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Influence of Sugar and Flavour on the Acceptability of Instant CSM: Trials on Young Children from an Urban Orphanage

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Manuscript Received: 11 May 1973

As a guide for the selection and development of food supplements suitable for young children, acceptability trials were conducted on 40 children, 4 to 7 years of age. In the first trial, subjects tested included ICSM (instant corn, soyflour, milk) porridge with 9 different flavours. The second trial investigated the relative importance of sugar and flavour in ICSM and optimum levels of sugar and flavour. Evaluation was based on plate waste, ranking, and scoring on a 3-point scale.

Results showed that sweetening increases intake of the porridge by 25 per cent. Among the flavours tested, Almond was rated best, although there were no statistically significant differences among any of the flavours added to sweet ICSM. While palatability improved substantially with the addition of sugar, no significant change occurred when flavour alone was added to the bland ICSM. Also, doubling sugar levels from 14.85 to 29.70 per cent, further improved the rating of the product. However, increasing flavour levels from 0.025 to 0.050 per cent did not result in any significant improvement. The nutritional significance of using food supplements, which have a high acceptability, in feeding programmes is discussed.

It is well documented that children find it difficult to consume enough of a cereal-based diet to fulfil their nutritional needs[1]. Distribution of high calorie-high protein supplementary foods like CSM (64 per cent degermed corn, 24 per cent defatted soyflour, 5 per cent dried skimmed milk, 5 per cent refined soy oil, vitamin mineral premix added) and Balahar has provided one approach to this problem. However, the effectiveness of such foods also is likely to be restricted if quantitative intakes of these foods are inadequate.

Ordinarily, CSM does not contain any sweetening or flavouring additives. In an attempt to raise acceptability, UNICEF suggested a formulation of instant (precooked) CSM which has 14.85 per cent sugar and vanilla flavouring added to it. Vanilla was used to make it widely acceptable in many different countries. No other flavours have been tested in CSM.

Our acceptability trials were undertaken to improve the acceptability of ICSM in a 'take-home' distribution study called Project POSHAK.[2] An earlier study on rural communities had shown that adults had specific taste preferences in ICSM, however, field conditions precluded any quantitative estimates of children's intakes.[3]

The objective of the study reported here was to determine whether any measurable change in intakes of young children would result from the addition of various flavours to ICSM. In the second part of this trial, the effect of sugar and flavour on acceptability and their optimum levels of incorporation were determined.

Materials and Methods

Part I: Instant CSM in the following formulations was tested: Bland (no sugar or flavour); sweetened (14.85 per cent sugar) with no flavour; sweetened (14.85 per cent sugar) with each of the following flavours (0.025 per cent): almond, banana, cardamom, chocolate, lime, orange and vanilla.

Part II: (*a*) Relative effects of sugar versus flavour on acceptability were determined using the following formulations of ICSM: (*i*) Bland ICSM; (*ii*) Bland ICSM+0.025 per cent flavour; (*iii*) Bland ICSM+14.85 per cent sugar; (*iv*) Bland ICSM+14.85 per cent sugar+0.025 per cent flavour.

(*b*) Optimum levels of sugar and flavour were determined using the following formulations of ICSM: (*i*) Bland ICSM+14.85 per cent sugar+0.025 per cent flavour; (*ii*) Bland ICSM+14.85 per cent sugar+0.050 per cent flavour; (*iii*) Bland ICSM+29.70 per cent sugar+0.025 per cent flavour; (*iv*) Bland ICSM+29.70 per cent sugar+0.050 per cent flavour.

The above trials (*a*) and (*b*) were repeated on three days and results were compared to determine whether young children are consistent in their preferences.

Panel: Forty apparently healthy boys and girls, 4 to 7 years old, were selected from the Arya Orphanage in Delhi. Of these, 36 participated in the first test (Part I). A week later, all 40 children were randomly divided into 2 groups of 20 each for the second test (Part II *a* and *b*).

Almond flavour was found to be the most preferred one in Part I, and hence was used for Part II tests.

