 LDPE	23.0	15.0	30.0	18.0	53.0	33.0
200 LDPE	24.0	17.0	32.0	20.0	56.0	37.0
120 PP	24.2	16.0	31.0	20.0	55.2	36.0
Blackgram alone (control)	(36.0)		(54.0)		(90.0)	
100 LDPE	28.1	18.3	49.8	25.0	77.9	43.3
200 LDPE	30.0	24.6	50.0	28.0	80.0	52.6
120 PP	30.2	25.2	51.6	30.0	81.8	55.2

LDPE – Low density polyethylene; PP – Polyproplene
Figures in parenthesis are the initial values

TABLE 4. ORGANOLEPTIC QUALITY OF DEEP-FAT-FRIED PAPADS DURING TWO AND FOUR MONTH STORAGE AT R.T. (13-42°C)

Package (gauge)	Colour (max. 10)		Texture (max. 15)		Flavour (max.60)	
	2	4	2	4	2	4
Blackgram:Bengalgram (70:30)	(9.0)		(13.0)		(54.0)	
100 LDPE	8.0	6.0	12.0	7.0	44.0	33.2
200 LDPE	8.0	6.2	12.0	8.0	44.0	35.6
120 PP	8.0	6.4	12.0	8.2	44.0	36.4
Blackgram:Lentil (80:20)	(8.0)		(13.0)		(42.0)	
100 LDPE	6.9	5.0	11.2	9.3	35.0	28.0
200 LDPE	7.0	5.2	11.8	11.1	36.0	29.5
120 PP	7.0	5.4	11.9	10.0	36.0	30.9
Blackgram:Redgram (80:20)	(9.0)		(13.0)		(50.0)	
100 LDPE	8.0	6.1	11.2	8.0	45.0	33.6
200 LDPE	8.0	6.3	12.1	7.9	46.2	34.2
120 PP	8.0	6.2	12.3	8.2	46.5	35.0
Blackgram:Greengram (60:25:15)	(8.00)		(11.0)		(44.0)	
100 LDPE	6.5	5.0	11.0	7.0	38.0	28.0
200 LDPE	7.0	5.0	12.5	7.2	38.3	29.3
120 PP	7.0	5.1	12.0	7.0	40.3	30.0
Blackgram alone (control)	(10.0)		(13.0)		(54.0)	
100 LDPE	8.0	7.0	12.2	10.0	46.0	36.5
200 LDPE	8.0	7.0	12.2	10.5	46.0	36.8
120 PP	8.0	7.0	12.6	11.0	46.0	37.0

LDPE – Low density polyethylene; PP – Polypropeylene
Figures in parenthesis are the initial values

flours were quite acceptable upto a storage period of 4 months at room temperature when packed in 100 and 200 gauge polyethylene and 120 gauge polypropylene bags. It was observed that papads prepared from these blends were bright in colour, and fairly uniform in shape and size. Raw papads from these blends were also flexible/pliable for safe handling and those prepared from blackgram and Bengalgram flours and blackgram and redgram flours were adjudged to be of quite high grade with respect to colour, appearance and overall quality characteristics (Table 3). Papads prepared from blends containing lentils showed relatively greater deterioration in colour and appearance during storage.

Fried papads prepared from various blends gave characteristic flavour of the raw materials used and had pleasant after-taste when compared with controls. After 2 months storage, papads gave products of acceptable colour (straw yellow with brownish tinge) upon frying. But after 4th month of storage, the colour of papads from blends containing lentils was not satisfactory while those from blends containing Bengalgram and redgram were of satisfactory colour (Table 4). The overall score of acceptability of papads made from these blends ranged from 47.2 to 60.3 per cent as compared to 63.5 per cent for control after 4 months storage. The minimum overall score of acceptability was taken as 40-60 per cent.

It may be concluded that the quality of papads made from blends containing Bengalgram or redgram was comparable to those made from blackgram. Papads remained quite acceptable in 200 gauge LDPE and 120 gauge PP bags during 4 months storage without much change in their quality.

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Application of Osmotic Technique in Plum Wine Fermentation: Effect on Physico-chemical and Sensory Qualities

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Osmotic treatment of plum fruits for wine fermentation was evaluated. Lye and water blanching as pre-treatments followed by osmosis were found to be quite effective in increasing the total soluble solids and decreasing the acidity of the fruits. But the treatments also resulted in some loss of anthocyanin and mineral contents. There were not much differences in rates of fermentation and the alcoholic contents of the wines. Compared to the untreated fruits, wines of acceptable acidities were produced from the musts of the pre-treated fruits. There was considerable improvement in various sensory quality attributes of wine obtained from the fruits pre-treated with water blanching followed by osmosis as compared to other treatments.

At present, plenty of plum (*Prunus salicina* L) fruits are grown in H.P. and these contribute about 60 per of the total stone fruits production of the state. Being a highly perishable crop, it calls for quick disposal/ immediate processing into suitable products including alcoholic beverages. These beverages have been prepared and consumed by man since times immemorial[1]. Like other fruits, plum can also be made into wine and methods for its preparation have also been investigated and reported elsewhere[2,3]. But a product of consistently high quality could not be produced due to high acid content, improper balance of tannins, astringency and lack of fruity flavour. The 'Osmosis Technique' – in which the fruits are submerged in a hypertonic solution of sugar, at a specific temperature and for a definite time[4], is known to remove water from the fruits along with some acid, thereby, concentrating the sugar and flavour in the treated fruits[5]. But, there is no report on the effect of this treatment on the physico-chemical and sensory qualities of wines. However, for reducing the acid contents of orange juice, a similar process called 'Electrodialysis' has been successfully employed[6]. The technique of 'Osmosis' as one of the approaches was applied after some pre-treatments of the fruits, to study its effect on physico-chemical and sensory characteristics of wines to be produced. The results obtained are reported in this paper.

Materials and Methods

Plum fruits of variety 'Santa Rosa' were procured from the orchard of the Department of Horticulture (PCDO Rajpura, Dist. Chamba, Himachal Pradesh). The fruits were harvested just before ripeness since over ripe fruits lose the semi-permeability properties[7]. The fruits were given the following treatments:

1) No treatment-control (PW_1), 2) Dipping of fruits in hypertonic solution of 70% sugar for 4 hr at 50°C – without pre-treatment (PW_2). 3) Blanching in hot 1.5 per cent lye solution (NaOH) for 30 sec followed by osmosis as above (PW_3), and 4) Blanching in plain boiling water for one min followed by osmosis (PW_4).

After the lye treatment, the fruits were washed in dilute acidic water to neutralise the adhering lye before submerging in hypertonic solution. The ratio of fruit to the solution was kept at 1:3. After the osmotic treatment, the fruits were taken out, drained till the surface of the fruits was free of any adhering syrup. The juice from the treated fruits was extracted, after destoning, with a hand operated juice extractor. The fruits as well as the juices were analysed for total soluble solids (TSS), acidity, pH, minerals, anthocyanin, etc. as per the standard methods[8,9].

For the preparation of musts, to every 1300 ml of juice obtained, 500 ml of water and enough sugar were added to raise the total soluble solids to 24°B, wherever necessary. Analysis of the musts for various characteristics was made according to the methods recommended[9,10]. Fermentations were carried out in glass containers at 24±1°C by a pure 24 hr old culture of *Saccharomyces cerevisiae* var. *ellipsoideus* added to

the musts at the rate of 5 per cent. The fermentation was carried out to dryness and the reduction in °B was recorded for 10 days. The rates of fermentation (Fall °B/24 hr) were reported. After the completion of fermentation, the wines were siphoned, racked and filtered. These were, then, ameliorated to a TSS. of 10°B (direct refractometer reading) with sugar syrup. The wines were bottled and pasteurized at 62.5°C for 15 min. Physico-chemical analysis of wines for various attributes was carried out according to the methods recommended[10,11]. The sensory evaluation of wines was carried out after six months of storage in bottles by a panel of semi-trained judges. Samples were presented in coded form and on the the proforma with weightage to the characteristics/attributes given. Scores of the judges and their remarks were recorded.

Results and Discussion

Physico-chemical characteristics of the juices, obtained after different treatments, is presented in Table 1. It is evident from the data that highest decrease in acidity and increase in TSS. have occurred in the juices with water blanching, as a pre-treatment (PW-4), closely followed by the lye pre-treatment (PW_3). The small changes in TSS. and acidity in PW-2 were observed as compared to PW-3 and PW-4 treatments. This may be due to the presence of wax layer which acts as a barrier to the movement of water externally and conserving it internally[12]. The marked changes in these characteristics in PW-3 and PW-4 could be attributed to the effects of lye and water blanching treatments which are known to disrupt or alter the wax layer of plums leading to the increased rates of osmosis and thereby, causing an increase of total soluble solids and a decrease in the acid contents[7]. However, increase in acidity of PW-2 could be due to the fact that since no treatment was given in this case and the skin did not alter enough to permit acid molecules to leach out along with water and the acid got concentrated[12].

TABLE 1. CHARACTERISTICS OF TREATED PLUM FRUITS, THEIR MUSTS AND THE FERMENTATION RATES

Characteristics	PW-1	PW-2	PW-3	PW-4
Fruits				
T.S.S. (°Brix)	10.00	15.0	30.0	32.50
Acidity (% malic acid)	1.78	2.24	0.88	0.82
pH	3.17	2.92	3.43	3.50
Anthocyanin (mg/100 g)	5.35	5.47	1.27	3.18
Nitrogen (p.p.m.)	840	840	56	56
Phosphorus (p.p.m.)	177	155	100	90
Potassium (p.p.m.)	1700	1550	570	570
Plum Musts				
T.S.S. (°B)	6.00*	10.00*	20.00*	24.00
Acidity (% malic acid)	1.50	1.33	0.71	0.71
pH	3.03	2.89	3.00	2.99
Rate of fermentation				
(°B/24 hr)	1.60	1.40	1.60	1.50

*°B raised to 24.0, Values are means of 3 replications.

Lye and water blanching as pre-treatments affected the anthocyanin and mineral contents of the fruits compared to the fruits which were not given any pre-treatments (Table 2). As regards anthocyanin contents, the decrease is more in case of lye blanched plum fruits (PW-3) than the water blanched ones (PW-4). It may be due to the action of lye on fruit pigments and waxy layer, making it more susceptible to leaching of pigments as well as minerals (Table 1). Analysis of the musts (Table 1) showed that the desired decrease in acidities could be achieved in PW-3 and PW-4 compared to other treatments. Hence, pre-treatments coupled with osmosis could successfully be used for lowering the acid content of the high acid fruits like plums for the production of wines. However, between the two, water blanching seems to have an edge over lye blanching because of retention of more of anthocyanins responsible for colour. Due to the concentration

TABLE 2. PHYSICO-CHEMICAL ANALYSIS OF WINES FROM DIFFERENT TREATMENTS

Treatments	pH	Acidity (% malic acid)	Alcohol (% v/v)	Colour value		Volatile acid (% A.A)	Colour* (mg/100 ml)
				A	B		
PW-1	3.51	1.05	11.45	1.49	1.66	0.011	186
PW-2	3.39	1.21	11.90	1.32	1.44	0.008	159
PW-3	3.56	0.61	11.20	0.99	1.35	0.011	93
PW-4	3.49	0.62	11.46	1.06	1.12	0.011	106

A = Sum of O.D. at 420 nm and 535 nm
B = Ratio of O.D. 420 nm/O.D. 535 nm
T.S.S.°B = 10.0 in all the treatments
*Includes total colouring matter and tannins.
A.A: Acetic acid

TABLE 3. SENSORY EVALUATION OF PLUM WINES FROM DIFFERENT TREATMENTS

Treatments	Colour/ appearance	Acid/sugar/ Alcohol blend	Astringency	Flavour	Overall score	Remarks
PW-1	4.0	2.3	2.6	3.8	12.7	Good colour, more bitter and acidic taste.
PW-2	4.2	2.6	2.8	3.7	13.3	Good pink colour, more bitter and acidic taste.
PW-3	2.1	4.4	4.2	4.2	14.9	Colour, pale, good acid/sugar/ alcohol taste and less bitter.
PW-4	2.8	4.6	4.4	4.2	16.0	Colour, light pink, good acid/sugar/ alcohol taste and desirable bitterness

Max. score is 5 for each aspect. Average score of 5 judges for 3 sittings.

of nutrients reportedly taking place in osmotic dehydration[4] it was expected that fermentation could be quite faster in case of PW-3 and PW-4, yet the results (Table 1) show that all the musts fermented at almost the same rate.

The results of chemical and sensory evaluation of the wines are presented in Tables 2 and 3 respectively. The data (Table 2) show that there were appreciable differences in acidities and colour values of the wines; however, PW-3 and PW-4 were comparable with each other in these characteristics. As compared to PW-1 and PW-2, there was a decrease in tannins and total colouring matter and acidity in PW-3 and PW-4 wines. The colour value – an indispensable attribute of wines, was minimum in PW-3 followed by PW-4, PW-2 and PW-1. Although there were significant differences in acidities of the wines, the differences in pH were insignificant. The alcohol contents of the wines remained almost the same in all the wines, irrespective of the pre-treatments employed and also corroborated with the fermentation behaviour discussed earlier. Volatile acidities of all the wines were also found to be quite comparable.

A reference to the data on sensory evaluation of wines produced (Table 3) showed that among the individual sensory attributes, the highest score was awarded to PW-2 for colour, PW-4 for acid/sugar/ alcohol, astringency and flavour while lowest scores were received by PW-3, PW-1 and PW-2 wines respectively. On the basis of over-all scores (Table 3), although all the wines had minimum acceptability, yet PW-4 was adjudged as the best wine due to the desirable levels of acid, astringency and acceptable colour. Wine from PW-3 treatment though acceptable with regard to acid and astringency, lacked in colour. Further, PW-1 and PW-2 had a very good colour but due to acidic and astringent taste, could not score well (Table 3). Overall, wine from treatment PW-4 was better than wines produced from other treatments.

In terms of economy of the process, utilization of the left-over hypertonic solution should not be a problem as this can be used in many ways. It can be reconcentrated at least five times without any loss of properties[13]. Since the syrup has a fruity flavour, acid and colour, it can also be utilised in canning of fruits or can be used for the preparation of ready-to-serve or carbonated beverages[4].

It is concluded that water blanching of plums followed by osmotic treatment can successfully be used to improve the sensory qualities of plum wine by reducing excessive acidity and astringency.

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Preservation of *Paneer* by Sorbic Acid

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Paneer **made from standardised milk had 56-60% moisture and pH 5.5 to 5.8. The initial microbial profile of the product was total plate count 2.3×10^3, proteolytics 7.4×10^2, lipolytics 4.9×10^2 and fungi 10 per g. The shelf life varied from few hr at 25-35°C. room temperature to 10 days at 5°C. Addition of 0.15% sorbic acid to the milk before preparing *paneer* or wrapping the *paneer* in sorbic acid coated butter paper (2 g/m^2) extended the shelf life to 36 days at ambient temperature. The same shelf life at 5°C was achieved by the addition of 0.05% sorbic acid in milk. The sorbic acid content in *paneer* varied from 0.15% to 0.3%.**

Paneer is an indigenous acid and heat coagulated milk product and is extensively used in culinary preparations and snacks. The product is very popular in northern India and is gaining popularity in the south also.

Paneer is a rich source of good quality protein since it normally contains all the essential amino acids present in milk and also appreciable amounts of minerals and most of the vitamins excepting vitamins B and C which are mostly lost during processing.

Armed Forces are located at far off places under different climatic conditions. Under these circumstances, variety in the menu as also nutritional adequacy assumes great significance. Because of its high nutritive quality, *paneer* has a high potential to be considered as a component of Service rations. However, the major drawback is its short shelf life due to high moisture content and low acidity[1-3]. For inclusion in the Service rations, its shelf life needs to be extended, particularly at ambient temperature. Some preservation methods such as dehydration[4,5], freezing[2] or use of additives[6] have been attempted; but there is lack of information on microbiological aspects during storage, which is the main cause of its spoilage. This study was undertaken to find out how the addition of sorbic acid affected the microbiological keeping quality of *paneer* at ambient as well as refrigeration temperatures.

Materials and Methods

Standardised and pasteurised milk (20 l) from a local milk dairy (Karnataka Milk Federation, Mysore) with 4.5 per cent fat and 8.5 per cent solid-non-fat (SNF) was used. *Paneer* was made by the method outlined by Bhattacharya *et al*[3]. The pressed blocks of *paneer* were cut into small pieces each weighing about 50 g and wrapped separately in butter paper coated with 2 g sorbic acid/m^2. The sorbic acid treated butter paper was made as per the method of Ghosh *et al*[7]. Final packing was done in 400 gauge high density polyethylene pouches with 5 pieces in each packet.

In another experiment, *paneer* was made from milk containing sorbic acid. Sorbic acid (0.1 per cent) solution was heated to 70°C and required amount added to hot milk (70°C) to get 0.05, 0.1 and 0.15 per cent concentrations. Samples without sorbic acid served as control.

Storage: The samples were stored in Walk-in cooler (5°C) and at ambient temperature (25° – 35°C).

Analysis: *Paneer* was analysed initially as well as on storage for pH, moisture and microbiological profile as per standard methods. Moisture was determined by keeping 10 g sample at 80°C until it attained constant weight. Steam distillation method[8] was followed to estimate the sorbic acid content of *paneer*. Sensory evaluation was conducted on random samples by a panel of not less than seven untrained members for colour, texture, taste and aroma on a 9 point Hedonic scale.

For microbiological analysis, 10 g *paneer* samples were homogenised aseptically using sterile mortar and pestle. Serial dilutions were made using quarter strength Ringer's solution as diluent. Samples were analysed for total plate counts (TPC), proteolytic bacteria, lipolytic bacteria, coliforms and yeasts and moulds as per standard methods[9,10]. Dextrose Tryptone Agar (DTA) was used for TPC and Violet Red Bile Agar (VRBA) for coliforms. Lipolytic bacteria

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were counted on Tributyrin Agar (TBA), yeasts and moulds were observed on Potato Dextrose Agar (PDA) supplemented with 100 p.p.m. chloramphenicol[11] and proteolytic bacteria on nutrient agar (NA) with 10 per cent skim milk.

Results and Discussion

Freshly prepared *paneer* showed a pH of 5.5.–5.8 and had 56-60 per cent moisture. Initially, it had 2.3×10^3 TPC, 7.4×10^2 proteolytics, 4.9×10^2 lipolytics and 10 yeasts and moulds per g sample (Table 1). Coliforms were absent; being more heat sensitive they might have got killed during pasteurisation of milk and subsequent heating during *paneer* preparation. Addition of 0.15 per cent sorbic acid to milk caused a reduction in microbial population from 2300, 740 and 490 to 100, 350 and 300 respectively for TPC, proteolytic and lipolytic bacteria per g. Fungi were not found to be present in treated samples.

There was slight decrease in microbial population in *paneer* (wrapped/unwrapped) at 5°C initially (Tables 2 and 3). But rapid increase in their number after 4 days (Table 2) in unwrapped *paneer* and after 6 days (Table 3) in wrapped *paneer* at 5°C was observed. The wrapped samples showed comparatively lower microbial growth than the unwrapped samples. The samples visibly in good condition were organoleptically acceptable for 8 days. The acceptability could be enhanced by 2 more days by wrapping the *paneer* in sorbic acid coated paper. Arora and Gupta[2], and Bhattacharya *et al*[3]. reported that *paneer* in cold storage (10°C) could be preserved for 6 days whereas the shelf life of 10 days in the present study has been possible due to comparatively lower temperature of storage (5°C) and wrapping of *paneer* in sorbic acid coated paper. There was no significant change in moisture content; however the pH decreased steadily for 12 days and increased thereafter (Fig 1). This can be attributed to the initial formation of lactic acid as metabolite, subsequent liberation of amino compounds by proteolytic bacteria. *Paneer*, made from milk containing 0.05 per cent sorbic acid, did not show any significant increase in

TABLE 1. EFFECT OF SORBIC ACID ON MICROBIAL PROFILE OF *PANEER*

Sorbic acid (% on milk basis)	Colony forming units per g		
	TPC	Proteolytic	Lipolytic
0	2.3×10^3	7.4×10^2	4.9×10^2
0.05	1.6×10^3	5.1×10^2	4.0×10^2
0.10	1.3×10^3	4.5×10^2	3.5×10^2
0.15	1.0×10^2	3.5×10^2	3.0×10^2

Yeasts and moulds : 10 CFU/g respectively

TABLE 2. STORAGE STUDIES OF UNWRAPPED *PANEER* AT 5°C

Storage period (days)	Moisture (%)	pH	TPC	Proteolytic	Lipolytic	Yeasts and moulds
0	58.0	5.6	2.3×10^3	7.4×10^1	4.9×10^1	1.0×10^1
2	58.5	5.6	2.0×10^3	7.0×10^1	4.9×10^1	1.0×10^1
4	59.0	5.5	2.5×10^3	7.5×10^1	5.8×10^1	1.0×10^1
6	58.8	5.5	4.0×10^3	8.0×10^1	6.0×10^1	2.0×10^1
8	57.8	5.4	7.0×10^3	1.5×10^2	8.0×10^1	4.0×10^1
10	58.0	5.2	5.5×10^4	3.0×10^3	1.0×10^3	8.0×10^1
12	57.0	5.1	9.0×10^4	7.0×10^3	4.0×10^3	5.0×10^2
15	56.9	5.5	2.0×10^5	1.5×10^4	9.0×10^3	7.5×10^2
18	56.8	6.0	8.0×10^5	5.0×10^4	3.0×10^4	1.0×10^3

TABLE 3. STORAGE STUDIES OF *PANEER* WRAPPED IN SORBIC ACID COATED PAPER AT 5°C

Storage period (days)	Moisture (%)	Colony forming units per g				
		pH	TPC	Proteolytic	Lipolytic	Yeasts and moulds
0	58.0	5.6	2.3×10^3	7.4×10^1	4.9×10^1	1.0×10^1
2	57.2	5.6	2.1×10^3	7.0×10^1	4.7×10^1	1.0×10^1
4	57.5	5.5	2.2×10^3	7.2×10^1	4.5×10^1	1.0×10^1
6	57.6	5.4	2.5×10^3	7.5×10^1	5.0×10^1	1.5×10^1
8	58.0	5.4	4.0×10^3	8.0×10^1	6.0×10^1	2.0×10^1
10	56.8	5.3	5.0×10^3	1.0×10^2	9.0×10^1	3.0×10^1
12	57.0	5.2	1.0×10^4	1.5×10^3	5.0×10^2	6.0×10^1
15	56.8	5.6	4.0×10^4	5.0×10^3	2.0×10^3	1.0×10^2
18	57.1	5.9	7.5×10^4	1.0×10^4	9.0×10^3	4.0×10^2

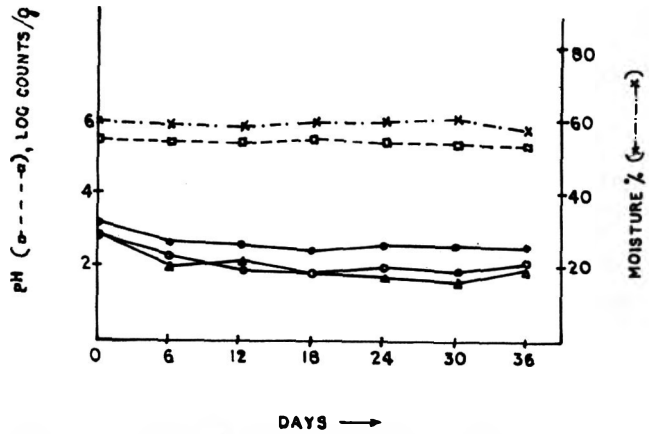

Fig. 1. Storage studies of paneer added with 0.05% sorbic acid at 5°C
●——● TPC,, o——o Proteolytics, △——△ Lipolytics.

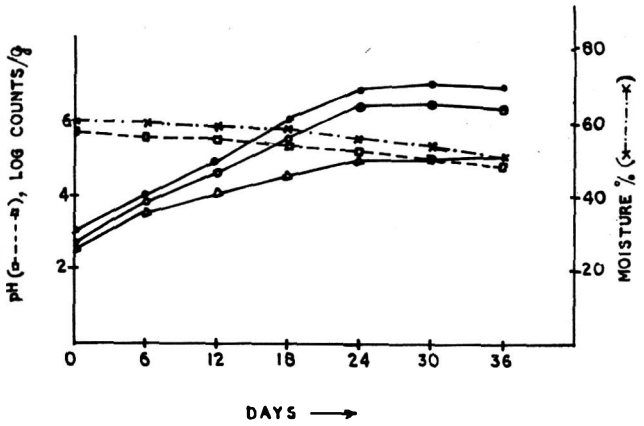

Fig. 3. Storage studies of paneer added with 0.1% sorbic acid at ambient temperature.
●——● TPC, o——o Proteolytics, △——△ Lipolytics.

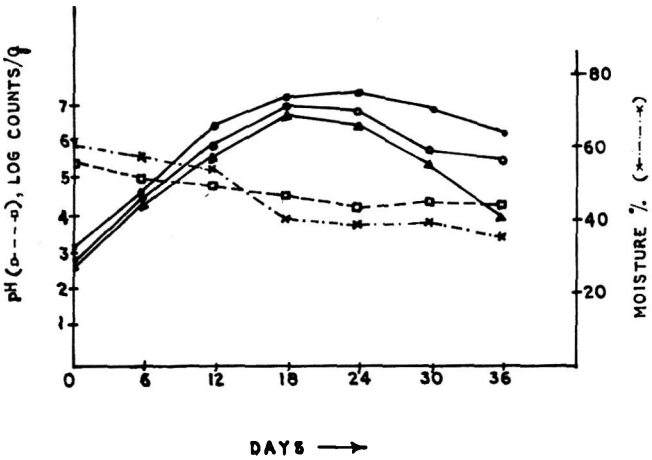

Fig. 2. Storage studies of paneer added with 0.05% sorbic acid at ambient temperature.
●——● TPC,, o——o Proteolytics, △——△ Lipolytics.

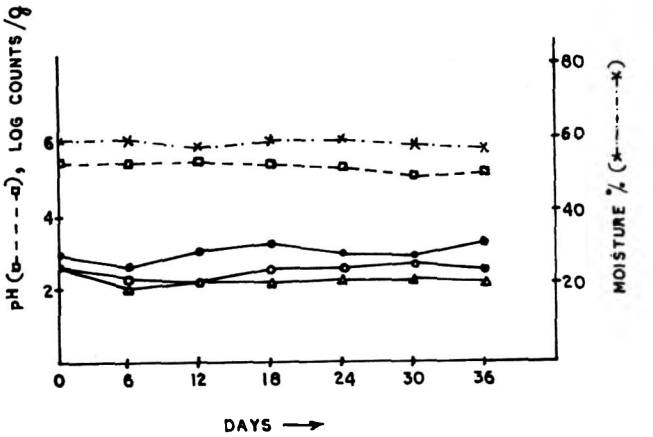

Fig. 4. Storage studies of paneer added with 0.15% sorbic acid at mbient temperature.
●——● TPC,, o——o Proteolytics, △——△ Lipolytics.

the microbial population during the storage period at 5°C. Moisture content and pH of *paneer* did not show any significant change during the period of study.

For use in Armed Forces' ration, the *paneer* should have acceptable shelf life at ambient temperature (25-35°C) for which preservation by sorbic acid was investigated. While *paneer* without additives did not last even for 24 hr at ambient temperature, addition of 0.05 per cent sorbic acid was not of much help either (Fig 2). The microbial population in the latter case increased steadily till 18th day, when the samples harboured 7.4 logs TPC, 7.1 logs proteolytics and 6.8 logs lipolytics per g. Subsequently, there was a tendency in reduction in all the microbial groups in number which may be due to exhaustion of nutrients and/or accumulation of toxic materials in the samples[12]. The pH of samples decreased to 4.5 from initial value of 5.5. Moisture loss was observed at slow rate initially but during the period of 12 to 18 days, plenty of water was collected in the pouches.

Addition of 0.1 per cent sorbic acid to milk slowed down the multiplication of bacteria, although it could not check completely. The maximum counts were seen at the end of 24 days (Fig 3). Sorbic acid at a concentration of 0.15 per cent (in milk) extended the shelf life for a period of atleast 36 days at ambient temperatures (Fig 4). Microbial counts did not show any significant change during storage. pH showed a slight decrease but no significant change could be seen in moisture content of *paneer*.

From the present studies, it is evident that addition of sorbic acid to the milk and subsequent wrapping of *paneer* in sorbic acid coated paper can extend the shelf life of the product. At low temperature (5°C), a minimum concentration of 0.05 per cent sorbic acid (milk basis) is required for extending the shelf life. Both these samples when subjected to sensory evaluation after 36 days of storage were found in the acceptable range on a Hedonic scale of 9 points.

Sorbic acid analysis of *paneer* samples showed that

0.05 per cent and 0.15 per cent sorbic acid in milk leads to residual levels of 0.15 and 0.3 per cent respectively in *paneer*. The latter is above the limits laid down by PFA[13]. Although frying of *paneer* resulted in about 20 per cent reduction in the residual level of sorbic acid, it still did not bring it down to the levels permitted in India although such levels are permitted elsewhere.

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Studies on the Blends of Different Pulses (Bengalgram, Greengram, Lentils and Arhar) in the Preparation of North Indian Spiced *Papads*

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The possibility of preparation of papads from different dhal (dehusked split pulses) flours with or without the addition of blackgram dhal (*Phaseolus mungo* L.) flour has been examined. Only blends of dhal flours comprising blackgram:Bengalgram (70:30), blackgram:arhar (80:20), blackgram:greengram:lentils (60:25:15) and blackgram:lentil (80:20) yielded papads with acceptable physico-chemical and sensory quality attributes. The papads prepared from the blend of blackgram:greengram: lentils (60:25:15) showed better colour, aroma, taste and texture.

Papad, also known as *Appalam* is a popular snack item of India. It is consumed either as such after frying or roasting or as adjunct along with vegetable soups and curries.

Papads are usually made from a blend of cereal flour, edible starch and pulse flour with common salt, spices, edible oil, alkaline and mucilagenous additives. Some varieties of papads are made from jackfruit (*Artocarpus heterophyllus*), banana or sweet potato; shrimps have also been used in papad recipes. The main varieties of papads are those made from (1) blackgram (spiced/unspiced) (2) greengram (spiced/unspiced) (3) sago, and (4) potato.

The market survey[1] and the available information[2] have revealed that in Northern India, papads are mainly prepared from blackgram dhal, blends of greengram and blackgram or blends of greengram, blackgram dhals and starch. The use of lesser known and cheaper dhals for the preparation of papads has, however, not been explored. The present studies were undertaken to explore the possibility of preparing papads from blends of dhals other than those used conventionally.

Materials and Methods

Raw materials: Good quality blackgram (*Phaseolus mungo* L.) greengram (*Phaseolus aureus* Roxb), Bengalgram (*Cicer arietinum* L.), lentil (*Lens culinaris* Medic) (Masoor), red gram (*Cajanus cajan* L.) or tur dhal (split pulse, dehusked), procured from local market were ground in a laboratory hammer mill into flour of 80 mesh. Blends of blackgram flour with other 'dhal' flours were prepared in the ratio of 100:0 (control), 80:20, 70:30, 60:40, 50:50 and were used for preparing papads using the recipe described elsewhere[3].

Common salt, spices (cumin seeds, black pepper, asafoetida, red chilli powder) and refined groundnut oil were purchased locally. Black pepper was coarsely ground in a laboratory mill. Sodium carbonate and sodium bicarbonate of L.R. grade (BDH) were used in place of *sajji khar* commonly employed.

Preparation of dough: One hundred parts blended dhal flour with 8 parts common salt, 1 part mixture of sodium carbonate and sodium bicarbonate (2:1)[3], cumin seeds 1.2 parts, asafoetida 0.2 parts, black pepper 2.4 parts, were kneaded with water to give dough of optimum characteristics. Groundnut oil (refined) 2-3 parts was added during kneading and rolling. Kneading time (manual kneading) for different blends of papads varied from 3-7 min.

Rolling of papads and drying: Dough was divided into small balls of 25-30 g and rolled on circular plate having smooth surface with a wooden pin (roller) to give disc of about 0.6-0.8 mm thickness and 150-200 cm diameter. Papads were dried in the sun/crossflow drier to a desired moisture level and packed in 120 gauge polypropylene bags till fried and evaluated for qualities.

Physico-chemical characteristics of papads: Physico-chemical analysis of papads prepared from selected blends was carried out by using ISI methods[4].

Sensory quality evaluation of papads: Raw (dried) papads and those fried in refined groundnut oil at 190±5°C were examined by a panel of judges comprising of scientific staff as per the method described by Govindarajan *et al.*,[5] for their quality parameters like colour, aroma, taste, texture and overall quality. Based on these results final blends were selected.

Results and Discussion

Bengalgram dhal flour when used alone absorbed nearly 25 parts of water and gave a very soft dough

without any elasticity. This dough was difficult to roll and gave papads with cracked edges, having yellow colour and brittle texture. Upon frying, papads had brown colour, were hard with brittle texture and had distinct Bengalgram flavour. Hence, it was not possible to get papads of desirable quality from Bengalgram flour alone (Table 1) Blends of blackgram and Bengalgram in 50:50, 60:40 ratio also gave unacceptable product due to difficulty in dough making, rolling and formation of crack edged papads with poor sensory quality after deep-fat-frying. Papads after frying had perceptible Bengalgram dhal flavour. But the blend containing flours of blackgram and Bengalgram dhals in the ratio of 70:30 gave a pliable dough which was easy to roll and yielded papads of acceptable sensory quality without any distinct flavour of Bengalgram dhal. The papads prepared from this blend compared well with those conventionally prepared from 100 per cent blackgram dhal (Table 1).

Blend of blackgram and redgram (tur) dhal flour in 80:20 ratio was found to be appropriate with respect to kneading quality of dough, easy rolling of papads and

TABLE 1. DOUGH CHARACTERISTICS, ROLLING PROPERTIES AND QUALITY OF DEEP-FAT-FRIED 'PAPADS' PREPARED FROM BLENDS OF DIFFERENT PULSES

Pulse blends	Water (ml)	Dough character	Dough colour	Rolling property	Expansion (%)	Colour	Texture	Aroma	Taste	Overall quality
Black gram: Lentil										
0. : 100	35	Less opt.	Brown	Impossible	—	—	—	—	—	—
60 : 40	35	"	Light brown	Easy	10.5	Light brown	Hard	Lentil dhal	Lentil	Not acceptable
70 : 30	35	Just opt.	"	Easy	22.5	Br. yellow	Hard	Fried pulse	Spicy and balanced	Acceptable
80 : 20	35	Opt. elastic	Pale brown	Opt.	37.4	Straw yellow brown	Crisp dissolving	Spicy & balanced	"	"
Black gram: Tur										
0 : 100	35	Less opt.	Deep yellow	Difficult	3.0	Reddish Yellow	Brittle & hard	tur dhal	Tur dhal	Not accept
60 : 40	37	Just opt.	Light yellow	Easy	9.0	Straw yellow	Fairly brittle	"	"	"
70 : 30	37	Opt.	Cream yellow	Optimum	11.9	"	Fairly crisp	Fried pulse	Fried pulse	"
80 : 20	37	Opt.	"	"	16.0	"	Crunchy	Spiced and balanced	Spiced and balanced	Acceptable
Black gram: Bengalgram										
0. : 100	25	Less opt.	Yellow	Difficult	2.0	Brown	Hard and brittle	Gram dhal	Gram dhal	Not acceptable
50 : 50	27	"	"	"	3.3	Golden yellow	"	"	"	"
60 : 40	27	Just opt.	"	Easy	11.6	"	Slight brittle	"	Fried pulse	"
70 : 30	30	Opt.	Cream yellow	Opt.	15.4	Straw yellow	Crisp and dissolving	Spicy and balanced	Spicy and balanced	Acceptable
Blackgram:Lentil:Greengram										
0:20:80	30	Less opt.	Light brown	Impossible	—	—	—	—	—	—
0:30:70	30	"	Brown	"	—	—	—	—	—	—
0:40:60	30	"	"	"	—	—	—	—	—	—
60:15:25	35	Opt.	Pale brown	Easy	24.8	Straw yellow & brownish tinge	Crisp	Spicy and balanced	Spicy and balanced	Acceptable

the sensory quality of the fried papads. These papads on frying possessed a straw yellow colour and a crunchy texture with slight blistering. There was no perceptible flavour of Tur dhal in the fried papads prepared from this blend. Expansion of papads after frying was 16 per cent compared to 21 per cent for papads prepared from blackgram. Other blends of these dhals were rejected on the basis of poor dough quality, difficulty in rolling of papads and poor sensory quality of the fried papads as these had hard and brittle texture and distinct flavour of Tur dhal (Table 1).

Blends of greengram dhal and lentils in different ratios gave dough of very poor quality having no rolling properties and was hence rejected (Table 1). But blend of blackgram, greengram and lentil flours in the ratio of 60:25:15 gave papads of very good quality. These papads on frying had straw yellow colour with brown tinge, crisp texture and without any distinct flavour of constituent dhals. The texture of these papads was even better than papads prepared from 100 per cent blackgram dhal (control).

It is evident from Table 1 that a blend of blackgram and lentil dhals at 80:20 ratio was considered quite acceptable for papad making based on the dough characteristics, rolling properties, quality of raw papads and fried papads. The quality of fried papads was considered very close to the control prepared from blackgram flour with respect to colour (straw yellow with brownish tinge), aroma (spicy, balanced), and texture (crisp and crunchy) and taste (fried pulse, balanced). There was no perceptible flavour of lentil dhal. Expansion of papad was better (37.4 per cent) than that of the control sample. Other blends containing higher proportion of lentil dhal were found unacceptable due to poor rolling properties of the dough and poor sensory quality of the papads as they were having distinctly perceptible lentil dhal flavour. Papads could not be rolled with 100 per cent lentil dhal flour as the dough lacked required elasticity.

Papads prepared from selected blends were almost round/circular discs with a diameter ranging from 18.5 cm (blackgram and lentils blend 80:20) to 21.0 cm in 100 per cent blackgram papads and thickness from minimum 0.6 to 0.80 mm (maximum) well within the

TABLE 2. DOUGH CHARACTERISTICS, ROLLING PROPERTIES AND QUALITY OF PAPADS PREPARED FROM SUITABLE BLENDS OF PULSES

Particulars	Blackgram (control)	Blackgram: lentil	Blackgram: tur	Blackgram: Bengalgram	Blackgram Bengalgram: Greengram
		Dough characteristics			
Water (ml)	40	35	37	30	35
Dough character	Optimum	Optimum	Optimum	Optimum	Optimum
Colour	Cream	Pale brown	Creamish	Cream yellow	Pale brown
Rolling property	Optimum	Optimum	Optimum	Optimum	Optimum
		Raw papads			
Av. unit wt (g)	30.00	24.00	24.30	23.80	28.9
Av. dia. (cm)	21.00	18.50	20.10	19.90	19.5
Av. thickness (mm)	0.80	0.60	0.65	0.70	0.8
Moisture (%)	12.80	13.50	13.50	13.10	13.2
Crude fat (%)	3.20	3.60	3.30	3.40	3.4
Crude protein (%)	19.00	17.90	18.40	19.70	18.2
Total ash (%)	9.70	8.80	9.20	8.20	9.9
Alkalinity (%)	2.00	2.10	2.20	2.10	2.0
pH	8.02	7.82	8.07	7.98	8.0
		Fried papads			
Expansion (%)	21.00	37.40	16.00	15.40	24.8
Colour	Straw yellow	Straw yellow with brownish tinge	Straw yellow	Straw yellow	Straw yellow with brownish tinge
Texture	Crisp	Crisp and dissolving	Crunchy	Crisp and dissolving	Crisp

Fried papads prepared from all the above blends had aroma and taste of fried pulse with balanced spices. All the fried papads were acceptable.

ISI specification[4]. The average weight of these papads ranged from 23.8 to 30.0 g per unit (Table 2).

All the papads had a moisture of 13.5 per cent or less as compared to 15.0 per cent (maximum) recommended by ISI. The protein and ash contents of these papads ranged from 17.9 to 19.7 and 8.2 to 9.9 per cent respectively, the highest in papads' made of blackgram, greengram, lentils (60:25:15) and the lowest in papads made of blackgram:Bengalgram (70:30). The alkalinity of these papads was about 2 per cent with pH ranging from 7.82 to 8.07.

From these observations, it may be concluded that lentils and Tur dhal flours blended to the extent of 20 per cent with blackgram dhal flour gave papads of acceptable quality. While Bengalgram dhal flour may be incorporated upto 30 per cent level in the blackgram dhal flour for making acceptable quality papads, lentils and greengram dhal flours alone or in combination could not be used to produce papads due to lack of mucilage in these pulses. However, papads could be made satisfactorily from blends of dhal flour of blackgram, greengram, lentils in the ratio of 60:40 and 60:25:15 respectively.

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Effect of Starch Dipping on Quality of Dehydrated Tomato Slices

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Tomato slices turn brown during drying and storage and their rehydration is poor. In this study, pre-treatments were developed to overcome these problems. Whole tomatoe of 'Pant T-2' variety was blanched for 60 sec in boiling water, 2.5% brine or 0.25% KMS, slices dipped in various solutions and dried to about 4% moisture in a cabinet drier. Blanching reduced pectin methyl esterase activity by 31.6%. Dipping brine blanched slices in sulphited starch for 2.0 min at room temperature before drying improved the rehydration ratio and colour as indicated by lycopene content and extent of browning. Ascorbic acid decreased from 582.5 to 28.0 mg/100 g solids during drying. Dried product contained 233.5 mg SO_2 per 100 g solids. There was negligible change in total lycopene of product during drying and the red colour was retained well during 6 months storage under ambient conditions.