Sample preparation and presentation: One part of the dry ICSM was mixed with 2 parts of boiled hot water by weight. Approximately 100g of each ICSM porridge was spooned out into steel cups (*Katoras*). A set of 3 such cups for Part I arranged in Balanced Incomplete Block Design[4] and a set of 4 cups for Part II arranged in randomized Complete Block Design were given to each child.

Evaluation: The investigators filled in the following information:

1. Preference ranking.
2. Hedonic rating (3 point scale: 1—Dislike, 2-Indifferent, 3-Like).
3. Plate-waste. Leftovers from all children were combined for each product and measured.

All children were able to answer the investigator's questions either verbally or by pointing to the various cups to indicate their preferences.

Data analysis: Analysis of variance technique was adopted to test the null hypothesis that the formulations did not differ significantly in preference/hedonic rating/plate waste. Duncan's Multiple Range Test[5] was used to test the significance between any two mean scores for acceptability difference analysis and also for the subject consistency in the 3 replications. The hedonic rating was also expressed as percentages.

Mean ranks from all the 3 criteria were obtained as an overall assessment of acceptability of different formulations.

Results

Flavour preferences: Almond was the most preferred flavour when ranked according to plate waste, preference ranking and the 3-point scale (Table 1). Bland was consistently ranked lowest by all 3 criteria. Sweet ICSM (without flavour) was less liked than almond, but was preferred over banana, lime, orange and vanilla flavours.

TABLE 1

FLAVOUR PREFERENCE OF CHILDREN FOR ICSM PORRIDGE (MEANS)

Flavours	Daily plate waste (g/child)	Mean rank	Mean score on a 3-point scale	Mean rank from all 3 criteria used
Almond	1.2	1.50	2.94	1.3
Cardamom	1.4	1.70	2.97	2.0
Chocolate	2.1	1.63	2.73	4.3
Sweet (unflavoured)	1.9	2.07	2.91	4.0
Vanilla	2.1	2.07	2.87	5.0
Orange	2.1	1.87	2.78	4.7
Lime	2.7	2.07	2.90	5.7
Banana	2.8	2.00	2.65	7.0
Bland	6.6*	3.00*	1.93*	9.0

* Significantly different ($p<0.05$) from others

TABLE 2. RELATIVE INFLUENCE OF SUGAR AND FLAVOUR ON THE ACCEPTABILITY OF ICSM (MEANS)

Products	Like %	Dislike %	Rank	Means		
				Score	Plate-waste g	Mean rank
Bland	26.7	63.3	3.62	1.59	27.0	4.0
Flavour	45.0	45.0	3.33	2.00	18.4	3.0
Sugar	88.3	...	1.71	3.00	6.4	1.7
Sugar/flavour	86.7	3.3	1.40	2.93	5.2	1.3

(*a*) *Influence of adding flavour* versus *sugar to bland ICSM:* All sensory parameters and plate waste showed that sugar improved acceptability of bland ICSM considerably more than flavour (Table 2).

The panel assigned statistically similar mean scores to the products during all 3 replications of the test (Table 3). However, the amount of plate waste and ranking differed significantly on the 3 days when the test was repeated.

(*b*) *Influence of increasing sugar and flavour levels:* Doubling sugar levels in ICSM from 14.85 to 29.70 per cent improved the rank, score and intakes of ICSM (Table 4). Doubling flavour levels from 0.025 to 0.050 per cent had no consistent effect on any of the evaluation criteria.

Analysis of variance for the different days showed that mean scores, amount of plate waste and ranking

TABLE 3. RELATIVE SIGNIFICANCE OF SUGAR VERSUS FLAVOUR—MEANS (PANEL CONSISTENCY IN 3 REPLICATIONS)

Sugar level %	Flavour level %	Mean rank			Mean score			Plate waste (g)			Likes (%)			Dislike (%)		
		day 1	day 2	day 3	day 1	day 2	day 3	day 1	day 2	day 3	day 1	day 2	day 3	day 1	day 2	day 3
0	0	3.79	3.43	3.64	1.86	1.71	1.14	16.7	13.3	14.1	40	30	10	85	55	80
0	.025	3.14	3.57	3.29	2.21	1.71	1.71	13.2	13.8	9.4	60	35	40	30	50	35
14.85	0	1.71	1.71	1.71	3.0	3.0	3.0	6.3	3.6	3.2	95	85	85	...	0	0
14.85	.025	1.36	1.28	1.57	3.0	3.0	3.0	5.6	2.5	2.8	95	80	85	...	5	5

TABLE 4. INFLUENCE OF VARYING SUGAR AND FLAVOUR LEVELS ON ACCEPTABILITY OF ICSM

Products				Means			
Sugar %	Flavour %	Like %	Dislike %	Rank	Score	Plate waste (g) ICSM /Child	Mean rank
14.85	0.025	81.64	16.67	3.16	2.65	3.9	3.0
14.85	0.050	80.00	20.00	3.39	2.60	4.3	4.0
29.7	0.050	93.33	6.67	1.56	2.87	1.2	1.0
29.7	0.025	90.00	10.00	1.58	2.80	1.8	2.0

TABLE 5. INTERACTION OF SUGAR AND FLAVOUR (PANEL CONSISTENCY IN 3 REPLICATIONS)

Products		Mean rank*			Mean score			Plate waste* (g)			Likes %			Dislikes %		
Sugar level (%)	Flavour level (%)	day 1	day 2	day 3	day 1	day 2	day 3	day 1	day 2	day 3	day 1	day 2	day 3	day 1	day 2	day 3
14.85	0.025	2.88	3.56	3.06	2.70	2.55	2.70	0.1	1.8	0.2	85.0	75.0	85.0	15.0	20.0	15.0
14.85	0.050	3.50	2.56	3.12	2.70	2.60	2.50	0.4	2.3	0.7	85.0	80.0	75.0	15.0	20.0	25.0
29.70	0.025	1.12	1.56	2.06	2.90	2.70	2.80	0.8	5.1	2.2	95.0	85.0	90.0	5.0	15.0	10.0
29.70	0.050	2.00	1.56	1.12	2.90	2.90	2.80	0.5	5.7	4.0	95.0	95.0	90.0	5.0	5.0	10.0

* Significantly different on days ($p<0.05$)

remained statistically similar on each of the three days on which the test was repeated (Table 5).

Discussion

Sweetness is more important than flavouring in increasing acceptability of ICSM. The addition of sugar at 14.85 per cent level made a substantial improvement whereas even the most preferred flavour without sweetness was not acceptable.

Among all flavours tested, almond was best liked. In a previous trial as well, which was conducted on a rural population, almond ranked the highest[3]. Sensory appreciation of the almond flavour *per se* by the adult group tested could not be concluded, since the association of almonds with high status and its reported therapeutic properties could have influenced the choice of the subjects. However, results from the present study suggest that the flavour of almonds *per se* is preferable to all others in ICSM.

Weighing the quantity of plate waste showed that almond flavouring (0.025 per cent) plus sugar (14.85 per cent) when added to ICSM succeeded in increasing intakes from approximately 20 to 25 g (Table 4). If a child were to consume this amount four times a day, the daily consumption would be 100g per child instead of 80g per child. This would afford a daily nutritional advantage of about 79 calories and 1g of protein (Table 6).

TABLE 6. NUTRITIONAL ADVANTAGE OF ADDING SUGAR TO ICSM

Product	Unflavoured unsweetened	Sweetened (14.85% sugar)	Difference (per day)
Intakes (g)	80	100	+20
Constituents			
Calories	298.10	377.00	+78.6
Protein (g)	16.00	17.00	+1.0
Fat (g)	4.80	5.10	+0.3

It is concluded that the addition of a sweetening agent can greatly increase the acceptability of a blended food like ICSM. When translated into nutritional benefits and greater community response, this would appear to be a significant factor to consider in formulating food mixes for feeding programmes.