In India, about 0.75 million tonnes of tomatoes are produced annually[1] but because of the perishable nature and seasonal production, their availability varies considerably and post-harvest losses are high. Tomatoes can be preserved by drying in the form of pieces,[2-5] and powder by foam-mat drying,[6-8] spray drying[9] or drum drying[2]. Gupta and Nath[5] sun-dried tomato slices after blanching them for 10 sec in 2.5 per cent brine and slicing. Since their residual moisture content was high, they turned brown during storage and rehydration was poor. In this study, slices were given various pre-treatments to overcome the above problems.

Materials and Methods

Firm ripe tomatoes of 'Pant T-2' variety were obtained from the Horticultural Research Centre of this University. They were washed well in water. Four tomatoes of uniform quality were tied loosely in muslin cloth and several such lots were immersed in boiling 2.5 per cent NaCl, blanched for 30, 45, 60, 75 or 90 sec, and dipped in cold water for quick cooling. Different lots were spread in petri dishes separately, checked for cracks in skins, cut into 3-4 pieces with sharp stainless steel knives and any change in the texture of their flesh was observed visually. Since there was extensive change during 75 and 90 sec blanching, tomatoes were blanched for 60 sec only in subsequent studies. This blanching period caused cracks in skin only without any visual change in the flesh.

Tomatoes blanched in brine or water were cut into 1.5 cm thick slices, kept on a sieve to drain-off free juice, and dipped for 2 min at room temperature in: (i) 5 per cent potassium metabisulphite (KMS), (ii) 2.5 per cent starch containing 5 per cent KMS (sulphited starch), or (iii) sulphited starch containing one per cent $CaCl_2$. Slices were spread on trays and dried in a cabinet drier at 78±1°C for first 4 hr and subsequently at 53±1°C. Dried tomatoes were packed quickly in polyethylene bags and heat sealed.

Dehydration and rehydration ratios were determined by the method described by Ranganna[10]. Extent of browning was estimated using 60 per cent ethanol and the results were expressed as optical density at 440 nm. Moisture percentage was estimated by oven-drying at 70±1°C for 24 hr and total soluble solids (TSS), pH and acidity of samples were determined by standard methods[10]. Reducing and total sugars were estimated by volumetric method, ascorbic acid by xylene extraction method, lycopene content spectrophotometrically at 503 nm using petroleum ether as blank, and total SO_2 by iodimetric titration following procedures outlined in the "Handbook of Analysis and Quality Control for Fruit and Vegetable Products"[10]. Pectin methyl esterase activity was measured by the method of Lineweaver and Jansen[11].

For drying rates, moisture percentage (on dry weight basis) of drying samples were measured at 30 min intervals and plotted against corresponding drying times.

Dried starch-dipped tomato slices were packed in

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polyethene bags (thickness 93 microns), heat sealed and stored for about 6 months under ambient conditions during the months of February to June (room temp. range 15° to 35°C, atmospheric RH 40 to 90 per cent.) The packets were kept away from direct sunlight. During storage, colour of dried slices was observed visually. At the end of storage period, rehydration ratio was determined.

Sensory evaluation was carried out by Monadic test using a five point scale[10]. Samples were distributed amongst 18 families. They were requested to rate the product for colour, texture, aroma, taste and overall acceptability on the evaluation card provided to them. Data so obtained were statistically analysed to determine consumer acceptability[10].

Results and Discussion

Chemical composition of tomato 'CV Pant T-2' is given in Table 1. Moisture content, TSS and pH of fresh tomatoes were in the range reported earlier from this laboratory[5] but acidity, total sugars and lycopene contents were lower and values for ascorbic acid were slightly higher. These differences may be due to variety.

Whole tomatoes were blanched for 30-90 sec in boiling 2.5 per cent brine. Blanching for 45 sec did not produce any visible change in peels or flesh of tomatoes. In 60 sec blanching, peels were moderately affected but flesh texture remained unchanged whereas 75 and 90 sec blanching damaged flesh texture in addition to causing severe cracks in peels and slicing them was difficult. Pectin methyl esterase (PME) activity in tomatoes got reduced by about 70 per cent during 60 sec blanching. As compared to this, there was not much further decrease in PME activity (2.30 PEU/100 g) during 75 sec blanching. Therefore, in further studies, whole tomatoes were blanched for 60 sec only.

Tomatoes were blanched in boiling brine or boiling water, sliced and dried to 3.5-5.5 per cent moisture along with unblanched samples as a control (Table 2). But these samples were dark brown (EB 2.000) indicating that blanching did not check browning. Their dehydration ratio (DR) and rehydration ratio (RR) were 18.5-20.5 and 3.4-4.2, respectively. Non-enzymatic browning in dried slices may be due to oxidation and polymerization of ascorbic acid, sugar caramelization or oxidation and polymerization of

TABLE 1. CHEMICAL COMPOSITION OF FRESH, BLANCHED AND DRIED TOMATO CV PANT T-2.

	Raw	Blanched**	Dried
Moisture (%)	94.90	94.80	4.20
T.S.S. (°Brix)	4.50	4.50	—
pH	4.20	4.25	—
Acidity* (% anhydrous citric acid)	7.99	7.67	5.43
Reducing sugars* (% dextrose)	18.00	13.70	—
Total sugars* (% dextrose)	63.30	62.80	48.30
Ascorbic acid (mg/100g solids)	582.50	522.40	28.00
Lycopene (mg/100 g solids)	53.30	53.20	52.30
Pectin methyl esterase (PEU/100g)	4.32	2.96	50.35

*On dry wt basis, **Blanched for 60 sec in boiling 2.5% brine and dipped in sulphited starch.

TABLE 2. STANDARDIZATION OF PRETREATMENTS FOR DRYING OF 1.5 CM SLICES OF TOMATOES CV PANT T-2.

Blanching media[1]	Dipping solution	Moisture in dried slices (%)	Dehydration ratio	Rehydration ratio	Browning (O.D.)
No blanching	No dipping	5.5	18.5	3.5	2.00
	Sulphiting[2]	4.8	20.2	3.6	1.70
	Sulphited starch[3]	4.1	20.3	4.0	1.46
Water	No dipping	3.6	20.1	3.4	2.00
	20% glycerol	3.5	15.2	3.2	1.70
	Sulphiting[2]	3.6	24.5	4.4	0.66
	Sulphited starch[3]	3.5	19.4	3.6	0.46
	$CaCl_2$ + sulphited starch[4]	3.3	21.8	4.3	0.27
2.5% NaCl	No dipping	3.3	20.5	4.2	2.00
	Sulphiting[2]	3.3	15.5	4.7	0.37
	Sulphited starch[3]	4.2	24.1	4.3	0.33
	$CaCl_2$ + sulphited starch[4]	3.6	21.8	3.8	0.28

[1]Blanching was done for 60 sec in boiling media [2]5% KMS [3]5% KMS in 2.5 starch. [4]1% $CaCl_2$ and 5% KMS in 2.5% starch.

lycopene[12]. The first and third reactions would require oxygen. Dipping blanched cauliflower before drying in different solutions, viz. glycerol, sucrose, etc was found to give light coloured dehydrated product[13,14]. But dipping water-blanched tomato slices in 20 per cent glycerol did not improve the colour (EB 1.699) of dried product and rehydration was also poor (RR 3.2) (Table 2). Dipping in 5 per cent KMS instead of glycerol solution improved the colour (EB 0.658). Srivastava and Nath[15] had obtained very light colour dried cauliflower by dipping blanched pieces in 2.5 per cent starch containing sodium sulphite (0.25 per cent) and sodium meta bisulphite (0.75 per cent) which retained colour and rehydration characteristics well during storage. Colour of water blanched tomato slices dipped in 2.5 per cent starch containing 5 per cent KMS and then dried was light (EB 0.456). The corresponding unblanched samples had darker colour (Table 2). Dried slices contained 233.5 mg SO_2 per 100 g solids.

Reconstituted tomato slices had poor shape and texture. Calcium chloride has been used to improve texture of many canned products[16]. Though addition of one per cent $CaCl_2$ to starch solution reduced browning (EB 0.268) in water-blanched samples, it did not improve their RR or shape (Table 2). Blanching in 2.5 per cent brine was found to give superior product possessing better colour and RR (Table 2) as compared to water-blanched samples. In brine-blanched samples dipped in sulphited starch and dried to 4.2 per cent moisture EB was 0.328 and RR was 4.3. Rehydrated samples were found to be of attractive red colour.

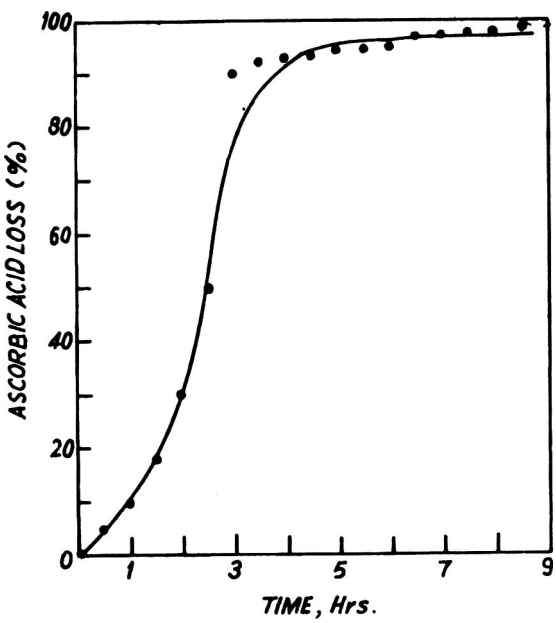

Fig. 1. Ascorbic acid loss at different periods of drying of tomato slices.

These samples were packed in polyethylene bags and stored for about 6 months under ambient conditions. They retained colour and rehydration characteristics well. Longer storage resulted in bleaching to red colour. This is contrary to the earlier observation of Gupta and Nath[5] who had observed extensive browning. Better colour retention observed in the present case may be due to formation of protective coating of sulphited starch.

Blanching in brine did not make any significant difference to the percentages of sugars, ascorbic acid or lycopene but drying reduced percentages of total sugars and ascorbic acid (Table 1). Ascorbic acid content reduced from 582.5 to 28.00 mg/100 g solids during the course of drying. The loss was slow during the first 3 hr of drying but it increased rapidly subsequently (Fig 1) when the moisture content of

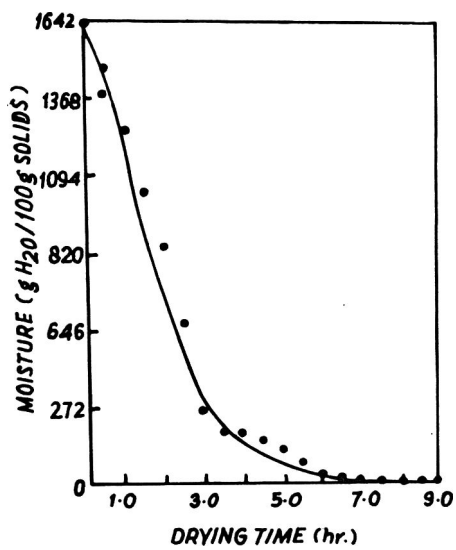

Fig. 2. Loss of moisture during drying of tomato slices.

TABLE 3. TOTAL SENSORY SCORES FOR DIFFERENT SENSORY ATTRIBUTES OF DRIED TOMATO SLICES

Sensory attribute	Water blanched sulphited starch dipped	Brine blanched sulphited starch dipped	Brine blanched and dipped in sulphited starch +1% $CaCl_2$
Colour	12	22	4
Texture	−3	0	6
Aroma	7	12	2
Taste	14	19	3
Overall quality	9	19	3

No of panelists 18; Numerical score for liked extremely +2, liked +1, Neither liked nor disliked 0, disliked −1, disliked extremely − 2

TABLE 4. ANALYSIS OF VARIANCE OF SENSORY RESPONSES FOR DRIED TOMATO SLICES

Source of variation	Degrees of freedom	Variance	Mean variance	F ratio calculated
For Colour				
Between samples	2	9.00	4.500	5.435**
Replicate error	51	42.21	0.828	
Total	53	51.21		
For Texture				
Between samples	2	2.33	1.165	1.071
Replicate error	51	55.50	1.088	
Total	53	57.83		
For Aroma				
Between samples	2	2.78	1.390	1.771
Replicate error	51	40.06	0.785	
Total	53	42.84		
For Taste				
Between samples	2	4.78	2.390	3.380*
Replicate error	51	36.06	0.707	
Total	53	40.84		
For Overall Acceptability				
Between samples	2	7.26	3.640	4.649*
Replicate error	51	39.94	0.783	
Total	53	47.20		

*Significant difference at 5% level **Significant difference at 1% level
Table value for 'F' ratio was 3.19 at 0.05 and 5.08 at 0.01 level of significance.

slices had considerably reduced (Fig 2). But drying did not reduce lycopene content (Table 1).

Moisture percentage (on dry weight basis) of drying slices against drying period is shown in Fig 2. It is similar to Fig 1 for ascorbic acid loss during drying.

Results of the sensory evaluation show that total scores for all the sensory attributes, except for texture, was highest for the brine-blanched sulphited starch dipped samples (Table 3). Statistical analysis of these data indicated significant differences between colour of samples at 1 per cent level (Table 4). Their taste and overall acceptability were found to be significantly different at 5 per cent level.

This study shows that dried tomato slices 'cv Pant T-2' possessing good colour can be obtained by blanching them whole in boiling 2.5 per cent brine for 60 sec, cutting into 1.5 cm thick slices, dipping for 2 min in 2.5 per cent starch solutions containing 5 per cent KMS and drying in a cabinet drier at 78° and 55°C. These slices retained their red colour well during 6 months storage as observed visually.

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Studies on the Use of Artificial Sweeteners in Sweet Bread and Biscuits

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Artificial sweeteners – saccharin, aspartame and acesulfame-K were used to replace sugar in the preparation of sweet bread and biscuits. The specific volume (3.57 to 3.61 ml/g) of loaves containing artificial sweeteners was comparable to that (3.59 ml/g) of control bread. The crust colour of breads was light brown as against brown of control. The adverse effects of artificial sweeteners on biscuit dough properties, colour, crispness and spread factor were overcome by using 20% sorbitol in place of sugar in the formula. The sweetness intensity of sweet bread and biscuits containing artificial sweeteners was comparable with that of control bread and biscuits.

The artificial sweeteners such as saccharin, aspartame and acesulfame-K which are respectively 500, 200 and 180 times sweeter than sucrose are permitted in various foods under different national statutory regulations, although these are not universally allowed[1]. These compounds are useful for diabetics and obese persons as substitutes for sugar. The FAO/WHO Joint Expert Committee on Food Additives has evaluated them and allocated an acceptable daily intake (ADI) of 0-2.5, 0-40 and 0-9 mg/kg b.w. respectively for saccharin, aspartame and acesulfame-K[2-4].

Materials and Methods

Commercial wheat flour and artificial sweeteners – namely saccharin, (S.D. Fine Chem. Pvt. Ltd., India), aspartame, (Searle (India) Ltd., India) and acesulfame-K (Brand name: Sunett, Hoechst AG, West Germany), were used in the studies. Total ash, sedimentation value, wet gluten, falling number, damaged starch and ether extractives of flour were determined according to AACC procedures[5]. Crude protein (N×5.7) was estimated by micro-Kjeldahl method. The flour colour was determined using a Kent-Jone Flour Colour Grader (Series III). Farinograph and extensograph curve characteristics were determined according to AACC procedures[5].

Breads were prepared according to remix procedure of Irvin and Mc Mullan[6] with a reduced fermentation period of 90 min. for the dough instead of 165 min. The recipe contained 10 per cent sugar or artificial sweetener equivalent in sweetness to 10 per cent sugar. Loaf volume was measured using rape seed displacement method of Malloch and Cook[7]. Evaluation of breads was carried out for crust and crumb characteristics after 24 hr by a panel of six judges.

Biscuits were prepared using the recipe: flour (64 g); sugar (18 g) or artificial sweetener equivalent in sweetness, fat (16 g), non-fat-milk-solids (1 g), glucose (1 g) salt (0.4 g), baking powder (0.2 g), ammonium bicarbonate (0.5 g), sodium bicarbonate (0.2 g), vanillin (0.05 g) and water (13-15 ml). Biscuits with artificial sweeteners were also prepared in combination with 10, 20 and 30 per cent sorbitol (Fluka Chem. AG, Switzerland). Evaluation of biscuits for colour, crispness, spread factor and sweetness was carried out by a panel of six judges.

According to Friedman[8], sorbitol can be used upto 30 per cent in baked goods.

Results and Discussion

Flour used in bread and biscuit was of medium strength. The values for protein (9.0 per cent), wet gluten (25.4 per cent), sedimentation value (21), water absorption (59.1 per cent), farinograph stability (5.5 min), extensograph maximum resistance to extension (480 BU), extensibility (173 mm) and energy (107 cm^2) were comparable to the average values reported by

TABLE 1. EFFECT OF ARTIFICIAL SWEETENERS ON THE QUALITY OF SWEET BREAD

Parameter	Control[a]	Saccharin[b]	Aspartame[b]	Acesulfame-K[b]
Crust colour	Brown	Light brown	Light brown	Light brown
Loaf wt (g)	147.70	133.30	135.40	134.40
Loaf vol (ml)	530.00	480.00	490.00	480.00
Specific loaf vol (ml/g)	3.59	3.60	3.61	3.57

a – Contains 10% sugar in the recipe
b – Added replacing sugar at the level equivalent to 10% sugar in sweetness.
Sweetness was normal in all cases.

Shurpalekar *et al*[7]., obtained for the medium strong wheat flours.

The change in crust colour of bread was from brown of the control to that of light brown of those made with artificial sweeteners (Table 1). This was the artifact of elimination of sugar in the formula. The reduction in loaf weight by 12.3 to 14.4 g of breads made with artificial sweeteners compared to that of control was attributed to the higher dough yield of control to which 10 per cent sugar was added. The reduction in volume (40-50 ml) observed for the loaves containing artificial sweeteners could be due to reduced gas production because of lack of enough fermentable sugar. However, specific volume (3.57 to 3.61 ml/g) of breads with artificial sweeteners was not affected because of lower dough yield and loaf weight which compared favourably with that of the control (3.59 ml/g). Also the crust, shape, crumb colour, grain and texture remained unaffected. The sweetness of breads with artificial sweeteners is perceptible and comparable to that of control sweet bread.

The biscuit doughs with artificial sweeteners were hard and difficult to roll. However, the rolling characteristics improved with the addition of 20 or 30 per cent sorbitol. The colour of biscuits with artificial sweetener was creamish white and improved to light brown similar to that of control with 20 per cent sorbitol (Tables 2,3 and 4). At 30 per cent sorbitol in the biscuit dough, the colour of biscuits was brown. The surface which was uneven and rough in case of biscuits with artificial sweeteners improved with the addition of 20 or 30 per cent sorbitol. The maximum score (8) for crispness of biscuits obtained with artificial sweeteners was at the 20 per cent sorbitol as against a score of 10 for the control. However with 30 per cent addition of sorbitol, the biscuits turned brittle. The spread factor value decreased from 100 for the control to 70.4 to 83.7 for biscuits with artificial sweeteners which gradually increased with the addition of sorbitol. The values obtained for 20 per cent sorbitol biscuits

TABLE 2. EFFECT OF SACCHARIN[a] AND SORBITOL ON THE QUALITY OF BISCUITS

Parameter	Control	Values at indicated sorbitol (%) levels			
		0	10	20	30
Colour	Light brown	Creamish white	Dull brown	Light brown	Brown
Surface	Even/smooth	Uneven/rough	Uneven/rough	Even/smooth	Even/smooth
Crispness[b]	10 ± 0	6 ± 0.71	7 ± 0.71	8 ± 0.32	6 ± 0.71
Weight (g)	7.0	7.1	6.4	6.3	6.2
Width (mm)	53.0	44.0	49.0	50.5	52.2
Thickness (mm)	5.4	5.4	5.2	5.0	4.7
Spread ratio	9.8	8.2	9.4	10.1	11.1
Spread factor	100.0	83.7	95.9	103.0	113.2

a = Added replacing sugar at the level equivalent to 30% sugar in sweetness
b = Maximum score – 10. values are means ± SD
Sweetness was normal in all the cases.