Acknowledgements

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Quality of AFD Meat during Storage

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Quality characteristics like bacterial counts and organoleptic and rehydration properties have been evaluated for several batches of AFD mutton during a storage period of 2 years. A very large proportion showed total counts of less than 400/g. No pathogens and coliforms were detected. Slight decrease in reconstitution was noticed on storage, but no off flavour or rancidity. Based on the results, it is felt that the warranted shelf life of AFD cooked mutton may be increased to one year.

The Department of Defence Production established an accelerated freeze drying factory for the production of precooked freeze dried mutton to cater to the needs of armed forces personnel under certain conditions, which offer logistic difficulties. In the factory the number of animals, (sheep/goat) processed per day has been stepped up from 150 per day to 700 in the course of last two years. The freeze drying method was adopted because of its well known advantages[1-3] over other methods of food preservation. The finished product is a ready to serve item with the nearest approximation to freshness, when reconstituted. It has excellent storage properties under all climatic conditions, when packed in moisture proof packages.

A high degree of hygienic control is required in a food manufacturing plant where a number of men, material, and equipment are involved. Since the prepared product is pre-cooked and dehydrated and may be consumed with/without any heat treatment, the assessment of quality and shelf life is of utmost importance. The normal shelf life assigned to it is nine months.[4] To gauge whether the shelf life expected of AFD cooked mutton can be raised to one year, 784 samples drawn from regular production during the year 1969 and 1970 were stored for a two year period and analysed systematically for assessment of changes brought about by storage at field conditions.

Materials and Methods

The mutton used was obtained from carcasses of sheep and goat slaughtered in the abattoir of the factory. The dressed and washed carcasses were chilled overnight at 5°C and then deboned. The deboned meat was cooked in steam at normal pressure. The cooked meat was pressed into a block, cooled and sliced 12 to 15 mm thick. The slices were steamed for a short while to minimise contamination caused during slicing and were frozen at -30°C in trays. The cooked out juices were not incorporated in the product. The frozen slices were dried at a pressure of 0.5 mm and a temperature of not more than 50°C. The energy for evaporation was supplied by radiant heating. The maximum temperature being 110°C. The drying time was nine hours. The dehydrated product was packed in A-2½ cans at the rate of 180 g/can under nitrogen.

Moisture, protein, fat, ash and peroxide value determinations were carried out according to Jacob.[5] Degree of rehydration was measured by the method adopted by Bedi and Labroo[6].

For microbial analysis 1g of the freeze dried material was homogenised in ringer solution at pH 7 in a sterile blender. Dilutions were made and 1 ml was inoculated in each medium. The nutrient media[7] employed were (*i*) nutrient agar; (*ii*) McConkey agar; (*iii*) manitol salt agar and (*iv*) lactose broth. Bacterial counts were obtained after incubation at 37°C for 72 hr.

Results and Discussions

Table 1 gives the proximate analysis of the raw, cooked and AFD mutton. The difference in moisture between the raw and cooked mutton is due to the

Table 1. Proximate analysis of raw, cooked and AFD meat

Constituents	Raw	Cooked	AFD meat
Moisture%	78.1	61–64	1–2.6
Fat%	2.0–3.5	3.0–5.7	8.0–15.0
Peroxide value m. eq/1000 g	Nil	...	8.0
Protein%	18.9	29–33.5	80–88
Ash%	1.00	0.8–1.1	2.0–3.0

inherent properties of muscle meat.[8] The moisture of cooked meat serves as standard (100 per cent) for expressing the rehydratability of the AFD meat. The low moisture of the AFD sample is an important characteristic of freeze dried products and is responsible for its stability. The fat in the raw meat is low because most of the trimmable fat is removed during deboning. On cooking a part of it goes out in cooked out juices which is not being incorporated and as such fat in the finished product ranges from 8-15 per cent. The partial removal of fat facilitates freeze drying by avoiding *in situ* melting during radiation heating[9, 10]. The deleterious effects of the removal of fat on flavour of the product is being investigated. Under the conditions of processing the peroxide value of the fat is not affected and is retained at 8 m.eq/1000 g. The mineral content in the finished product is low as a part of it is lost in the cooked out juices.

Total counts obtained at the start of the storage period in all the batches have been grouped into 7 ranges and tabulated in Table 2. The bacterial counts in general are low. Eighty seven per cent of the batches examined showed a count of less than 400/g. In Table 3, the counts are re-grouped into the same 7 ranges but distributed into four quarters of the year. No definite conclusion on density of microbial counts due to seasonal variation can be drawn from the present data.

The microbial status of freeze dried mutton can be compared to other precooked dehydrated products or to precooked frozen products which do not require much heat treatment by the consumer. The Commonwealth Food Specifications[11] for products like precooked freeze dried ham, omellette, ham and beans, beef with rice, minced and sliced beef are as follows: total counts<20,000, coliforms<10, yeast and moulds<100, and coagulase positive staphylococci nil in one gram of sample. The standard for dehydrated foods proposed by HMSO[10] is total count<1000 at 22°C and<100 at 37°C. Since there was no increase in bacterial counts in AFD sample prepared during the summer months, the above standard specifications even though of countries with temperate and cold climates may be suitable for adoption in tropical countries also. The total bacterial counts obtained by Haberman[12] for freeze dried meat cubes with fat content of 35 per cent were 5×10^5/g. The proposal for bacterial counts for dried foods,[13] and for dried soups[14] is also of the same order; viz., 5×10^5. The counts obtained in the recent study are much below these limits and are also less than what is specified for precooked frozen foods.[15-18] These testifiy to the overall hygienic standards maintained in the processing area.

Table 2. Initial bacterial load of 784 AFD mutton samples

Counts/g	No. of batches	Percentage
0–100	211	26.9
101–200	221	28.2
201–300	148	18.9
301–400	103	13.1
401–500	47	6.0
501–1000	41	5.2
1001-2000	13	1.7

Table 3. Initial bacterial load of 784 AFD mutton samples distributed in quarter of the year

Counts/g	Samples (%) in different quarters							
	I		II		III		IV	
	1969	1970	1969	1970	1969	1970	1969	1970
0–100	19.1	17.3	18.9	19.0	14.5	17.8	70.0	39.0
101–200	21.3	32.7	29.7	30.0	34.2	28.2	17.0	32.6
201–300	7.0	19.1	27.0	19.0	26.3	21.0	6.5	15.6
301–400	15.0	18.0	12.2	16.0	15.8	18.5	4.0	5.6
401–500	8.5	7.3	5.4	7.6	2.6	11.3	1.3	3.6
501–1000	12.8	3.7	5.4	6.7	6.6	1.6	1.2	3.6
1001–2000	6.3	1.9	1.4	1.7	...	1.6	...	...

The samples drawn from time to time and analysed for gauging changes during storage are presented in Table 4. No peroxide oxygen was detectable initially. After storage of 11 to 27 months isolated cases of measurable peroxide oxygen have been noted.

Initial rehydratability varied from 100 to 79 per cent. The variation after storage is 87-78 per cent. No definite pattern is apparent. These observations show that more stringent regulation of operating condition of the freeze drier should be attempted. Total counts have decreased on storage. This is in conformity with the observation of Haberman.[12]

Moisture, protein and fat have shown no appreciable changes. Few samples analysed for amino nitrogen did not also show any change. No change in organoleptic characteristics like, colour, appearance flavour and odour were observed. The stability is due to low moisture and fat contents and packing under nitrogen