TABLE 3. EFFECT OF ASPARTAME[a] AND SORBITOL ON THE QUALITY OF BISCUITS

Parameter	Values at indicated sorbitol (%) levels			
	0	10	20	30
Colour	Creamish white	Dull brown	Light brown	Brown
Surface	Uneven/rough	Uneven/rough	Even/smooth	Even/smooth
Crispness[b]	5 ± 0.71	6 ± 0.63	8 ± 0.71	5 ± 0.71
Weight (g)	6.9	5.9	5.9	6.5
Width (mm)	49.0	47.8	50.3	52.4
Thickness (mm)	6.8	5.1	5.1	4.4
Spread ratio	6.9	9.4	9.9	11.9
Spread factor	70.4	95.9	101.0	121.4

a = Added replacing sugar at the level equivalent to 30% sugar in sweetness
b = Maximum score – 10. values are means ± SD
Sweetness was normal in all the cases.

TABLE 4. EFFECT OF ACESULFAME-K[a] AND SORBITOL ON THE QUALITY OF BISCUITS

Parameters	Values at indicated sorbitol (%) levels			
	0	10	20	30
Colour	Creamish white	Dull brown	Light brown	Brown
Surface	Uneven/rough	Uneven/rough	Even/smooth	Even/smooth
Crispness[b]	5 ± 0.71	7 ± 0.71	8 ± 0.63	6 ± 0.71
Weight (g)	7.4	6.7	6.9	7.5
Width (mm)	49.5	49.9	51.1	53.7
Thickness (mm)	6.9	5.3	5.1	5.5
Spread ratio	7.2	9.4	10.0	9.8
Spread factor	73.5	95.9	102.0	100.0

a = Added replacing sugar at the level equivalent to 30% sugar in sweetness
b = Maximum score – 10. values are means ± SD
Sweetness was normal in all the cases.

(101 to 103) compared well with that of control biscuits. The sweetness of biscuits with artificial sweeteners was perceptible and similar in intensity to that of control biscuits.

The results have shown the possibility of using artificial sweeteners in the preparation of sweet bread and biscuits for medical reasons. However, at present, addition of artificial sweeteners in bread and biscuits is prohibited under the provisions of Prevention of Food Adulteration Act of India.

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Intestinal Epithelial Cell Glycoproteins in Albino Rats in Retinol Deficiency

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Retinol deficiency in rats leads to changes in the intestinal epithelial cell membrane glycoproteins. A significant, quantitative decrease in one of the component glycoproteins was observed in the villus cell fraction. Lithium diiodosalicylate extraction of the cell membrane glycoproteins followed by electrophoretic analysis supported the above observation. Cell agglutination studies with lectins indicated a decreasing trend in the titer in the villus fraction in retinol deficiency. This suggests a cell surface change in the epithelial villus cells as they differentiate and move up the villi. Carbohydrate composition of isolated glycoproteins showed a significant reduction in mannose, which was supported by tracer studies indicating reduced incorporation of mannose. These results have been discussed in the light of the effects of retinol deficiency on epithelial cell glycoproteins in the process of differentiation of villus cells.

An impairment in the intestinal epithelial cell differentiation is suspected in retinol deficiency due to altered membrane structure[1]. The involvement of retinol in the biosynthesis of tissue-specific glycoproteins and the participation of mannosyl retinyl phosphate as the sugar donor is known[2]. This intermediate could also modify certain glycosylation reactions resulting in altered cell surface properties, specifically mediated by membrane glycoproteins. In the present study, the effect of retinol deficiency on the rat intestinal epithelial cell membrane glycoproteins has been reported.

Materials and Methods

Retinol deficiency was induced in weanling male albino rats as reported earlier[3]. The animals (140 g) were killed by cervical dislocation and the small intestines were processed immediately. The epithelial cells were fractionated into villus, middle and crypt fractions according to the method of Weiser[4].

Agglutination of intestinal epithelial cells: Intestinal epithelial cell fractions were separately tested for agglutination. Clean plexi-glass plates provided with wells were used to follow agglutination. Initially, 0.2 ml of saline was placed in these wells. Lectin (concanavalin A or wheat germ agglutinin, 1 mg/ml) was diluted serially in these wells. The last well containing only buffer, served as blank. Cell suspension (0.2 ml) was pipetted into each well and incubated at ambient temperature for 2 to 3 hr followed by 10 to 15 min at 37°C. The agglutination titer was scored against the blank without lectin as follows. Each value is computed thus. In a single experiment, the agglutination titer was scored 4+ (highest), 3+ to 2+ (medium) and 1+ to 0 (lowest). After completing six experiments the highest scores were added and taken as 100. Medium and lowest were also computed similarly and expressed as percentages in relation to the highest titer.

Cell membrane glycoprotein extraction and analysis: Cell membrane glycoproteins were extracted using lithium diiodosalicylate according to Marchesi[5]. The extract (1.0 ml) was hydrolysed in 4N hydrochloric acid at 105°C for 6 hr. The hydrolysate was fractionated on Dowex H^+, 200-400 mesh. The alditol acetates of neutral sugars were analysed by gas liquid chromatography in a Packard Model 427 gas chromatograph with a column of 3% OV-225 on chromosorb W(HP) according to Sawardekar *et al.*[6] .

Sialic acid content of glycoproteins and the cell sonicate supernatant (in the neuraminidase assay) was determined by the thiobarbituric acid method[7].

Neuraminidase and β-glucuronidase assay: Neuraminidase activity in the cell sonicate and β-glucuronidase activity in the cell homogenate were assayed according to Heijlman and Roukema[8] and Gianetto and de Duve[9] respectively.

Electrophoresis of membrane glycoproteins: Glycoproteins were solubilised in 1% sodium dodecyl sulphate, electrophoresed on polyacrylamide gels and stained with Periodate-Schiff's stain according to

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Fairbanks *et al.*[10] The gels were scanned at 520 nm in a Beckmann Model 26 recording spectrophotometer provided with a gel-scanning attachment. Protein was assayed using the Folin-phenol reagent according to Lowry *et al.*[11]

Tracer studies in vitro: The incorporation of U-^{14} C-mannose into intestinal epithelial cells was done as follows. Isolated epithelial cells suspended in Krebs-Henseleit buffer were incubated with 7.5 μCi of U-^{14} C-mannose (sp. act. 36.5 μCi/m mole, Bhabha Atomic Research Centre, Bombay, India) at 37°C for 45 min. The reaction was stopped by the addition of 4 ml of 5 per cent cold trichloroacetic acid (TCA) followed by centrifugation at 2000 **g**. The pellet was washed with ether. The dry residue was hydrolysed with 0.4 ml of 1 N NaOH at 80°C for 30 min. Aliquots (0.5 ml) were taken for counting in a Beckman LS-100 Counter.

Tracer studies in vivo: The animals were injected (ip) with 15 μCi of D-1-^{14}C glucosamine hydrochloride, New England Nuclear, USA. After three hours, the animals were sacrificed by cervical dislocation and the intestinal epithelial cells were fractionated. Aliquots (0.5 ml) of cell suspension were treated with 5 per cent TCA and sedimented at 2000 **g**. The pellets were washed once again with TCA followed by ether. The dried samples were hydrolysed in 1 N NaOH at 80°C for 20 min. Aliquots (0.2 ml) were counted as above.

Results and Discussion

The intestinal epithelial cells were chosen as a model system to study the changes induced at the membrane level during differentiation since the epithelium comprises differentiated cells in the villus and undifferentiated cells in the crypt zone. It has been observed that in retinol deficiency the activity of alkaline phosphatase is decreased in the villus fraction[1]. Alkaline phosphatase has been suggested as one of the markers of cell differentiation. In order to determine whether the observed decrease in alkaline phosphatase activity is reflected in changes in glycoprotein pattern, the cell membrane glycoproteins were solubilized and analysed by electrophoresis on polyacrylamide gel. Four distinct bands and an occasional hazy, fifth band could be seen. Among the four bands in the villus fraction, the first band is reduced significantly in retinol deficiency (Table 1). There is no significant change in the other cell fractions.

Using lithium diiodosalicylate, the cell membrane

TABLE 1. DENSITOMETRIC ANALYSIS OF RAT INTESTINAL EPITHELIAL CELL MEMBRANE GLYCOPROTEINS IN RETINOL DEFICIENCY

Band No.	Intestinal epithelial cell fractions					
	Villus		Middle		Crypt	
	Control	Retinol deficient	Control	Retinol deficient	Control	Retinol deficient
1	28.2 ± 2.6	19.0 ± 1.9*	22.3 ± 2.8	22.5 ± 1.3	21.2 ± 2.1	25.2 ± 2.7
2	27.7 ± 3.7	30.2 ± 3.1	25.5 ± 2.1	25.2 ± 2.4	23.0 ± 2.6	25.3 ± 4.2
3	21.5 ± 1.7	23.5 ± 1.9	22.5 ± 1.5	25.2 ± 2.0	30.6 ± 2.4	23.8 ± 2.0
4	22.5 ± 3.6	27.2 ± 4.7	29.5 ± 4.2	27.3 ± 5.0	25.4 ± 3.7	25.5 ± 4.8

Values are relative percentages and mean ± SEM of six independent determinations.
*Significant, $P<0.05$.

TABLE 2. DENSITOMETRIC ANALYSIS OF ISOLATED INTESTINAL EPITHELIAL CELL GLYCOPROTEINS IN RETINOL DEFICIENCY

Band No.	Intestinal epithelial cell fractions					
	Villus		Middle		Crypt	
	Control	Retinol deficient	Control	Retinol deficient	Control	Retinol deficient
1	39.0 ± 2.8	45.0 ± 2.5	43.2 ± 5.0	50.4 ± 6.0	36.0 ± 6.9	37.5 ± 4.3
2	51.0 ± 1.9	43.6 ± 2.2*	46.2 ± 4.0	38.8 ± 4.0	52.4 ± 2.5	52.5 ± 3.8
3	11.2 ± 1.2	11.2 ± 2.6	10.6 ± 1.9	10.6 ± 3.1	10.6 ± 1.9	9.8 ± 1.4

Values are relative percentages and mean ± SEM of six independent determinations.
*Significant, $P<0.05$.

glycoproteins were partially purified for electrophoretic analysis. Only three bands could be seen which on densitometric analysis revealed that (Table 2) the second band in the villus zone is significantly lowered in retinol deficiency. The second band did not show any significant change in the crypt and middle fractions. The above observations support the results on alkaline phosphatase in the cell membrane as reported earlier[1].

In an attempt to investigate whether the observed differences in glycoproteins in retinol deficiency is the result of lysosomal damage, the activities of β-glucuronidase and neuraminidase were determined in the homogenates of epithelial cell fractions. The results indicated no significant differences in the activities in retinol deficiency (Table 3). This is suggestive of the non-involvement of lysosomes in glycoprotein changes.

Alkaline phosphatase and its isoenzymes are reported to be glycoprotein in character[12]. In view of this and to investigate the ensuing cell surface changes and the nature of the carbohydrate involved, the effect of plant agglutinin on the cell agglutination in retinol deficiency was studied. The results with concanavalin A (Con A) and wheat germ agglutinin (WGA) are given in Table 4. The agglutination titer of villus cells in retinol deficiency appeared to be lower than in controls. On the other hand, the agglutination of middle and crypt cells is unaltered by Con A. With WGA, the villus cells did not reveal any marked change in the titer in retinol deficiency, except at low concentration of the lectin, a decreasing trend in titer is indicated. In the middle and the crypt fraction, the titer in the deficient group was lower than that in the villus fraction at 200 μg lectin. This indicates that the villus celis are more responsive to WGA than the other two

TABLE 3. LYSOSOMAL ENZYME ACTIVITIES IN INTESTINAL EPITHELIAL CELLS IN IN RETINOL DEFICIENCY

	Intestinal epithelial cell fractions					
	Villus		Middle		Crypt	
	Control	Retinol deficient	Control	Retinol deficient	Control	Retinol deficient
β-Glucuronidase[a]	41.0 ± 4.0	42.0 ± 4.6	31.1 ± 2.3	28.5 ± 2.5	34.2 ± 5.1	32.8 ± 2.7
Neuraminidase[b]	0.106 ± 0.02	0.109 ± 0.02	0.116 ± 0.01	0.120 ± 0.02	0.106 ± 0.01	0.120 ± 0.02

[a] μ moles naphthalene released/mg/60 min.
[b] μ moles sialic acid released/mg/60 min.
Values are the mean ± SEM of five independent determinations.

TABLE 4. AGGLUTINATION OF EPITHELIAL CELLS IN RETINOL DEFICIENCY

Lectin (μg)	Titers of intestinal epithelial cell fractions					
	Villus		Middle		Crypt	
	Control	Retinol deficient	Control	Retinol deficient	Control	Retinol deficient
Con A						
0.195	22	16	16	—	13	20
0.781	38	26	40	36	28	30
3.125	48	38	46	40	45	33
12.50	55	48	46	—	51	54
50.00	79	74	70	78	70	80
200.0	100	84	90	88	80	86
WGA						
0.195	10	3	6	9	14	10
0.781	24	15	30	26	20	29
3.125	37	27	32	42	29	34
12.50	60	54	—	—	40	58
50.00	84	92	70	84	—	—
200.0	95	100	100	84	60	66

TABLE 5. MONOSACCHARIDE COMPOSITION OF INTESTINAL EPITHELIAL CELL GLYCOPROTEINS

Mono-saccharide	Intestinal epithelial cell fractions					
	Villus		Middle		Crypt	
	Control	Retinol deficient	Control	Retinol deficient	Control	Retinol deficient
Glucose	1.94 ± 0.3	1.67 ± 0.5	1.74 ± 0.3	0.97 ± 0.3	1.84 ± 0.5	1.79 ± 0.7
Mannose	1.55 ± 0.4	0.60 ± 0.1*	0.56 ± 0.0	0.77 ± 0.2	0.70 ± 0.1	0.62 ± 0.0
Galactose	3.93 ± 0.9	3.64 ± 1.5	3.52 ± 0.7	2.41 ± 0.6	3.50 ± 0.8	4.00 ± 1.3
Fucose	1.62 ± 0.3	0.93 ± 0.2	2.44 ± 0.3	2.07 ± 0.5	1.63 ± 0.5	2.80 ± 0.7

Values are relative percentages and mean ± SEM of six independent determinations.
*Significant, $P<0.05$.

fractions. The decreasing trend in the agglutination titer in the villus fraction in retinol deficiency indicates a surface change in the epithelial villus cells through a change in the cell surface carbohydrate in the differentiating villus and middle cells in retinol deficiency.

The carbohydrate composition of the isolated epithelial cell glycoproteins was analysed in order to correlate the changes observed in the membrane glycoproteins in relation to the retinol status of the animal. The results are shown in Table 5. A specific significant reduction in mannose content is observed in the villus fraction in retinol deficiency. Glucose appears to be reduced whereas galactose is unaffected. In the middle fraction, fucose, galactose and glucose reveal a decreasing trend in retinol deficiency, mannose being unaffected. The crypt fraction showed increased level of glucose and galactose in relation to middle and villus fractions in other groups whereas fucose and mannose showed a gradual increment from crypt to villus. The content of sialic acid was not affected (Table 6).

TABLE 6. SIALIC ACID CONTENT OF INTESTINAL EPITHELIAL CELL GLYCOPROTEINS IN RETINOL DEFICIENCY

	Intestinal epithelial cell fractions		
	Villus	Middle	Crypt
Control	0.084 ± 0.01	0.040 ± 0.01	0.05 ± 0.01
Retinol deficient	0.084 ± 0.01	0.034 ± 0.01	0.05 ± 0.01

μ moles of sialic acid/mg protein.
Values are the mean ± SEM of five independent determinations.

TABLE 7. *IN VIVO* INCORPORATION OF 1-^{14}C GLUCOSAMINE INTO INTESTINAL EPITHELIAL CELL IN RETINOL DEFICIENCY

	Incorporation (cpm/mg) into intestinal epithelial cell fractions		
	Villus	Middle	Crypt
Control	2638 ± 265	1931 ± 334	1579 ± 260
Retinol deficient	3163 ± 392	2182 ± 376	1880 ± 283

Values are mean ± SEM of five independent determinations.

It has been suggested that the epithelial cell differentiation depends on the direct involvement of retinol in certain glycosyl transfer reactions during specific glycoprotein synthesis. Hence, it was of interest to investigate whether glycosylation proceeds simultaneously with the development of cell membrane from crypt to villus. The results shown in Table 7 indicate no significant change in the incorporation of 1-^{14}C-D-glucosamine into the intestinal cells in retinol deficiency. Further, the extent of incorporation increases from crypt to the villus in both the groups and the latter could incorporate twice as much as the former.

Studies on *in vitro* incorporation of U-^{14}C-mannose revealed reduced incorporation into villus fraction (70 per cent compared to controls) in retinol deficiency (data not shown), that was compatible with the significantly reduced mannose content observed in extracted glycoproteins.

In conclusion, a significant quantitative reduction of a glycoprotein component of the rat intestinal villus cell membrane in retinol deficiency is observed. Cell agglutination studies using plant lectins suggested cell surface changes in the villus fraction. Carbohydrate analysis and tracer studies revealed reduced mannose in the cell membrane glycoproteins.

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Intestinal Epithelial Cell Alkaline Phosphatase in Retinol Deficient Albino Rats

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The activity of rat intestinal epithelial cell alkaline phosphatase revealed a significant decrease in the villus cells in retinol deficiency. There was no recognisable decrease in activity from the cells of the crypt region. Non-specific esterase activity also exhibited a similar trend. A significant decrease in activity was noted in the middle fraction in retinol deficiency. The cell count showed a significant difference in the lower villus-middle zone in retinol deficiency indicating a reduced ability of the cells to migrate out of the crypt zone. An assessment of the activity of the soluble and membrane forms of alkaline phosphatase suggested a vectorial gradation in activity. Electrophoretic analysis of alkaline phosphatase isoenzymes revealed minor changes only in the villus and middle fraction in retinol deficiency.

It is universally accepted that retinol is required for growth and differentiation. Studies by De Luca *et al.*[1] and Corey and Hayes[2] on rat intestinal mucosa in retinol deficiency, indicated that the goblet cells are significantly reduced in number in addition to altered biochemical functions. It was also shown that retinol influences the direction of differentiation in epithelial cells[3]. The importance of retinoids have been emphasized in their requirement for the normal urothelial differentiation[4] and for the maintenance of normal mucociliary epithelium[5]. Intestinal epithelial cells are continuously renewed once in 72 hr and are formed from a principal cell that multiplies inside the crypt region. These cells will gradually move up the villi, differentiate into different types of cells. During the process of differentiation, the cells acquire morphological[6], enzymatic[7] and transport[8] characteristics of mature enterocytes. Weiser[10] studied alkaline phosphatase, a membrane-bound enzyme as an indicator of intestinal epithelial cell differentiation.

In this paper, the effect of retinol deficiency on rat intestinal epithelial cells at different stages of differentiation has been reported by following alkaline phosphatase activity as a marker for cell differentiation.

Materials and Methods

Retinol deficient rats: Weanling female albino rats were made retinol deficient by feeding retinol-free diet as reported earlier[9]. Rats were killed by cervical dislocation and the intestinal epithelial cells were fractionated according to Weiser[10].

Intestinal epithelial cell fractionation: Epithelial cells were fractionated from non-fasted female rats. Intestine was excised and rinsed once with 0.154 M sodium chloride containing 1 mM dithiothreitol (100-125 ml/intestine). They were filled with solution A (potassium chloride 1.5 mM, sodium chloride 96 mM, trisodium citrate 27 mM, potassium hydrogen phosphate 8 mM, disodium hydrogen phosphate 5.6 mM, pH 7.3) and incubated at 37°C for 15 min. The contents were discarded. The intestine was filled with solution B (Potassium dihydrogen phosphate 8 mM, disodium hydrogen phosphate 5.6 mM, EDTA 1.5 mM and dithiothreitol 0.5 mM, pH 7.3) and incubated at 37°C for varying periods of time to detach the cells sequentially from the villus tip to the crypt base. Fractions 1, 2, 3 and 4 were obtained sequentially by incubating separately for 4, 2, 2, and 3 min. respectively. Fractions 5 and 6 were collected after 4 and 7 min. respectively and fractions 7 and 8 were collected after 10 and 15 min respectively. Fractions 1 to 4, 5-6 and 7-8 representing the villus, middle and crypt regions respectively were pooled and cells collected by centrifuging at 900 g for 10 min. in polyallomer tubes. Cells were suspended in appropriate buffer before use. Cell viability was tested by Trypan blue dye exclusion method. The number of viable cells were counted in all the individual fractions.