TABLE 4. CHANGES DURING STORAGE OF AFD MUTTON

Duration of storage (month)	Peroxide value m.eq/1000 g		Rehydratability %		Total counts	
	Initial	Final	Initial	Final	Initial	Final
4	...	...	80.7	78.4	800	133
5	...	...	79.2	79.0	83	...
9	...	...	95.4	81.0	233	200
11	...	34.0	94.1	86.8	566	233
13	...	12.8	86.9	81.2	166	166
15	...	13	100	81.54	183	133
17	...	...	88.8	84.5	66	nil
19	...	...	96.1	82.3	150	100
21	...	14.3	94.0	87.1	216	166
23	...	...	82.7	83.6	216	33
24	...	...	83.9	74.7	450	nil
25	...	...	94.8	79.2	149	33
26	6.0	6.4	100	83.3	233	66
27	10.0	10.5	90.4	78.5	66	566

which inhibit[10, 19] the spoilage caused by micro-organisms, maillard reaction and autooxidation during storage. As such the warranty period may be increased to one year.

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Changes in the Carbonyl Composition of a Milk Based Sweetmeat—*Burfi* during Preparation and Storage

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During the conversion of open pan evaporated milk (*khoa*) to *burfi* there was a two fold increase in the total carbonyls. Analysis of the carbonyls indicated no quantitative change in the monocarbonyls of *khoa* and *burfi*. The major classes of carbonyl compounds identified in *khoa* and *burfi* were methyl ketones and saturated aldehydes. There was no quantitative difference between *khoa* and *burfi* in the methyl ketones but there was qualitative difference, *khoa* being rich in a lower carbon chain length methyl ketones while *burfi* was rich in higher methyl ketones. Similar difference was also noticed in the saturated aldehydes. During storage of *burfi* there was quantitative decrease in methyl ketones and increase in the saturated aldehydes. The methyl ketones appear to be associated with the pleasant flavour of fresh *burfi* and the saturated aldehydes with the off flavours which develop during storage.

Burfi is one of the popular Indian sweetmeats based on milk. There are many varieties of *burfis* made from various nutmeats and flavourings but their base, which also contributes to texture, taste and aroma, is milk. The simplest of *burfis* contains only milk solids and sugar and its preparation consists in evaporating milk in an open pan to obtain a thick pasty mass known as *khoa* and subseqently cooking the *khoa* with an adequate quantity of sugar for a brief period. During these two repeated heat treatments the *burfi* acquires its characteristic aroma and taste. However, these sensory attributes deteriorate in a few days and distinct stale and waxy flavours develop on longer storage.

The principal class of compounds responsible for the desirable or undesirable flavour in milk and milk products is known to be carbonyl compounds[1,2]. Studies on heat treated milk products like conventionally sterilised concentrated milk have revealed methyl ketones and saturated aldehydes as the dominant class of compounds contributing to the aroma of the products. The off flavour associated with such heated milk products has been ascribed to the formation of saturated aldehydes and methyl ketones[1,2]. Information regarding the compounds associated with the flavour of a milk product like *burfi* which has undergone extensive heat treatment with complete exposure to atmosphere is not available. Since such information would be useful in understanding the flavour profile of *burfi* aroma, the carbonyls formed during the preparation of *burfi* and the subsequent changes during storage were examined.

Materials and Methods

Khoa was prepared using buffalo milk by the traditional method. Milk (2 litres) was taken in a shallow round bottom iron pan (stainless steel pan was used by us) and boiled over a slow fire (bunsen burners were used instead of the usual coal hearth) and was stirred continuously with a circular motion by the flattened end of an iron stirrer called *khunti*. During the operation all parts of the pan with which the milk comes in contact were lightly scraped. The milk thickened progressively and at a certain stage the thickened mass showed an abrupt change in colour. Vigorous stirring and desiccation was continued till the viscous product reached a pasty consistency and began to dry up. The *khoa* was ready when it showed signs of leaving the sides of the pan. At this stage the pan was removed from the fire and the product was well worked with the flattened end of the scraper by alternately spreading into thin layers and collecting it repeatedly until the product retained its own shape. The mass had then the consistency of butter but after cooling it became more solid like hard dough. This is customarily shaped into large circular pats or balls weighing 500-700 g. The time taken for the preparation of *khoa* was about 1 hr. However, the time taken depends on the size of the pan, the heat source and it may well extend to 3-4 hr depending on the quantity of milk. The yield of *khoa* from 2 l. of milk was 340 g. The composition of *khoa* is given in Table 1.