Assay of alkaline phosphatase activity: Pooled

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epithelial cell fractions were sonicated for 60 seconds in cold phosphate buffered saline without Ca^{++} and Mg^{++}. The sonicate was centrifuged at 27000 g for 20 min. at 4°C. The activity was determined in the supernatant using p-nitrophenyl phosphate according to Weiser[10]. The assay system consisted of 0.5 M tris HCl buffer, pH 9.4, containing 0.3 mM zinc chloride, 10 mM magnesium chloride, and 0.23 mM p-nitrophenyl phosphate substrate in a total volume of 1.0 ml taken in a test tube. Reaction was initiated by adding 0.1 ml of enzyme extract. Contents were incubated at 37°C for 15 min. Reaction was terminated by adding 0.5 ml of 0.5 N sodium hydroxide. The contents were clarified by centrifugation at 4000 rpm/15 min. The product, p-nitrophenol is estimated in the supernatant by measuring the absorbance at 420 nm. with reference to a standard curve for p-nitrophenol.

Assay of non-specific esterase activity: Esterase activity was determined essentially according to Hipps and Nelson[11] except that fast blue BB was used instead of fast garnet.

A tube containing 0.42 mM 1-naphthyl acetate, 2.4 per cent acetone, 40 mM sodium phosphate buffer (pH 7.0) and enzyme in a total volume of 2.5 ml was incubated at 29°C for 10 min. The reaction was terminated by adding 0.5 ml fast blue BB solution (0.075 g in 15 ml of 10 per cent (w/v) sodium dodecyl sulphate). After 15 minutes, the absorbance at 560 nm was measured. The activity in terms of α-naphthol liberated was calculated with reference to a standard curve for α-naphthol.

Electrophoresis of alkaline phosphatase: The method of Smith *et al.*[12] was followed for the electrophoretic analysis of isoenzymes of alkaline phosphatase on 5 per cent polyacrylamide gels. The gels were stained for alkaline phosphatase activity using α-naphthyl phosphate and fast blue BB before scanning at 595 nm. Membrane forms of alkaline phosphatase were analysed by including 0.1 per cent (w/v) Triton-X-100 in the above procedure.

Results and Discussion

The specific activity of alkaline phosphatase in the soluble fraction of intestinal epithelial cells is shown in Table 1. A reduction in the activity is observed in the first fraction representing the cells from the villus tip in retinol deficiency. Fractions two to four representing the cells from the villus region also revealed reduced activity. There was no recognisable change in the activity in fraction six to eight corresponding to the cells originating predominantly from the crypt region.

Non-specific esterases have also been suggested as markers of cell proliferation and differentiation in the intestinal epithelial cell renewal system[13]. An assessment of the activity indicated an elevated activity in the differentiated villus cells and low activity in the undifferentiated crypt cells (Table 1). It further revealed that in retinol deficiency, the activity at the villus/crypt zone, viz., the middle zone is reduced. This could be due to the presence of undifferentiated crypt cells in the functional villus zone with inherent lower esterase activity.

The cells in each fraction were counted to get an idea of the cell migration along the villi in terms of the cell number. Results (Table 1) revealed that in each fraction, the pattern of migration was unaltered in the crypt (7 and 8) and upper villus (1 and 2) zones. A significant difference is observed in retinol deficiency

TABLE 1. ENZYME ACTIVITIES IN RAT INTESTINAL EPITHELIAL CELL MEMBRANE FRACTIONS IN RETINOL DEFICIENCY

Cell fraction	Cell number[a]		Alkaline phosphatase[b]		Esterase[c]	
	Control	Retinol deficient	Control	Retinol deficient	Control	Retinol deficient
1	3.7	3.1	3.7 ± 0.7	1.7 ± 0.1	14.4 ± 2.6	14.1 ± 1.0
2	5.9	7.1	2.4 ± 0.6	1.0 ± 0.5	15.4 ± 1.9	12.9 ± 1.2
3	9.1	3.1	1.6 ± 0.3	0.6 ± 0.1	14.1 ± 1.5	11.2 ± 0.8
4	12.4	6.7	1.6 ± 0.5	0.9 ± 0.2	11.7 ± 1.3	10.5 ± 1.2
5	8.1	5.1	1.7 ± 0.5	0.9 ± 0.2	14.5 ± 1.1	9.9* ± 0.9
6	11.9	4.9	1.2 ± 0.2	1.0 ± 0.1	12.9 ± 2.1	13.8 ± 1.6
7	8.8	8.1	1.0 ± 0.1	1.2 ± 0.2	11.5 ± 1.8	11.5 ± 1.5
8	8.6	9.4	1.0 ± 0.1	0.7 ± 0.3	12.7 ± 1.9	8.4* ± 2.1

Values are the mean ± SEM of five independent determinations.
*$P<0.05$.
a $\times 10^6$
b μ moles p-nitrophenol liberated/mg/15 min.
c μ moles α-naphthol liberated/10 min/mg.

TABLE 2. RAT INTESTINAL EPITHELIAL CELL ALKALINE PHOSPHATASE IN RETINOL DEFICIENCY

	Intestinal epithelial cell fractions					
	Villus		Middle		Crypt	
	Soluble	Membrane	Soluble	Membrane	Soluble	Membrane
Control	0.22 ± 0.02	5.7 ± 0.9	0.3 ± 0.03	5.4 ± 0.9	0.11 ± 0.03	2.9 ± 0.6
Retinol deficient	0.14 ± 0.02 (63)	3.5 ± 0.4 (61)	0.09 ± 0.01 (45)	1.8 ± 0.2 (38)	0.09 ± 0.02 (81)	1.7 ± 0.3 (58)
p value	<0.05	<0.01	<0.005	NS	NS	NS

Values are the mean ± SEM of five independent determinations.
Activity expressed as μ moles p-nitrophenol released/mg/15 min.
Figures in parentheses indicate per cent change
NS – not significant

in the number of cells from lower villus (fractions 3 and 4) and the middle zone (fractions 5 and 6). Thus in retinol deficiency, the cells exhibit their inability or reduced efficiency to migrate out of the crypt zone. Thus, as the intestinal epithelial cell differentiates from crypt to villus, the specific activity of the marker enzyme also increases concomitantly. Reduced activity in the villus zone indicates the specific action of retinol on the differentiating cells whereas the activity in the crypt fraction comprising the undifferentiated cells is not affected.

Alkaline phosphatase, in rat, is present in the soluble as well as the membrane-bound form[14]. These two activities were assayed and the results are given in Table 2. The activity in the soluble fraction is significantly reduced in the villus and middle zones in retinol deficiency without affecting the activity in the crypt zone. The activity is in the same range in the retinol deficient group whereas in the control group, the villus and middle fractions had two fold activity than the crypt fraction. The activity in the membrane is also reduced in retinol deficiency in the middle and villus fractions.

The decrease in the alkaline phosphatase activity in the soluble and membrane forms is higher in the villus compared to the middle fraction suggesting a vectorial gradation in activity. Thus, the results reveal that both forms of alkaline phosphatases are diminished in the villus and middle fractions indicating that these enzyme activities show a common property of being influenced by retinol. It has been reported that these two activities are antigenically identical but distinct biochemically (indicating marker of differentiation). Reduced alkaline phosphatase activity in the villus zone indicates the specific action of retinol on the differentiating cells whereas the activity in the undifferentiated crypt cell is not affected at all. Recent studies by Sasak *et al.*[15] showed that changes in cell surface are the result of altered glycosylation mechanism that could affect the process of differentiation. Hence, the results are suggestive of an impairment in the cell extracellular matrix interaction in the retinol deficient

TABLE 3. RAT INTESTINAL EPITHELIAL CELL ALKALINE PHOSPHATASE IN RETINOL DEFICIENCY (RELATIVE PERCENTAGES)

Activity Band No. (Iso-enzyme)	Intestinal epithelial cell fractions					
	Villus		Middle		Crypt	
	Control	Retinol deficient	Control	Retinol deficient	Control	Retinol deficient
1	19.3 ± 2.9	11.5 ± 1.8	14.8 ± 1.8	9.6 ± 1.3	ND	ND
2	12.2 ± 1.5	11.4 ± 0.8	14.5 ± 2.8	13.2 ± 2.4	12.1 ± 2.2	8.3 ± 1.9
3	43.4 ± 6.6	54.0 ± 3.3	49.5 ± 5.7	62.5 ± 5.1	70.0 ± 5.0	79.8 ± 5.5
4	20.0 ± 2.9	18.3 ± 2.9	19.8 ± 3.2	11.0* ± 2.0	19.0 ± 2.4	14.5 ± 3.0
5	9.8 ± 2.9	6.1 ± 1.8	3.2 ± 1.5	4.2 ± 1.4	ND	ND

Values are the mean ± SEM of five independent determinations. The relative percentages were computed after densitometry.
*Significant $P<0.05$; ND – Not detectable

status hindering the cell migration along the villi.

The isoenzymes of alkaline phosphatase were identified on 5 per cent polyacrylamide gels after electrophoresis and enzymatic staining. The soluble form of the enzyme showed five bands with varying intensities in the villus and middle zones whereas only three bands could be seen in the crypt fraction constantly. The intensities of these forms were analysed by densitometry and the results are given in Table 3. The first band in the villus and middle zones showed a significant decrease in activity in retinol deficiency, supporting the results in Table 2. The crypt fraction did not reveal any change in activity between the two groups. The isoenzymic activity of the membrane form of alkaline phosphatase as analysed by staining on electrophoretic gels revealed only two activity and protein bands, which did not show any change in activity in retinol deficiency. To lend further support to the biochemical observations, the alkaline phosphatase activity was followed histologically in cryostat sections of the intestinal duodenum, jejunum and ileum. The control group revealed characteristic high enzyme activity in different parts of the intestines. Significant to moderate reduction in activity is observed in duodenal, jejunal and ileal sections of retinol deficient group.

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DETERMINATION OF PHENOLIC ACIDS IN DIFFERENT SOYBEAN VARIETIES BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Phenolic acids in different soybean varieties were determined by reverse phase HPLC using a Bondapack C_{18} column. The soybean varieties contained gallic 0.16-2.89, protocatechuic 0.20-0.54, chlorogenic 1.70-8.30, p-hydroxy benzoic 0.72-2.70, vanillic and caffeic 1.49-7.88, syringic 4.12-4.70, p-coumaric 1.79-4.30 and ferulic 1.71-4.16 μg/g. Most of the varieties contained 2 to 6 phenolic acids except 'PK-416' which had all the nine phenolic acids.

Soybean is a good source of calories and proteins. Varieties of acceptable foods such as soy-milk[1], soy-yoghurt[2], soy-paneer[3] and extruded products[4] have been developed from soybeans. Besides this, soybeans can be effectively used to fortify traditional foods such as chapati[5] and bakery products[6]. However, greater utilization of soybeans as food is primarily limited because of development of beany flavour during processing. Soybeans contain a number of phenolic compounds including phenolic acids and their esters. In addition to lipoxygenase activity, a key relationship between these compounds and flavour defects of soybean based foods has been demonstrated[7].

Maga and Lorenz[8] detected a number of phenolic acids in defatted soy flour and demonstrated that presence of p-hydroxybenzoic, vanillic, ferulic, and p-coumaric acids imparted astringent flavour to the protein supplement. Greuell[9] observed that p-coumaric and ferulic acids undergo thermal decarboxylation to form 4-vinyl phenol and 4-vinyl guaicol during processing which adversely affect the flavour of soy based foods.

The present investigation was undertaken to screen Indian soybean varieties for phenolic acids and quantify them using high performance liquid chromatography (HPLC), as gas liquid chromatography causes their thermal decomposition. Results obtained by using modified solvent system isocratically for effective separation of phenolic acids from different soybean varieties are reported in this paper.

Soybean varieties: Samples of soybean varieties 'PK-262', 'PK-327', 'PK-426', 'PK-564', 'PK-566', 'PK-749', 'T-49', 'D-5-76-37-1', 'Kalitur' and 'Bragg' were kindly supplied by Dr. H.H. Ram, Dept. of Plant Breeding of this University. The soybeans were cleaned manually and ground into 100 mesh flour which was stored in air tight plastic containers at ambient temperature until use.

Methanol and acetic acid HPLC grade) were obtained from M/S Spectrochem Pvt. Ltd. Bombay. Deionized and triple distilled water was used throughout this study. Phenolic acids obtained from M/S Sigma Chemical Co. U.S.A. were used as standards.

HPLC: Beckman Model 322 HPLC equipped with 100 A pumps, 410 injector, 420 micro-processor-controller, 160 U.V. detector at a fixed wave length of 254 nm and Kipps and Zonen BD-40 recorder was used in this study. The absorbance of the samples as well as of standards was recorded at 0.5 second time constant and AUFS 0.02. Column used was 250 mm × 4.6 mm I.D.μ Bondapack C_{18} (0.5 μm, Altex, U.S.A.). The mobile phase was 4 per cent aqueous acetic acid and methanol in the ratio of 73:27 (v/v) at a flow rate of 0.8 ml/min.

Extraction: The phenolics were extracted according to the procedure of Charpentier and Cowler[10] with the modification that ethyl acetate was replaced with ether[11] for extraction and shorter heating time was used. Five grams of soybean flour were transferred to a conical flask containing 100 ml of 2 N HCl. The contents were heated for 30 min. on a boiling water bath, cooled to room temperature and filtered. The filtrate was transferred to a separating funnel and extracted with 150 ml ether (3×50 ml). The combined ethereal layer was washed with distilled water and dried over anhydrous sodium sulfate. It was then filtered and evaporated under nitrogen. The residue thus obtained was dissolved in 2 ml methanol and kept at 5°C until use. The samples were filtered and 5 μl was injected into the HPLC system.

Among the phenolic acids determined, gallic acid was present in six varieties in detectable amounts whereas in the remaining either it was altogether absent or present only in traces (Table 1). It was found in significant amounts in varieties 'PK-416' (2.89 μg/g) and 'PK-262' (2.56 μg/g). Protocatechuic acid was present only in varieties 'PK-327' (0.21 μg/g) and 'PK-416' (0.54 μg/g). Chlorogenic acid was detected in 8 varieties with maximum content in 'T-49' (8.29 μg/g) and minimum in 'Bragg' (1.70 μg/g).

TABLE 1. PHENOLIC ACIDS (μG/G) IN DIFFERENT SOYBEAN VARIETIES*

Variety	Gallic	Chlorogenic	p-hydroxy-benzoic	Vanilic + Caffeic	p-coumaric	Ferulic
PK-416	2.89	4.25	2.22	5.50	3.58	4.16
PK-749	0.54	2.76	1.59	3.58	1.79	1.96
PK-262	2.56	5.74	2.70	7.89	4.30	1.96
PK-564	0.71	1.95	2.06	—	2.51	—
T-49	0.16	8.29	0.72	1.49	1.79	—
PK-566	0.76	2.55	1.50	2.87	2.55	—
Kalitur	—	7.23	—	1.49	1.79	1.71
D5-76-1-37-1	Tr	—	0.96	—	Tr	—
Bragg	Tr	1.70	—	4.84	—	—
PK-327	—	—	—	4.24	—	—

*Values are average of three replications
Tr = Traces

P-hydroxybenzoic acid was present in only 7 varieties and its content in these varieties ranged from 0.96 to 2.70 μg/g. Vanillic and caffeic acids were major acids in 'PK-262' (7.89 μg/g), 'PK-416' (5.50 μg/g), 'PK-327' (4.24 μg/g) and 'Bragg' (4.84 μg/g).

Syringic acid was detected only in varieties 'PK-416' (4.12 μg/g) and 'PK-564' (4.70 μg/g). P-coumaric acid was found in detectable amounts in the range of 1.79 to 4.30 μg/g in 7 varieties only. The maximum concentration of ferulic acid was detected in 'PK-416' (4.16 μg/g) whereas its range in 'PK-749' 'PK-262' and 'Kalitur' was from 1.71 to 1.96 μg/g (Table 1).

Arai *et al.*[12] reported that syringic acid was the principal phenolic acid found in defatted soybean flour. However, in the present investigation, this acid was found to be present only in two varieties out of ten tested. Seo and Morr[13] found syringic, ferulic and sinapic acids to be major phenolic acids present in defatted soybean flour. In contrast, results of this investigation showed that chlorogenic acid and combined vanillic and caffeic acids were present in majority of the soybean varieties in maximum amounts followed by P-coumaric and P-hydroxybenzoic acids.

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EFFECT OF PRE-HARVEST SPRAYS OF TRIACONTANOL ON THE STORAGE LIFE OF LETTUCE (*LACTUCA SATIVA* L.) cv. GREAT LAKES

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Preharvest sprays of triacontanol(TRIA) reduced the loss of ascorbic acid and bacterial decay in prepacked lettuce heads, but had no effect on physiological loss in weight and storage life. But TRIA sprays improved the storability of lettuce heads packed in polyethylene bags (7.22 and 6.66 days in polythene bags without vents and with 0.20% vents respectively).

Lettuce is the most widely cultivated vegetable crop in the world for its rich source of vitamins and minerals[1]. Lettuce prepacked in polyethylene bags can be stored upto 21 and 14 days at the temperatures of 5 and 10°C. However, due to the non-availability of such facilities under local conditions, an attempt was made to extend the storage life of lettuce by using triacontanol (TRIA), a growth stimulant, known to delay the senescence[2].

Field grown lettuces were sprayed once and twice with TRIA (2,4,6 and 8 ppm at an interval of 15 and 30 days after transplanting). Control treatments received water sprays. Lettuce harvested and packed in polyethylene bags of 100 gauge thickness and the content in each bag ranged from 1.15 to 1.3 kg. The treatments consisted of; no ventilation, 0.20 per cent ventilation and unpacked. Observations were recorded on the loss of ascorbic acid, physiological loss in weight, spoilage due to decay and storage life of lettuce heads.

Ascorbic acid content was determined by 2,6-dichlorophenol indophenol method[3]. Physiological losses in weights of packed and unpacked lettuces were calculated by weighing the produce daily until, they were judged unsuitable for marketing. The spoilage due to decay of packed lettuces was calculated by weighing the rotten portion. The economic storage life of packed lettuce was determined based on the extent of unusable portion ≥ 30 per cent of the total sample weight, whereas, the economic storage life of unwrapped lettuces was calculated based on the PLW ≥ 10 per cent of the total weight[3]. The results are presented in Tables 1,2 and 3.

The results indicate that the TRIA treatments reduced the loss of ascorbic acid (62.20 and 64.69 per cent) of packed lettuce heads on the 8th day of storage compared to water spray treatments (80.92 and 83.64 per cent), whereas, the differences were very small on 2nd day (Table 1). This observed differences could be attributed to the delay of senescence of TRIA similar to that of Benzyl adenine[2].

The physiological loss in weight was unaffected by the TRIA sprays, but prepackaging reduced it significantly (Table 2). The reduction in physiological losses in

TABLE 1. EFFECT OF TRIA ON THE PER CENT LOSS OF ASCORBIC ACID CONTENT OF POLYTHENE BAG PACKED LETTUCE HEADS, ON THE 8TH DAY OF STORAGE

Treatments	Bag without vents	Bag with 0.2% vents
Water sprays (control)	80.92	83.64
TRIA sprays	62.20	64.69

TABLE 2. EFFECT OF TRIA SPRAYS ON PHYSIOLOGICAL LOSS IN WEIGHT (%) AND STORAGE LIFE (AV. DAYS) OF LETTUCE HEADS, PACKED IN POLYTHENE (PE) BAGS

Treatments	Physiological loss in wt(%)		Storage period (days)	
	T sprays	Water sprays	T sprays	Water sprays
Unpacked	11.78	11.34	1.20	0.89
PE packs without vent	0.14	0.15	7.22	6.66
PE packs with 0.2% vents	3.48	3.34	6.66	6.00

'F' test showed highly significant difference only for prepackaging under physiological loss in wt and storage days.

TABLE 3. EFFECT OF TRIA ON SPOILAGE OF POLYTHENE (PE) PACKED LETTUCE HEADS STORED FOR EIGHT DAYS

Type of package	Spray	Spoiled portion (%) after indicated period (days) of storage			
		5 days	6 days	7 days	8 days
PE without vents	Water	3.88	25.59	32.34	40.50
"	TRIA	6.29	19.03	27.44	36.53
PE with vents	Water	14.01	28.76	33.59	37.63
"	TRIA	8.44	24.82	30.03	34.87

weights of packed lettuces may be attributed to impermeability of polyethylene bags to moisture loss[4]. Spoilage due to decay was observed in packed lettuces from the 5thday of storage (Table 3). TRIA sprays reduced the decay. Unventilated lettuces recorded comparatively less decay compared to ventilated ones. Similar results were reported by Midon and Lam[4] and Lipton and Ceponis[5].

Storage life of lettuce heads was unaffected by the preharvest sprays of TRIA (Table 2). Prepackaging increased the storage life significantly compared to unpacked lettuces. Similar results due to prepackaging were observed by Midon and Lam[4] and Aharoni and Yehoshua[6].