Burfi: *Khoa* (200 g) was grated and mixed with 80 g sugar. The powdery mix was taken in a steam jacketted kettle. The powdery mix was briskly worked

TABLE 1. PROXIMATE COMPOSITION OF *khoa* AND *burfi*

Constituent g/100 g dry matter	*Khoa*	*Burfi*
Moisture (fresh weight basis)	26.5	15.1 5.6*
Fat	39.11	24.70
Protein (N×6.38)	20.54	13.34
Total sugars	28.57	59.63
Sucrose	...	40.36
Lactose	28.57	19.27
Free fatty acids, g oleic acid/kg fat	3.44	3.41

* Moisture content of *burfi* stored for 15 days at ambient temperature and humidity.

up with a stainless steel ladle. In a few minutes it became a pasty mass and the steam was then shut off. The plastic mass was worked up for a further minute and then poured on a wooden table and rolled with a wooden rolling pin to about 1 cm thick slab. It was allowed to cool and then cut with a knife to 5-6 cm square blocks. The composition of the *burfi* prepared as above is given in Table 1. A portion of the *burfi* was taken directly and the other was stored at ambient conditions of temperature (25-30°C) and humidity (50-70 per cent R.H.) for 15 days for carbonly analysis.

Carbonyl free solvents: Hexane (BDH) was rendered carbonyl free as follows: 3 litres of hexane were refluxed with 20 ml concentrated sulphuric acid for 4 hr. The hexane was decanted and washed twice with 500 ml distilled water and then distilled over sodium hydroxide pellets.

Chloroform (Analar, BDH) and benzene (BDH) were made carbonyl free by distilling over 2,4-dinitrophenylhydrazine.

Extraction of carbonyls: A mixture of 100 g *khoa* and 100 g Hyflo Super-cel (previously washed with carbonyl free hexane) was packed in a glass column and was eluted with about 3 lit of carbonyl free hexane. The effluent from the column was allowed to drip directly on to a 2, 4-dinitrophenylhydrazine (DNPH) impregnated bed of Hyflo Super-cel (instead of the celite bed described by Schwartz and Parks[3]) to convert the carbonyls to DNP-hydrazones. The absorbance of effluent was read at 340 nm in a Beckman DU Spectrophotometer and the total concentration of carbonyl derivatives in the extract was calculated using E= 22,500[4].

Isolation, class separation and identification of carbonyls: The monocarbonyl DNP-hydrazones were freed from lipids and ketoglycerides using essentially the procedure described by Schwartz *et al.*,[4,5] except that Hyflo Super-cel and magnesium oxide (E. Merck) were used in place of Celite 545 and Seasorb and the sequence of solvents used for final elution in class separation was 100 ml each of 1, 5 and 10 per cent methanol in chloroform. The class authenticity of each fraction was established on the basis of the following absorption maxima in chloroform (nm): methyl ketones, 365; saturated aldehydes, 355; 2-enals, 373; and 2, 4-dienals, 390.

For the separation of individual methyl ketones within the class, the method of Badings and Wassink[6] using Kieselguhr G thin-layer plates impregnated with carbowax-400 and methyl cyclohexane as solvent was followed. The resolution of the isolated saturated aldehydes was not as good as that of the methyl ketones (Fig. 1) perhaps due to some interfering compounds described by Schwartz, *et al*[4]. Hence, the saturated aldehydes were separated on the acetonitrile-hexane-celite column of Corbin, *et al.*[7]

The carbonyls from fresh and stored *burfi* were similarly extracted and identified.