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VARIATION IN CURD FIRMNESS OF COLD-STORED RAW MILK FROM INDIVIDUAL COWS

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A decrease in curd firmness occurred among chymosin-coagulated milk samples with and without added 0.02% calcium chloride on cold storage (4°C for 24/48 hr). Raw milk samples from individual Holstein cows were used in this study. Milk samples were coagulated with chymosin in the Formagraph instrument at 37°C. Milk samples with poor coagulation characteristics after storage showed an average decrease of 27.7% in curd firmness as compared to 6.1% decrease among good coagulating samples after cold storage for 48 hr. Blended good-and poor-coagulating samples with $CaCl_2$ showed less decrease in curd firmness than those without added $CaCl_2$.

Cold storage is widely used to preserve raw milk, if there is delay in further processing. This practice may enable the survival and propagation of psychrotrophic bacteria[1]. Slow disruption of casein micelles due to reversible loss of temperature-sensitive β-casein into the serum phase is also known to occur[2,3]. These factors bring about reduction in the yield of cheese due to poor curd development and a concomitant loss of fat in whey[2,4].

In view of the reports[4,5] about milk samples from individual cows exhibiting poor chymosin-coagulation characteristics, very little has been documented on the effects of cold storage on coagulation properties of these samples. It has also been shown that 38 per cent of cows in a herd produced milk that did not coagulate with chymosin in 30 min one month prior to their dry periods[4]. In this study, an attempt has been made to study the effects of cold storage on curd firmness of chymosin-coagulated milk samples from individual cows.

Selection of milk samples: Twenty-five cows were randomly selected from each of two pens of mid-and late-lactation cows in the Utah State University Holstein herd. These stages of lactation were used because the widest variation in curd firmness was observed between them in an earlier study[4]. Evening milk samples were collected from each cow in August 1983. The samples were equilibriated at 4°C for 24 hr to insure a uniform temperature history for all the samples. Ten milliliters of each sample were tempered at 37°C for 90 min and were then coagulated with 200 μl of 0.4 rennet unit (RU)/ml of chymosin (Chris Hansens Laboratories Inc, Milwaukee, WI) at 37°C without replication in a Formagraph[6]; an instrument which has good repeatability with a standard deviation of ±0.15 on ten replicate coagulated milk samples[4]. Curd firmness was expressed in millimeters, and it was the width of Formagraph tracing recorded 30 min after chymosin addition. Milk with good chymosin coagulation characteristics (GCM) and milk with poor chymosin coagulation characteristics (PCM) were selected on the basis of curd firmness[7] and the issuing cows were identified.

Cold storage and coagulation of milk samples: Aliquots of GCM and PCM samples were stored at 4°C as follows: (a) 24 hr with and without added 0.02 per cent $CaCl_2$, (b) 48 hr with and without added 0.02% $CaCl_2$.

Two GCM and two PCM samples (50 per cent each) were separately blended and their aliquots stored at 4°C as follows: (a) 24 hr with and without added 0.02 per cent $CaCl_2$ (b) 48 hr with and without added 0.02 per cent $CaCl_2$.

Aliquots of GCM and PCM samples were stored at 4°C for 24 hr and at −1°C for 30 days.

All samples were coagulated after storage with chymosin without replication as described during sample selection.

Effect of cold storage on curd firmness: Decrease in curd firmness occurred among the samples after cold storage (Table 1). After 48 hr storage, PCM samples showed an average decrease of 27.7 per cent while in the GCM samples the decrease was 6.1 per cent except for two samples with identification numbers *5198* and *4532* in which curd firmness remained unchanged and increased by 12.5% respectively. Decrease of curd firmness also occurred in samples that were stored at −1°C for 30 days (Table 1). Significantly, greater decreases were observed among PCM (68.2 per cent) than GCM (7.6 per cent) samples. One PCM sample could not clot in 30 min after chymosin addition in consequence to prolonged storage. General decrease in curd firmness that

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TABLE 1. CURD FIRMNESS (mm AT 30 MIN) OF CHYMOSIN-COAGULATED RAW MILK SAMPLES FROM INDIVIDUAL COWS

Cow identity	Curd firmness (mm) when stored 24 hr	at 4°C 48 hr	Storage at −1°C for 30 days	Decrease in curd firmness (mm)	% decrease in curd firmness
Good chymosin-coagulating milk					
5270	56	51		5	8.9
5198	44	44		0	0
5330	41	39		2	4.9
5318	40	38		2	5.0
4532*	40	45		5	12.5
5012	37	35		2	5.4
5270	56		48	8	14.3
5326	56		51	5	8.9
5518	55		53	2	3.6
5484	55		53	2	3.6
Poor chymosin-coagulating milk					
58	33	31		2	6.1
4800	30	28		2	6.7
5186	19	12		7	36.8
5068	18	7		11	61.1
5278	17		7	10	58.8
5186	19		1	18	94.7
5138	12		0	12	100
5448	18		13	5	27.7

*Curd firmness increased in this sample

TABLE 2. EFFECT OF .02% CALCIUM CHLORIDE ON CURD FIRMNESS (MM AT 30 MIN) OF CHYMOSIN-COAGULATED RAW MILK SAMPLES FROM INDIVIDUAL COWS

Sample	Curd firmness (mm) when stored 24 hr	at 4°C 48 hr	Decrease in curd firmness (mm)	% decrease in curd firmness
A (GCM)	57	35	22	38.5
B (GCM)	44	27	17	38.6
C (PCM)	30	10	20	66.7
D (PCM)	30	30	0	0
Blend* (A+C)	38	27	11	28.9
Blend (A+C**)	40	44	4	+10***
Blend (B+D)	39	24	15	30.8
Blend (B+D**)	46	46	0	0

*Blended samples consisted of 50% each of milk with GCM and PCM characteristics

**0.02% $CaCl_2$ added before storage

**0.02% $CaCl_2$ added before storage

occurred with cold storage indicates slower aggregation rate of casein micelles[6,8]. This may be partly due to extreme proteolysis of casein[1,9]. Greater decrease in curd firmness observed in PCM than in GCM samples suggests greater proteolytic rates in the former. In a previous study[5], PCM samples exhibited higher levels of proteolysis as indicated by a high content of γ-caseins[10,11] as compared to GCM. Greater proteolytic activity that was observed in PCM than in GCM[5] may be related to the plasmin content of PCM. Plasmin activity is high in late lactation milk[12] and most PCM samples are late lactation milk[5]. Pasteurization of milk before cold storage would reduce microbial count, thus minimizing bacterial proteolysis. However, there are doubts as to the effectiveness of pasteurization in limiting other forms of proteolysis, especially those associated with plasmin group of proteinases[9].

Calcium ions are known to maintain the integrity of casein micelles by forming $Ca_9(PO_4)_6$ linkages between submicelles and this limits their disruption[13]. It is also known that β-casein dissociates from micelles into milk serum primarily because of its hydrophobicity and changes in salt equilibrium[11]. The effect of addition of $CaCl_2$ to cold stored milk on curd firmness is shown in Table 2. The trend of decrease of curd firmness in individual samples after 48 hr storage did not vary much from results in Table 1, but the decreases in Table 2 generally were of greater magnitude. For blended samples, however, those containing $CaCl_2$ showed less decrease in curd firmness than the corresponding blends without $CaCl_2$. Bulk storage tanks contain blended milk from individual cows and its storage for greater than 24 hr before cheesemaking is common. Since it has been established[2,3] that a decrease in curd firmness occurs in cold stored mixed milk, it may, therefore, be advantageous to add 0.02 per cent $CaCl_2$ to cheesemilk before cold storage. This probably would minimize curd firmness losses and therefore improve cheese yield[14].

Technological implications: Larger average decrease in curd firmness that occurred among PCM than GCM samples after cold storage further supports previous observations of this author[15]. In that study, it was recommended that periodic tests of individual cow's milk to detect poor chymosin-coagulation characteristics be done, and such milk should be excluded from cheese-milk. Long term achievement of this objective may be accomplished by selective breeding of lactating cows. Cows which produce milk that coagulate with chymosin throughout their lactation would, possibly, give offsprings with similar genetic characteristics.

Reimerdes[10,11,16] showed that 3 distinct proteolytic reactions occur in cold stored milks. First is the reversible disruption of casein micelles. This reaction could be counteracted by warming milk before chymosin-coagulation[2] and this was done in this study by tempering the milk samples at 37°C for 90 min. Secondly, the caseins are proteolyzed irreversibly by plasmin, a trypsin-like enzyme that converts β-, k- and α_S-caseins[17] into products that have proved

detrimental to curd formation[5]. This appears to be a problem without an easily discernible solution. However, since it was established that PCM contains more casein degradation fragments than GCM[5], exclusion of PCM from mixed milk for cheese-making appears to be an acceptable solution. Thirdly, enzymes produced by psychrotrophic bacteria proteolyse milk proteins non-specifically into soluble short peptides which impart undesirable taste to processed dairy products. Pasteurization or thermalization of cheese-milk before cold storage would reduce bacteria-derived proteolysis of milk proteins.

Further research is needed to compare proteinase activities of psychrotrophic bacteria and plasmin group of enzymes in cold stored raw milk.

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THE INFLUENCE OF STRAIN, AGE AND SEASON ON CHOLESTEROL, VITAMIN A AND FATTY ACID CONTENTS OF EGG

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Changes in cholesterol, vitamin A and fatty acid contents of egg due to strain and age of birds and season of the year were investigated. Control had higher egg weight and contained more cholesterol and vitamin A than the selected strains. Cholesterol level in the egg decreased while vitamin A content increased with increase in age of hens and advancement of season from winter through fall. Of all the fatty acids studied, only stearic acid varied among strains. Oleic acid content was higher in pullet eggs whereas linoleic acid increased continuously with age. Winter eggs had more oleic and arachidic acids and less palmitoleic and linoleic acids than summer and fall eggs.

Current interest in the cholesterol as related to human health has stimulated the study of various factors which influence the cholesterol level in the egg. Breed and strain differences in cholesterol[1], vitamin A[2] and fatty acid[3] contents of egg have been reported, although very limited data are available to ascertain the extent of genetic differences in vitamin A and fatty acid contents. Conflicting reports are available on the effect of advancing age of hens on cholesterol content[4,5] and possibly no attempts have been made so far to study the influence of age and season on vitamin A and fatty acid contents. The present investigation was thus, undertaken to study the influence of strain, age and season on cholestrol, vitamin A and fatty acid contents of egg.

Eggs were sampled from 5 different laying strains of White Leghorn ('IWG', 'IWH', 'IWI', 'IWJ' and Control) at 20-24, 28-32, 38-40, 50-54 and 60-64 weeks of age in winter, summer and fall seasons of the year. Control was a random-bred population, closed flock with pedigree mated, while others were selected strains maintained by closed flock selection for high part period egg production.

A total of 2,433 eggs laid by at least 50 birds in each strain per age group were weighed individually to the nearest 0.01 g on the same day of lay to determine the egg weight. Twenty eggs per group were broken out on the next day of lay, yolk separated and analysed for total lipid[6], cholesterol[7], and vitamin A[8] contents using 5 replicates of composite samples. Fatty acids of egg yolk lipid were estimated after preparing the methyl esters[9] by gas liquid chromatograph (Chemito 3800 of Toshniwal Instruments, Bombay) using flame ionization detector and 8 per cent diethylene glycol succinate (DEGS) column, having oven, injection and detection temperatures of 200, 220 and 230°C. Data were analysed statistically[10] and means were compared for significant differences[11].

Changes in egg weight, total lipid, cholestrol and vitamin A contents of egg are shown in Table 1. Significant ($P<0.05$) differences between strain, age and season were observed for egg weight. Control had higher egg weight than the selected strains. Among the selected strains, IWI was found superior over others. Egg weight increased significantly with increasing age of hens except at 50-54 weeks, where it reduced slightly. Since egg weight is the result of interaction of age and environmental temperature, this reduction may be attributed to the effect of high environmental temperature during that period. Seasonal trend indicated a significant gradual increase in egg weight from winter through fall seasons.

Total lipid and cholestrol levels in the egg varied significantly between strains with higher levels in control eggs. Significant strain differences for lipid and cholesterol contents have been reported[1]. Cholesterol content per g of yolk did not change between the onset of lay and peak production period (38-40 weeks) but decreased significantly thereafter as the bird aged. On the contrary, when expressed on per egg basis it was significantly higher during peak production period and then decreased. These results agree with Bair and Marion[5]. Cholesterol levels were significantly higher in winter than in summer and fall eggs which tally with those of Harris and Wilcox[12].

Control eggs were significantly richer in vitamin A content than those of selected strains. On individual egg basis, it increased gradually with increasing age and advancing seasons from winter through fall. No concrete evidence is available on changes in vitamin A level due to strain, age and season except, breed differences[2]. The increase in vitamin A level with increasing age and advancing season may partly be attributed to increasing egg size as the birds aged.

The results of fatty acid composition of egg yolk lipid (Table 2) revealed that the strain effect was significant only for stearic acid as observed by

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TABLE 1. INFLUENCE OF STRAIN, AGE AND SEASON ON EGG WEIGHT, TOTAL LIPID, CHOLESTEROL AND VITAMIN A CONTENTS OF EGG

Effect	Egg wt (g)	Total lipid (%)	Total cholesterol		Vitamin A	
			mg/g yolk	mg/egg	IU/g yolk	IU/egg
Strain						
IWG	48.72^{ac} ± 0.23	33.26^{a} ± 1.20	3.31^{bc} ± 0.13	47.85^{bc} ± 2.86	17.59^{a} ± 0.58	259.82^{a} ± 6.60
IWH	49.15^{a} ± 0.25	32.65^{a} ± 1.02	3.33^{bc} ± 0.21	45.22^{abc} ± 5.56	18.63^{ab} ± 0.48	260.86^{a} ± 7.42
IWI	50.33^{b} ± 0.26	35.62^{ab} ± 1.10	2.74^{a} ± 0.23	40.23^{a} ± 4.94	18.78^{b} ± 0.71	280.56^{b} ± 10.81
IWJ	48.30^{c} ± 0.23	33.91^{a} ± 0.86	2.98^{ab} ± 0.14	41.59^{ab} ± 1.90	18.44^{ab} ± 0.56	261.92^{a} ± 8.34
Control	52.05^{d} ± 0.27	37.31^{a} ± 1.07	3.62^{c} ± 0.23	50.24^{c} ± 5.12	21.12^{c} ± 0.69	303.89^{c} ± 10.62
Age (week)						
20-24	43.00^{a} ± 0.16	35.34 ± 0.90	3.45^{b} ± 0.21	41.53^{ab} ± 4.79	16.22^{a} ± 0.61	194.49^{a} ± 13.43
28-32	47.65^{b} ± 0.17	36.30 ± 1.02	3.66^{b} ± 0.23	47.39^{b} ± 5.77	14.99^{b} ± 0.36	194.61^{a} ± 9.84
38-40	51.88^{c} ± 0.19	31.18 ± 1.67	3.96^{b} ± 0.15	59.17^{c} ± 4.03	20.48^{c} ± 0.38	304.66^{a} ± 5.80
50-54	51.57^{c} ± 0.22	34.39 ± 0.80	2.60^{a} ± 0.15	40.12^{ab} ± 4.39	19.22^{d} ± 0.27	304.97^{a} ± 6.74
60-64	53.39^{d} ± 0.21	35.54 ± 0.44	2.32^{a} ± 0.05	36.13^{a} ± 1.30	23.76^{c} ± 0.28	371.32^{b} ± 6.45
Season						
Winter	47.66^{a} ± 0.14	34.27 ± 0.76	3.69^{a} ± 0.12	49.36^{a} ± 4.91	17.20^{a} ± 0.34	231.25^{a} ± 10.17
Summer	51.57^{b} ± 0.22	34.39 ± 0.80	2.60^{b} ± 0.15	40.92^{b} ± 4.39	19.22^{b} ± 0.27	301.97^{b} ± 6.75
Fall	53.39^{c} ± 0.21	35.54 ± 0.44	2.32^{b} ± 0.05	36.13^{b} ± 1.30	23.76^{c} ± 0.28	371.32^{c} ± 6.45

Effect-wise means in a column bearing atleast one common letter are not significantly ($P<0.05$) different.

Edwards[13]. The palmitoleic acid increased gradually with hens age upto 50-54 weeks and then reduced slightly, whereas oleic acid was higher in pullet eggs than that of hens. Linoleic acid increased continuously with layer's age. Winter eggs contained more oleic and arachidic acids and less palmitoleic and linoleic acids than summer and fall eggs. The predominant order of concentration of various fatty acids was observed to be 18:1 > 16:0 > 18:2 > 18:0 > 16:1 > 20:0, indicating that fatty acids consisted mainly of 16– and 18– carbon

TABLE 2. CHANGES IN FATTY ACID COMPOSITION (% OF TOTAL FATTY ACIDS) OF EGG YOLK LIPID DUE TO STRAIN, AGE AND SEASON

Effect	Palmitic acid (16:0)	Palmitoleic acid (16:1)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Arachidic acid (20:0)
Strain						
IWG	27.45 ± 1.45	5.43 ± 0.98	7.70^{ab} ± 0.47	51.59 ± 1.05	7.15 ± 0.88	1.95 ± 0.32
IWH	25.84 ± 0.73	4.32 ± 0.42	9.73^{c} ± 0.57	51.16 ± 1.29	7.72 ± 0.72	1.55 ± 0.20
IWI	25.69 ± 0.86	5.37 ± 0.76	8.11^{bc} ± 0.79	51.84 ± 1.68	9.01 ± 1.06	0.79 ± 0.21
IWJ	27.01 ± 1.03	6.64 ± 0.72	6.33^{a} ± 0.36	50.77 ± 1.38	8.52 ± 0.82	1.23 ± 0.05
Control	26.09 ± 0.87	6.02 ± 0.87	9.18^{bc} ± 0.61	49.95 ± 1.67	8.84 ± 0.75	1.17 ± 0.43
Age (week)						
20-24	26.32 ± 0.85	3.93^{a} ± 0.40	8.48 ± 0.57	55.39^{c} ± 1.35	6.18^{a} ± 0.55	1.14 ± 0.22
28-32	26.88 ± 0.91	4.20^{a} ± 0.57	8.30 ± 0.79	52.55^{bc} ± 1.38	7.02^{a} ± 0.79	1.53 ± 0.28
38-40	26.91 ± 1.25	5.56^{ab} ± 0.43	8.87 ± 0.65	49.39^{ab} ± 1.15	7.88^{ab} ± 0.44	1.50 ± 0.19
50-54	25.25 ± 1.34	7.73^{c} ± 0.74	7.63 ± 0.58	49.36^{ab} ± 1.03	9.72^{bc} ± 0.76	0.79 ± 0.39
60-64	26.71 ± 0.90	6.46^{bc} ± 1.00	7.76 ± 0.79	48.61^{a} ± 0.90	10.45^{c} ± 0.89	—*
Season						
Winter	26.70 ± 0.57	4.53^{a} ± 0.29	8.55 ± 0.38	52.44^{b} ± 0.85	7.03^{a} ± 0.36	1.39^{a} ± 0.12
Summer	25.25 ± 1.34	7.73^{b} ± 0.74	7.63 ± 0.58	49.36^{ab} ± 1.03	9.72^{b} ± 0.75	0.79^{b} ± 0.39
Fall	26.71 ± 0.90	6.46^{b} ± 1.00	7.76 ± 0.79	48.61^{a} ± 0.90	10.45^{b} ± 0.89	—*

*Not detected

Effect-wise means in a column bearing atleast one common letter are not significantly ($P<0.05$) different.

acids, which agree with those of Bitman and Wood[14].

It is evident from the above results that strain and age of birds and season of the year do have definite influence on the cholesterol and vitamin A contents of eggs, while strains have little role to play in fatty acid composition of yolk lipid.

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A SIMPLE TECHNIQUE TO REMOVE UREA FROM SHARK (*CARCHARHINUS* SP)

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Urea content of shark muscle is mainly responsible for its unacceptability for human consumption as its slow degradation by bacterial action results in persistent ammoniacal odour. The urea in shark muscle could be rapidly eliminated by mixing the minced muscle with unroasted soya flour which contains urease. A 10% concentration of unroasted soya flour could eliminate the urea in shark muscle within 5.5 hr.

Trash fish constitutes about 30-40 per cent of the total marine catch in India[1]. Among the trash fish, shark (*Carcharhinus* sp) has poor consumer acceptability because of its high urea content (1–2.5 per cent W/V), though it is a rich source of protein (22.9 per cent)[2,3]. Besides being harmful, the urea in shark is slowly degraded to ammonia by the action of bacterial urease as the bacterial load gradually builds up during the storage of fish[4-6]. The resultant ammoniacal odour makes shark muscle unpleasant for human consumption. Urea content in shark meal has been reduced to 16 mg/100 g by cooking and pressing, followed by soaking in 1 per cent acetic acid overnight[7]. The unpleasant odour in shark could be eliminated if urea is rapidly removed from shark muscle using a source of urease. Unroasted soya flour has been reported to be a good source of urease[8]. This paper deals with a simple technique to eliminate urea in shark muscle for the preparation of sausages.

Freshly caught shark was purchased from Sasson Dock, Bombay, eviscerated and washed. The muscle was separated and minced mechanically by using a deboning machine[9]. The minced muscle was then mixed with unroasted soya flour in different proportions (0-20 per cent) (total weight of minced muscle and soya flour was 100 g) and incubated at ambient temperature (26-28°C). The samples (10 g) were withdrawn at regular intervals to determine the quantity of ammonia released. The sample was then suspended in 100 ml water and 10 ml of this suspension was treated with 10 ml of trichloroacetic acid (10 per cent W/V) to precipitate out proteins. After centrifugation at 5000 rpm. for 30 min, the supernatant

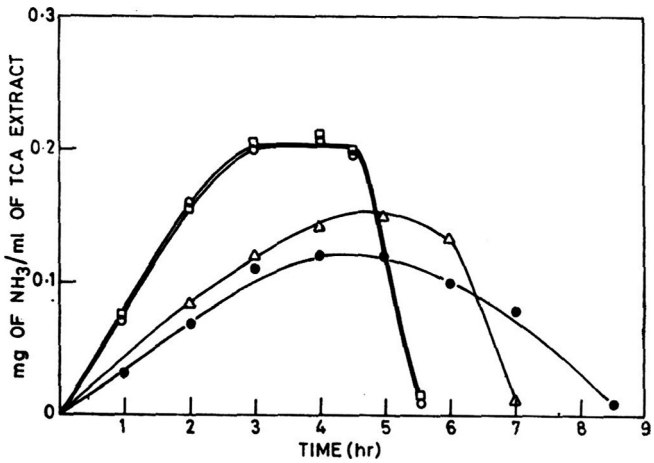

Fig. 1. Comparison of ammonia formation in minced shark meat with respect to time at different concentrations of soya flour.

●——● 1% – soya flour
△——△ 5% – "
o——o 10% – "
□——□ 20% – "

was utilised for the determination of ammonia by using the modified Nessler's reagent[10]. The determinations were carried out in triplicate and the final value was the average of three readings.

Fig. 1 depicts time dependent formation of ammonia with different proportions of unroasted soya flour. With an increase in the time of incubation, there was a concomitant increase in the formation of ammonia followed by a plateau and sharp decrease thereafter. The increase in soya flour proportion from 1-10 per cent showed an increased rate of formation of ammonia. However, the differences were not appreciable when the concentration of soya flour was increased beyond 10 per cent. The results showed that the incubation of shark muscle with 10 per cent soya flour for 5.5 hr eliminated the urea and consequently the subsequent development of ammoniacal odour. The unmixed shark muscle at the beginning of the experiment contained negligible quantity of ammonia (.0003–.0005 mg of NH_3/ml of TCA extract). The ammonia content in the control unmixed samples did not show a noticeable increase with the increase in incubation upto 5.5 hr, the period in which the minced shark muscle showed the maximal release of ammonia. The ammonia release from the soya flour mixed muscle stopped after 5.5 hr of incubation. Since it takes more than 6 hr for the building up of bacterial load in stored fish, the process of ammonia formation through bacterial action is negligible to start with. However, with the increase in storage periods beyond 6 hr, bacterial degradation of urea starts resulting in

persistent ammoniacal odour in shark. Since unroasted flour is a rich source of urease, mixing of shark muscle with soya flour resulted in quick release of ammonia and thereby depleted the shark muscle of its urea. Incubation of shark muscle with soya flour thus resulted in obtaining odour free product. Soya flour also serves as an excellent extender and binder for making sausages.

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A NOTE ON THE DISTRIBUTION OF *ALTEROMONAS PUTREFACIENS* IN MARINE FISHES LANDED AT COCHIN

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The study reveals that *Alteromonas putrefaciens* forms 0.11 to 3.28% of the total plate count (TPC) on fish and 0.88 to 31.6% of the total hydrogen sulphide producers. There was no correlation between TPC and count of hydrogen sulphide producers/*A. putrefaciens*. The study shows that the prevalence of *A. putrefaciens* is quite low in freshly landed fish. Important biochemical characteristics useful for identification of this species are also reported.

Several groups of microorganisms appear to be particularly associated with the production of sulphide like odours in spoiling flesh foods and the prominant among these is the microorganism referred to as *Alteromonas putrefaciens*[1]. This organism, originally isolated from putrid butter[2], has been isolated from many other sources such as milk, fish and poultry[3-6]. There are also several reports of its isolation from clinical materials[7-10]. Although it has been isolated from many clinical samples, its pathogenicity has not been proven. On the other hand, this organism has gained importance for its ability to cause spoilage of proteinaceous foods at low temperature.

There have been some confusion regarding the grouping of this bacteria. Originally, they had been grouped in the genus *Pseudomonas* by Long and Hammer[11]. This continued to be so until in 1977, Lee *et al.*[12] transferred them to a separate genus *Alteromonas* on the basis of numerical taxonomic studies. More recently, MacDonell and Colwell[13] placed them in a separate genus *Shewanella* which was composed of *Shewanella putrefaciens*, the type species and two related species, *S. hanedai* and S. *benthica*.

This is the first report of the distribution of *A. putrefaciens* in marine fishes from the west coast of India. Some of its biochemical properties which are of use for identification are also discussed.

Thirty six samples of fishes collected from the landing centres at Cochin during the period April 1986 to September 1986 were studied (Table 1).

The total plate count was determined using sea water agar (SWA) containing peptone, 10g, ferric phosphate, 50 mg; agar agar, 15 g and full strength sea water to make one litre. The medium was adjusted to pH 7.2 and autoclaved at 15 lb for 15 min.

Ten grams of the muscle with skin were removed aseptically from about 6 individual fishes, homogenized well in saline and one ml of the appropriate serial dilution was pour plated. Duplicate plates were incubated at room temperature for 48 hr and colonies were counted.

The number of hydrogen sulphide producing bacteria was also determined simultaneously on tryptone soytone agar (TSA), a medium developed in this laboratory. The medium contained, tryptone, 10g, soytone 5g, beef extract, 5g, sodium chloride, 5g, cysteine hydrochloride, 500 mg, magnesium sulphate ($MgSO_4$ $7H_2O$), 300 mg, ferric ammonium citrate, 200 mg, sodium thiosulphate, 300 mg, agar agar powder, 15 g and distilled water to make 1 l; pH was adjusted to 7.2. Sodium thiosulphate was added to the rest of the ingredients only after adjusting the pH. Ferric ammonium citrate was added separately as sterile solution just before pouring into the plates. These steps reduced the possibility of the medium getting blackened after sterilization. Details regarding inoculation, incubation etc. were the same as those of TPC. Black colonies indicating hydrogen sulphide production were counted after 48 hr and the count per g. in the muscle of hydrogen sulphide producers calculated.

The same plates that were used for the enumeration of hydrogen sulphide producers were used to determine the number of *Alteromonas putrefaciens*. Smooth, glistening, pinkish to reddish brown colonies of 1-3 mm diameter having a black centre could be easily distinguished from the rest of the bacterial flora. Such colonies were counted and the count of *Alteromonas putrefaciens* determined.

Biochemical characteristics: Typical colonies of *A. putrefaciens* were taken onto slants of soyabean casein, digest agar (Himedia), purified by repeated streaking on the same medium and maintained at room temperature.

Formation of salmon pink pigment and hydrogen sulphide production were tested on TSA. Sensitivity to vibriostatic compound 0/129 (2, 4-diamino-6, 7 diisopropyl pteridine phosphate) and penicillin was determined by the method of Van Spreekens[6]. Hugh and Leifson's[14] method was used to study the mode of attack on various sugars. Growth at various temperatures was tested by holding nutrient broth tubes inoculated with the organism at the respective temperatures. The effect of sodium chloride on growth was determined in nutrient broth to which different

TABLE 1. DISTRIBUTION OF *ALTEROMONAS PUTREFACIENS* IN FRESHLY LANDED MARINE FISHES

Fish	No. of samples	% *Alteromonas putrefaciens* in Total H_2S producers	Total count	% of H_2S producers in total count
Mackerel (*Rastrelliger kanagurta*)	6	6.09+	0.05	0.78
Vatta (*Decapterus russelli*)	4	28.40	0.12	0.40
Parava (*Lactarius lactarius*)	4	5.96	0.18	2.95
Jew fish (*Otolithes argenteus*)	4	2.42	0.11	4.51
Kathiran (*Sillago sihama*)	2	Nil	Nil	2.44
Mullet (*Mugil cephalus*)	4	0.88	0.07	7.52
Malan (*Mugil parsia*)	1	5.68	0.12	1.74
Sardine (*Sardinella longiceps*)	6	31.6	3.28	10.3
White sardine (*Kowata coval*)	1	27.47	1.19	4.32
Johnius sp.	4	8.80	0.50	5.68

+Average values of 3 replicates

quantities of sodium chloride were incorporated. All other tests such as catalase, oxidase, indole etc. were carried out as per the procedures outlined by Harrigan and McCance[15].

In the freshly landed fish samples, the percentage of hydrogen sulphide producers in the total microbial population varied from 0.40 to 10.3 per cent (Table 1). The total plate count in these samples ranged from 8.39×10^4/g to 8.82×10^7/g of muscle and the count of hydrogen sulphide producers from 1.41×10^3/g. to 8.4×10^5/g, *A. putrefaciens* formed 0.88 to 31.6 per cent of total hydrogen sulphide producers and 0.11 to 3.28 per cent of the total bacterial population in freshly landed fish. The count of *A. putrefaciens* ranged from 1.1×10^2/g to 8.9×10^3/g of the muscle with skin. But in two samples of 'Kathiran' (*Sillago sihama*), no *A. putrefaciens* could be detected.

TABLE 2. RELATIONSHIP BETWEEN TOTAL PLATE COUNT AND *A. PUTREFACIENS* COUNT

% of *A. putrefaciens*	No. of fish samples at indicated total plate count/g 10^4	10^5	10^6	10^7
Nil	Nil	Nil	5	Nil
<0.1	1	4	2	Nil
0.1 – 0.49	Nil	10	2	2
0.5 – 0.99	Nil	3	Nil	Nil
1.0 – 1.99	2	3	Nil	Nil
2.0 – 2.99	Nil	Nil	Nil	Nil
3.0 – 3.99	Nil	2	Nil	Nil
Total	3	22	9	2

A closer study of the results given in Table 1 shows poor correlation of total plate count with the count of hydrogen sulphide producers and *A. putrefaciens*.

The results are further highlighted by the data given in Table 2. Maximum number of samples had *A. putrefaciens* in the range of 0.1 per cent to 0.49 per cent. This shows that the prevalence of this bacterial species is negligible in the freshly landed marine fishes.

Chai *et al.*[6] noticed that the number of *Pseudomonas (Alteromonas) putrefaciens* in freshly caught haddock fillets were mostly below 1 per cent and never exceeded 4 per cent. The present data support this finding. The study also reveals that in more than 80 per cent of the samples, hydrogen sulphide producers constituted less than 10 per cent of the total microbial population (Table 1). These results also indicate that the fishes collected from waters in and around Cochin have a lower incidence of hydrogen sulphide producers compared to the retail fish samples from Jakarta[16] or Melbourne[17].

The biochemical characteristics of forty two cultures isolated from typical colonies were compared with those of the typical strain of *A. putrefaciens* (60423) received from National Institute of Public Health at Bilthoven.

All the isolates were motile, Gram negative rods producing pink or reddish brown pigment in medium containing beef extract, soytone, peptone or casein. The intensity of the pigment varied from strain to strain.

In Hugh and Leifson's medium with glucose, there was no action under anaerobic condition, but under aerobic condition, an alkaline reaction was observed at the top of the tube. Forty out of forty two isolates gave this type of reaction within 24 hr. and two gave delayed reaction. This type of reaction was noted by Van Spreekens for some of her isolates. There was also no acid production from other sugars such as fructose, sucrose, maltose, lactose, mannitol and arabinose.

All isolates were distinctly cytochrome oxidase and catalase positive. They reduced nitrate, but did not produce indole. All the cultures liquefied gelatin. Eighty five per cent of the cultures hydrolysed casein and 80 per cent peptonized milk. Citrate utilization and starch hydrolysis were absent in all isolates. None of them also showed any sensitivity to vibriostatic compound 0/129 or 2.5 IU of pencillin.

Using Moller's decarboxylase medium, all the cultures showed a negative reaction for arginine dihydrolase and lysine decarboxylase. However, all were ornithine decarboxylase positive. All except one were also urease negative. According to Lee *et al*[12]., the presence or ornithine decarboxylase can be considered as a test specific to *A. putrefaciens*.

All the strains in this study grew well at 5°C as well at 37°C, but failed to grow at 44°C. In the study carried out by Owen *et al.*[10] all the members of group III to IV which he grouped as clinical isolates failed to grow at 5°C. While all the cultures grew at 0.5 per cent sodium chloride, only 40 per cent of them grew at a concentration of 6.5 per cent sodium chloride. Growth at 6.5 per cent sodium chloride was noticed for some clinical isolates[9,10].

The study shows close similarities in biochemical properties of the isolates with the typical strains of *A. putrefaciens*. The only deviation was that typical culture failed to grow at 35°C. It did not grow in presence of 6.5 per cent sodium chloride also.

The study reveals that in freshly landed marine fishes, the number of hydrogen sulphide producers in general and *A. putrefaciens* in particular is very low. This results in the failure to detect this organism in the dilutions used for plate count of fresh fish specimens, when qualitative studies are undertaken.

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A NOTE ON THE MICROBIAL QUALITY OF SELECTED SPICES

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Microbial quality of seven spices – Omum asafoetida, fenugreek, cardamom, cinnamon and clove, collected from the market of Chandigarh were studied for antibiotic resistance, enterotoxigenicity and hydrophobicity of isolates of *Escherichia coli* and *Salmonella* obtained from the spices. The maximum resistance was towards Pencillin (77.5%). Nearly 40% of *E. coli* and 62% of *Salmonella* isolates were positive for enterotoxigenicity.

Spices used in various foods are known to harbour a wide variety of microorganisms, several of which may be hazardous to the health of the consumers. Several genera of enterobacteriaceae have been isolated from spices[1,2]. Many of them are known to be pathogenic and resistant to antibiotics like Pencillin, Tetracycline, Streptomycin and Kanamycin[3,4]. Besides, hydrophobicity is an important determinant of pathogenicity of an organism[5]. Though spices are widely used, their microbiology has not been well studied. In this study, an attempt is made to study the microflora of spices with special reference to antibiotic resistance, hydrophobicity and enterotoxigenicity of enterobacteriaceae members isolated from the spices.

Seven spices – Omum (*Cardum copticum*), asafoetida (*Ferula asafoetida*), fenugreek (*Trigonella foenumgraecum*), Cinnamon (*Cinamonum zeylanicum*, big and small cardamom (*Elettaria cardamomum*), and cloves (*Caryophyllus aromaticus*), were obtained from different grocery stores in different sectors of Chandigarh and stored in sterile, stoppered bottles. Five or six samples of each spice were taken.

The total bacterial count of spices was enumerated by taking one gram of the sample, diluting it in normal saline and pour plating it using nutrient agar. The spice samples were streaked on Mac Conkey's agar; the isolated colonies of these gram negative organisms were tested biochemically by the following tests: Fermentation of carbohydrates, indole production, Methyl-Red Test, Voges-Proskaur Test, Citrate Utilisation Test, Nitrate Reduction Test, Urease Test and Motility Test. *E. coli* and *Salmonella* were then identified with the help of Bergey's Manual[6]. The antibiotic resistance of *E. coli* and *Salmonella* isolates was determined by the method of Bauer *et al.*[7] while enterotoxigenicity and hydrophobicity were tested by the method of Wadstrom *et al.*[8] and Lindahl *et al.*[9] respectively.

The average counts obtained from the various spices are shown in Table 1. The counts vary among different spices as well as among different samples of the same spice. The total bacterial load is high when compared to the I.S.I. standard of 5.0×10^4 CFU/g. The large variation in count both between spices and samples of the same spice is in accordance with the results obtained by Christensen *et al.*[10], who reported a wide variation in bacterial counts of spice samples.

Table 2 shows antibiotic resistance patterns of the isolates of *E. coli* and *Salmonella*. They showed maximum resistance towards Pencillin (77 and 93 per cent respectively) while Chloramphenicol was the antibiotic to which least resistance was shown (nil and 18 per cent respectively). The frequency of multi drug resistance was fairly high (39.04 per cent). The common resistance patterns were P (22.6 per cent), T and PTE (9.7 per cent). There existed a relation between drug resistance and source of isolation, wherein majority of the resistant strains were from

TABLE 1. MICROBIAL QUALITY OF SPICES

Spice	Total bacterial count (CFU/g)			No. of isolates obtained	
	Min	Max	Average	*E. coli*	*Salmonella*
Omum	3.2×10^4	5.5×10^7	2.7×10^7	3	2
Asafoetida	2.5×10^2	3.0×10^5	1.5×10^4	2	5
Fenugreek	1.8×10^7	8.4×10^7	5.1×10^7	2	5
Cardamom (Big)	1.0×10^3	1.0×10^5	5.0×10^4	3	5
Cardamom (Small)	7.0×10^2	2.6×10^5	1.3×10^5	2	7
Cinnamon	2.0×10^4	4.8×10^4	3.4×10^4	2	4
Cloves	7.0×10^4	9.9×10^7	4.9×10^7	1	2

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TABLE 2. ANTIBIOTIC RESISTANCE PATTERN, ENTEROTOXIGENICITY AND HYDROPHOBICITY OF ISOLATES OF *E. COLI* AND *SALMONELLA* OBTAINED FROM SPICES

Spices	Antibiotic resistance pattern (%)							Entero-toxige-nicity	Hydro-phobicity
	PI	TC	EM	NA	SM	KM	CP		
				Salmonella*					
Omum	100	50	50	0	50	0	50	100	100
Asafoetida	100	60	100	40	40	40	20	66	100
Fenugreek	90	80	60	60	10	40	20	25	100
Cardamom (Small)	75	30	15	15	0	0	0	71	100
Clove	100	0	0	0	0	0	0	50	100
				E. coli**					
Omum	35	70	35	100	0	0	0	35	100
Asafoetida	100	50	50	50	50	50	0	0	100
Fenugreek	100	100	0	0	0	0	0	60	100
Cardamom (Small)	50	0	0	50	0	0	0	100	100
Clove	100	0	0	0	0	0	0	0	100

PI = Pencillin, TC = Tetracycline, EM = Erythromycin, NA = Nalidixic Acid, SM = Streptomycin, KM = Kanamycin, CP = Chloramphenicol

*No. of isolates tested: 21

**No. of isolates tested: 10

asafoetida followed by fengugreek and small cardamom.

Enterotoxigenicity was seen in 40% of *E. coli* and 62 per cent of *Salmonella* isolates. Maximum number of enterotoxigenic isolates were from cardamom, followed by fenugreek and small cardamom.

Enterotoxigenicity was seen in 40 per cent of *E. coli* and 62 per cent of *Salmonella* isolates. Maximum number of enterotoxigenic isolates were from cardamom, followed by omum, asafoetida, fenugreek and cloves. Hydrophobic character of these strains was determined using Salt Aggregation Test (SAT). Positive results were obtained in all cases (100 per cent hydrophobicity). The results compare favourably with those by Ljungh and Wadstrom[5], who obtained 71.5 per cent hydrophobicity among urinary *E. coli*..

In conclusion, it can be said that the incidence of pathogenic gram negative organisms on spices is very low but the organisms present are highly pathogenic. Majority of the organisms present were bacilli, which suggests the possibility of the hazards of food poisoning due to these organisms rather than the possibility of gastro-intestinal infections.

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TOXIC EFFECTS OF EXCESS ONION EXTRACTS IN RATS

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When aqueous extracts of onions were fed to rats on a high fat diet (HFD) the triacylglycerols and the total cholesterol in the serum, liver and kidneys were reduced significantly, but there was a significant rise in urea, creatinine and total and direct reacting bilirubin. SGOT (EC 2.6.1.10) and SGPT (EC 2.6.1.2) rose marginally above normal levels, while LDH (EC 1.1.1.27) and alkaline phosphatase (EC 3.1.3.1). in the serum remained within normal limits. Total proteins and albumin in the serum, and blood glucose were significantly reduced by feeding onion extracts 2.5 g/100 g body weight. Thus, consumption of large quantities of onion extracts may produce undesirable side effects.

Onion possesses hypoglycemic, hypolipidemic and antifibrinolytic activities[1-3]. For hypolipidemic studies, usually aqueous extracts from 330 mg onion are administered per day per 100 g body weight[2]. Onion contains many organic disulphides and sulphoxides which could react with various SH group compounds in the body spontaneously near pH 7 [2,4-6]. Though onions are normal constituents of diet, excessive consumption of it may produce other side effects. Hence, the present work was undertaken to study the biochemical effects of excessive onion consumption in high fat diet fed rats.

Male albino rats weighing 100-150 g obtained from Ahmedu Belle University, Zaria, were divided into 5 groups of 6 each. Group 1 rats were fed *ad libitum* a standard rat diet (Pfizer, Kaduna). Groups 2-5 rats were fed *ad libitum* a high fat diet (HFD) containing 20 per cent fat. This diet was prepared by mixing 620 g whole wheat flour, 150 g whole milk powder (NIDO), 160 g beef tallow, 50 g millet husks, 20 g common salt and vitamin supplements containing 1 mg thiamine, 0.5 mg riboflavin, 250 IU vit. A and 300 IU vit. D. Onions (100 g) were crushed separately in 3 batches without water in a waring blender and filtered through a cheese cloth. The residue was squeezed well. It was mixed with about 10 ml water and squeezed again. The volume of the extract of each batch was diluted with water to 100 ml, 250 ml and 500 ml respectively. Group 1 rats were given tap water to drink. Rats of groups 2,3 and 4 were given the onion extracts prepared fresh every day in the above three concentrations respectively in the drinking water bottle. Each rat of groups 2-4 consumed about 2.5 ml fluid per 100 g body weight per day corresponding to extracts from 2.5 g, 1 g and 0.5 g onions, respectively. After 60 days, the rats were sacrificed by decapitation in the fed state. Glucose was estimated from a fresh sample of blood[7]. Total and direct reacting bilirubin[8], urea[9], total proteins and albumin[10], creatinine[11], glutamate pyruvate transaminase (SGPT) (EC 2.6.1.2)[12], glutamate-oxaloacetate transaminase (SGOT) (EC 2.6.1.10)[12], alkaline phosphatase (EC 3.1.3.1)[13] and lactate dehydrogenase (LDH) (EC 1.1.1.27)[14] were estimated from the serum. Triacylglycerols[15] and total cholesterol[16] were estimated from the serum and in the extracts of liver and kidneys[17]. *In vitro* addition of onion extracts to the serum of normal rats was found to give high values of creatinine but did not interfere with urea or bilirubin estimations. Differences between groups were evaluated according to students 't' test.

From Table 2, it is clear that group 2 rats fed HFD had higher levels of triacylglycerols and total cholesterol in the serum, liver and kidneys and total lipids in the liver ($p<0.01$), but the urea, bilirubin and creatinine levels were unchanged ($p<0.01$) compared to group 1 control rats. SGOT, SGPT, serum alkaline phosphatase and serum LDH levels were within normal limits in group 2 rats. Feeding of onion extracts in drinking water corresponding to 2.5 g, 1 g or 0.5 g per 100 g body weight per day lowered triacylglycerols and cholesterol levels in the serum, liver and kidneys ($p<0.01$), but resulted in significant rise in urea, creatinine and direct reacting and total bilirubin levels in the serum ($p<0.01$). Serum albumin and total proteins were also reduced significantly when onion extracts were fed at 2.5 g/100 g body weight per day ($p<0.01$).

Onion contains many organic disulphides and sulphoxides, which could react spontaneously with the various SH group proteins, some of which are necessary for their function[6,18-21]. Thus, many of the above results may be due to the changes in the cell membranes of RBC, kidneys and liver due to thiol – disulphide and thiol – sulphoxide exchange reactions, resulting from excessive consumption of onion extracts. However, SGOT and SGPT increased only

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TABLE 1. EFFECT OF FEEDING ONION EXTRACTS TO RATS FED A HIGH FAT DIET (HFD)

Parameter	Group No. 1	2	3	4	5
	Values are for below indicated onion extract in 100 ml drinking water				
	Nil+	Nil‡	100 g‡	40 g‡	20 g‡
Serum triacylglycerols (m mol/l)	0.54 ± 0.093	0.82 ± 0.054	0.31* ± 0.043	0.32* ± 0.057	0.43* ± 0.048
Liver triacylglycerols (m mol/kg)	12.36 ± 0.46	28.13 ± 0.76	14.37* ± 0.84	14.52* ± 1.37	18.92* ± 2.39
Kidney triacylglycerols (m mol/kg)	3.0 ± 0.20	5.0 ± 0.24	3.6* ± 0.63	3.6* ± 0.42	4.0* ± 0.47
Serum total cholesterol (m mol/l)	2.59 ± 0.14	4.00 ± 0.19	2.33* ± 0.12	2.40* ± 0.13	2.53* ± 0.13
Liver total cholesterol (m mol/kg)	2.98 ± 0.13	6.73 ± 0.62	3.10* ± 0.26	3.24* ± 0.18	4.01* ± 0.13
Kidney total cholesterol (m mol/kg)	1.17 ± 0.21	2.18 ± 0.19	1.30* ± 0.25	1.30* ± 0.16	1.29* ± 0.12
Blood glucose (m mol/l)	4.44 ± 0.31	6.67 ± 0.31	5.0* ± 0.19	6.11 ± 0.30	6.39 ± 0.38
Serum albumin (g/l)	34 ± 4	36 ± 5	28* ± 2	32 ± 5	36 ± 5
Serum total proteins (g/l)	70 ± 4	75 ± 2.5	52* ± 4	72* ± 7	70 ± 7
Serum urea (m mol/l)	4.0 ± 0.33	4.2 ± 0.20	10.0* ± 0.83	9.2* ± 0.57	8.7* ± 0.52
Serum creatinine (μ mol/l)	17.7 ± 0.00	17.7 ± 0.00	106.2* ± 17.7	111.0* ± 22.1	111.0* ± 18.6
Serum direct reacting bilirubin (μ mol/l)	1.7 ± 0.00	1.7 ± 0.00	22.3* ± 2.6	30.8* ± 8.6	6.9* ± 1.7
Serum total bilirubin (μ mol/l)	4.28 ± 0.00	4.28 ± 0.00	60.0* ± 1.7	47.9* ± 8.56	30.8* ± 8.56
SGOT (m mol/l/min))	15 ± 1.24	20 ± 1.6	20 ± 2.5	25 ± 2.5	26 ± 2.6
SGPT (m mol/l/min)	10 ± 1.5	16 ± 1.6	15 ± 1.4	20 ± 1.2	23 ± 1.3
Serum alkaline phosphatase (m mol/l/min)	10 ± 1.5	15 ± 1.4	15 ± 1.4	25 ± 1.2	20 ± 1.3
Serum LDH (m mol/l/min)	35 ± 1.5	45 ± 1.5	35 ± 2.2	48 ± 2.5	58 ± 2.5

Results are expressed as Mean ± SD, of 6 rats per group.
*$p<0.01$ compared to group 2.
+Normal diet; ‡ High fat diet
Concentration of onion extract (100 g, 40 g and 20 g) in 100 ml of drinking water.

marginally above the normal limits, while LDH and alkaline phosphatase in the serum remained within normal limits, indicating that liver function is not seriously affected in rats fed onion extracts in these concentrations. Thus, it may be concluded that though onion possesses hypoglycemic and hypolipidemic activities, excessive consumption of aqueous extracts of fresh onions can produce deleterious effects in rats.

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BOOK REVIEWS

Recent Advances and Developments in the Refrigeration of Meat by Chilling: Proceedings of meetings of Commission C2 of the International Institute of Refrigeration, held in Bristol (UK) on September 10-12, 1986. Issued by International Institute of Refrigeration, 177, Boulevard Malesherbes, F 75017, Paris (France); pp. 550; Price: Hard cover bound 220 FF.

When one considers the recent developments in the domain of meat and meat products, one will note that refrigerated protection remains a necessary or even indispensable condition. Although refrigeration of meat has been in vogue for well nigh a century, until 1970's the advances in refrigeration were concerned with design and operating efficiency of machinery rather than with product requirements.

The book under review is a compilation of the 63 papers from 17 countries presented at a symposium organised by the International Institute of Refrigeration in Bristol on September 10-12, 1986. The symposium, considered, inter alia, the biochemical behaviour of animal products, the action of refrigeration on meat and physical phenomena of cooling and heat transfers. The papers presented were distributed among the 10 sessions (or sections) of the symposium.

Section 1 on fundamental effects of meat chilling dealt with microbiological aspects of meat chilling, cold shortening, biochemical changes and conditioning of meat.

Papers on electrical stimulation, cold shortening and conditioning were presented at Section 2 and covered tenderising mechanism of ES, quality of ES meat and low voltage ES *vs* quality.

Five papers dealing with factors affecting the chilling rate of red meat and poultry were presented in Section 3.

In Section 4, 11 papers were presented, dealing with design principles, economics of chilling systems, continuous chilling, chemical tainting during chilling and waste energy recovery.

The four papers presented in Section 5 dealt with chilling of edible affals using cryogenics, carbon dioxide chilling of hot boned meat, application of liquid nitrogen to meat chilling and plate freezing of hot boned meats.

Section 6 on Storage of chilled meats had 6 papers covering microbiology of vacuum packaged pork, display of fresh meat packed in elevated oxygen/ carbon dioxide atmosphere, salt level of commercial frankfurters, time-temperature-tolerance concept for chilled meat and storage of ham at cryoscopic temperature.

Carbon dioxide cooling of meat and meat products, influence of gas composition, chilling of meat in hydroaerosol medium curing and aseptic packaging of meat dishes were covered in the 5 papers of Section 7.

Section 8 had 4 papers on prediction and modelling in meat chilling.

Eleven papers on display retail and catering were presented in Section 9 and covered all aspects of display including factors affecting retail display, weight loss, effect of gas atmospheres on colour, effect of lighting, colour changes, storage temperature and stability, microbiology and cook/chill catering.

Section 10 on transport of chilled meats dealt with experience with transport of carcasses, containers, packaging of chilled meat for remote markets, time/ temperature monitoring and use of liquid nitrogen for in-transit refrigeration.

The book is a useful compilation of papers presented by experts from various countries. Each paper is followed by a summary of the discussions that took place after the presentation.

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D.F.R.L., MYSORE - 11

Rice: Post Production Manual: by P. Pillaiyar; Wiley Eastern Ltd., New Delhi, 1988; pp: 437; Price Rs.45.

The book is a comprehensive treatise of a wide spectrum of subjects including not only the post-production but many of the pre-harvest factors related to rice. The nomenclature 'Post Production Manual' coined for this book, no doubt implies a modest sense but does not seem to mirror the entire spectrum of literature contained in it. Although several books written by contemporary foreign authors on the subject are very exhaustive but they neither contain the details of Indian work nor the descriptions given are directly relevant to Indian context. It is the first book written by an Indian author which provides such a huge wealth of information with due emphasis on Indian work especially on several indigenous technologics. It should not, however, mean that its international value is insignificant. In fact, the author has made a sincere effort to bring together most of the recent developments in rice research taken place in several parts of the world. It is indeed, a very useful

reference book not only for researchers but for anyone who is dealing with rice.

The book is divided into fifteen chapters. The chapter on "harvesting, post harvesting operations and processing" deals with development of grains, optimum harvesting stage, various kinds of losses during and after harvesting, shedding characteristics of the grain, its cracking and milling qualities, use of field desciccants, and its influence on quality characteristics. Next is a unique chapter on "changes in wet paddy and its preservaion" not commonly found in conventional books on rice. This chapter deals with the genesis of the problems associated with wet paddy preservation, its microbiological implication, extent of losses and several approaches made for the preservation of wet paddy. The information contained in this chapter is of very great practical significance in field level application. The chapter on "drying" deals with scientific principles involved in indigenous technologies such as, field-drying, sun-drying and conduction-drying of paddy, in addition to mechanical-drying and some of the recent innovations on solar-drying.

The next two chapters deal with milling, curing and parboiling. They describe exhaustively the optimum conditions of the raw materials, various factors which influence the processing and different methods of processing and their merits and demerits. The subjects are discussed both on a general scale as well as specific to Indian conditions,and as such they are highly informative. The inclusion of a separate chapter on storage is probably the most prominent feature of this book as this subject is generally not discussed in such details in conventional books on rice. Storage is an inseparable part of food technology with very great influence on food losses and food qualities. The chapter deals with factors influencing storage, phenomenon of ageing, losses during storage due to microorganisms, insects and rodents and various kinds of storage structures including indigenous and modern types. The two chapters on cooking and quality of rice are brief and contain some relevant information but do not take the issues to their full depth.

The last three chapters dealing with rice husk, bran and germ present the latest information on these topics and discuss the various technologies used in exploiting these resources to the maximum extent.

The tremendous efforts of the author in bringing out this book should be richly complimented. However, there are a few lacunae in it on which any reader should like to offer comments. First of all, there are certain prominent omissions of important references especially on grain structure, chalkiness and topography. The "elucidation of rice quality based on its type classification", a recent innovation, should have been dealt with in some greater detail. Some information on rice products such as beaten rice, popped paddy and puffed rice should have found some place in the book as they are important post production processings in rice. Lastly, it should be noted that any reader cannot help but pointing out the poor get up of the book caused due to using poor quality paper and prints, frequent spelling mistakes, and low perceptibility of the apparently hazy figures, all of which have very seriously counteracted the great value of the book. It should, thus, be suggested that there is enough scope for its improvement, may be in the next edition, to make it more attractive, complete and purposeful.

T. SRINIVAS
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Livestock Feeds and Diseases of Dietary Origin (Hindi Text): by Dr. S P. Arora, Directorate of Publication, G.B. Pant University of Agriculture and Technology, Pantnagar 1988; pp: 304; Price: Not mentioned.

In a single volume, there are 16 chapters coverin Introduction, Rumen digestion, Metabolism o Proteins, Metabolism of Carbohydrates, Fat metabolism, Diseases occurring due to dietary errors, Mineral requirements, Vitamin metabolism, Feeding systems, Calf raising, Feeding for milk production, Dietary requirements of sheep and goat, Poultry feeding, feeding for reproduction and draft, swine nutrition, Feeding under famine and drought conditions. The book contains 84 figures and 89 tables.

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Proceedings of the Symposium on Recent Advances in Mineral Nutrition: Department of Animal Nutrition, Haryana Agricultural University, Hissar, India. (1984); pp: 243; Price: Not mentioned.

In all, there were five sessions of the symposium. The original research papers including some review papers were presented for Mineral Requirements, Deficiencies and Toxicities in man and animal; Metabolism of different Elements; inter-relationships between mineral elements; Analytical techniques and their applications; Bioavailability, their safe and toxic levels and health hazards; Mineral Elements chain, soil-plant-animal relationship along with all other aspects of mineral nutrition. The proceedings also

included major recommendations of each session at the end as a guideline for further research work.

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Alternatives to Animal Use in Research, Testing and Education: Compiled by the Congress of the United States, Marcel Dekker Inc, 270, Madison Avenue, New York, N.Y. 10016, 1988; pp:456 Price: Bound illustrated $59.75 (US and Canada), $71.50 (all other countries).

The book on alternative to animal use in research, testing and education brought by the office of technology assessment congress of United States takes off very well with the opening chapter on summary, policy, issues, option for congressional action which is very well written. A full chapter devoted to patterns of animal use fails to bring out any concrete findings from the data provided. Chapter 6 on alternatives to animal use in research is written well providing information but again one is left with the impression that only the tip of ice berg is sighted. Chapter 8 on alternatives to animal use in testing provides good reading and is well presented. The chapter on public and private funding towards the development of alternatives is well presented and the suggestion that a strong alternative test method will attract funding readily is noteworthy. The chapters on federal and state regulations of animal use extensively cite regulations and rules showing thereby the interest that is already there in governing humane and proper treatment of animals. The chapter on regulation of animal use in selected foreign countries makes an interesting reading and it is indeed necessary to find out about other countries. A very useful feature of this book is the presentation of summary and conclusion at the end of each chapter. Though the material is very well covered and presented, one desires that a strong indication at least as to the real alternatives to animal use could have been elaborated instead of categorising many of them. Nevertheless, the book is well presented and would be useful to all the laboratories, that conduct research on animals. Special mention must be made to the extensive bibiliography to each chapter which should be an additional factor that adds value to the book.

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AFST (1) News

Nagpur Chapter

A Seminar on 'Prospects of Processed Foods' was organised on January 15, 1989 at the Central Agmark Laboratory. The Seminar was inaugurated by Hon'ble Sri Ranjeet Deshmukh, Minister of State for Housing, and Slum Improvement. A Souvenir was released by the minister. Importance of providing balanced diet to the population and the role of food processesors was stressed by the Hon'ble Minister. Dr. Naik Kurade delivered the keynote address.

Prof. D.K. Kawadkar, Secretary, presented a report about the activities of the Association and Mr. Ashok Bhiwapurkar, President of the Chapter made introductory remarks. Mr. I.H. Ali, Joint Secretary, proposed a vote of thanks.

Two technical sessions were organised. A total of 20 poster papers were presented.

Publications of the Association of Food Scientists and Technologists (India) CFTRI Campus, Mysore – 570 013, India

Title	India		Foreign	
	Price	Postage	Price	Air Mail
	Rs.	Rs.	US$	US$
Ist Indian Convention of Food Scientists and Technologists, 1978 (Proceedings)	25	9	12	6
Symposium on the Status and Prospects of the Confectionery Industry in India, 1979 (Proceedings)	30	13	12	8
Symposium on By-products from Food Industries: Utilization and Disposal, 1980 (Proceedings)	30	9	12	6
2nd Indian Convention of Food Scientists and Technologists, 1981 (Proceedings)	40	13	15	7
3rd Indian Convention of Food Scientists and Technologists, 1983 (Proceedings)	25	9	15	5
4th Indian Convention of Food Scientists and Technologists, 1984 (Proceedings)	35	9	25	6
Symposium on Recent Developments in Food Packaging, 1986 (Proceedings)	65	13	35	8
Prof. V. Subrahmanyan Commemorative Issue, 1980	30	13	12	8
Production and Processing of Meat and Poultry Products, 1986 (Proceedings)	100	9	45*	
2nd International Food Convention & Exhibition (IFCON'88) – Food Technology Overview	100	13	45*	
2nd International Food Convention & Exhibition, (IFCON' 88) – Abstract of Papers	100	13	45*	

*Includes postage

INSTRUCTIONS TO AUTHORS

1. Manuscripts of papers (in triplicate) should be typewritten in double space on one side of bond paper. They should be complete and in final form. The paper should not have been published or communicated for publication anywhere else. Research Notes should clearly indicate the scope of the investigation and the salient features of the results. Only *invited* review papers will be published.

2. The typescript should be arranged in the following order: Title (to be typed in capital and small letters for Research Papers and all capitals for Research Notes), Authors' names (all capitals) and Affiliation (capitals and small letters). Also give a short running title not exceeding 10 words as a footnote.

3. **Abstract:** The abstract should indicate the principal findings of the paper and typed in single space. It should not be more than 200 words and in such a form that abstracting periodicals can readily use it.

4. Use names of chemical compounds and not their formulae in the text. Methods of sampling, number of replications and relevant statistical analyses should be indicated. Footnotes especially for text should be avoided as far as possible.

5. **Tables:** Tables as well as graphs, both representing the same set of data, should be avoided. Tables should be typed on *separate* sheets. Nil results should be indicated and distinguished clearly from absence of data, which is indicated by '—' sign. Tables should not have more than *nine* columns.

6. **Illustrations:** Graphs and other line drawings should be drawn in Indian ink on tracing paper or white drawing paper preferably art paper not bigger than 20 cm (OY axis) × 16 cm (OX axis). The lettering should be twice the size of the printed letter. Photographs must be on glossy paper and must have good contrast; **three copies** should be sent.

7. **References:** Names of all the authors along with title of the paper should be cited. Abbreviations such as *et al.*, *ibid*, *idem* should be avoided. References should be serially numbered as superscripts in the order they are cited in the text and the same order should be maintained in the reference list. The titles of all scientific periodicals should be abbreviated in conformity with the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.

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

   (*a*) *Research Paper*: Jadhav S S and Kulkarni P R, Presser amines in foods, J Fd Sci Technol, 1981, 18, 156.

   (*b*) *Book*: Venkataraman K, The Chemistry of Synthetic Dyes, Academic Press, Inc, New York, 1952, Vol. II, 966.

   (*c*) *References to article in a book*: Joshi S V, in The Chemistry of Synthetic Dyes, by Venkataraman K, Academic Press Inc, New York, 1952, Vol. II, 966.

   (*d*) *Proceedings, Conferences and Symposia Papers*: Nambudiri E S and Lewis Y S, Cocoa in confectionery, Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India, Mysore, May 1979, 27.

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

   (*f*) *Unpublished Work*: Rao G, unpublished, Central Food Technological Research Institute, Mysore, India.

8. Consult the latest issue of the *Journal* for guidance. For "Additional Instructions for Reporting Results of Sensory Analysis" see **issue No. 1** of the Journal.

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Research Papers

Research Notes

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