Regeneration of carbonyls: For the regeneration of the carbonyls the technique used by Ralls[8] for gas-liquid chromatography was modified as follows:

The carbonyl DNP-hydrazone solution in chloroform or hexane was taken in a test tube and the solvent was driven off using a jet of nitrogen. A pinch of alpha-ketoglutaric acid was added and the test tube was heated on an oil bath (150°C) till alpha-ketoglutaric acid melted. The test tube was swirled if necessary to effect proper mixing of the carbonyl DNP-hydrazone with alpha-ketoglutaric acid. The tube was taken out of the oil bath, cooled, 2-3 ml of water was added and heated to boiling over a bunsen flame. The vapours ensuing from the tube were sniffed to record the odour of the regenerated carbonyls.

The proximate composition of *khoa* and *burfi* was determined according to standard AOAC methods[9].

Results and Discussion

Carbonyl distribution: The amounts and the pattern of carbonyls in *khoa* and fresh and stored *burfi* are presented in Table 2. The data reveal that there is a marked increase in the total carbonyls (column A) during conversion of *khoa* to *burfi*. This is significant for the reason that though during the preparation of *khoa* milk is boiled for over an hour the total quantity of carbonyls formed is far less than that of *burfi* which

TABLE 2. AMOUNTS (μ MOLES PER KILOGRAM FAT) AND PATTERN OF CARBONYL COMPOUNDS ISOLATED FROM *khoa* AND FRESH AND STORED *burfi*

Product	Total from DNPH column A	Carbonyls recovered from defatting column B	Carbonyls other than ketoglycerides and mono carbonyls A-B	Carbonyls as ketoglycerides B-C	Monocarbonyl class				
					Total monocarbonyls C	Methyl ketones	Saturated aldehydes	2-Enals	2, 4-Dienals
Khoa	1569	1106	463	628	478	266	144	19	nil
Burfi (fresh)	3573	1265	2308	741	524	292	56	10	2
Burfi (stored)	2471	1409	1062	959	450	34	391	34	nil

gets an additional heat treatment of only 5-6 min. There was no visible caramelization during conversion of *khoa* to *burfi* and hence it is difficult to attribute the increase in total carbonyls to caramelization of sugars. It also cannot be attributed solely to oxidation of milk lipids since they have already undergone heat treatment of over an hour prior to preparation of *burfi*, and therefore, further heat treatment of the fat for 5-6 min may not effect significant change in the total carbonyls. It is quite likely that the Maillard reaction between lactose and proteins and amino acids of milk, which was slow due to high moisture content during preparation of *khoa* is accelerated by the low water activity (more than lowering of moisture content, for it has been seen that lowering moisture level of *khoa* alone does not bring about the characteristic flavour of *burfi*) caused by addition of sugar during preparation of *burfi*, thus resulting in an increase in total carbonyls. However, it is difficult to single out any one mechanism responsible for the increase in total carbonyls and may be that all the above reactions are simultaneously operative.

It is significant that though there is marked difference in the quantity of total carbonyls between *khoa* and fresh and stored *burfi*, the quantity of carbonyls recovered from the defatting column (B) is approximately the same in all of them. There were several blue, violet and pink bands on the defatting column which could not be eluted with nitromethane-chloroform solvent. These bands apparently are the same as those described by Schwartz, *et al.*,[4] representing all the dicarbonyl DNP-hydrazones. It appears as though (column A-B) that during conversion of *khoa* to *burfi* the major amount of the carbonyls formed (or lost during storage of *burfi*) are carbonyls other than monocarbonyls. The carbonyl derivatives eluted from the defatting column were further fractionated into ketoglycerides and monocarbonyls comprising methyl ketones, saturated aldehydes, 2-enals and 2, 4-dienals. Like the amount of carbonyls recovered from the defatting column, the amount of ketoglycerides (B-C) and the total monocarbonyls (C) are approximately the same in the three samples of *khoa*, fresh and stored *burfi*, pointing as if there is no change in these carbonyls during conversion of *khoa* to *burfi* and during storage of *burfi*. However, it is interesting to note that though monocarbonyls are quantitatively the same in the three samples, qualitatively they are different as is shown by the class separation (Table 2).

During conversion of *khoa* to *burfi* there does not appear to be any quantitative change in the methyl ketones while slight reduction in the saturated aldehydes and 2-enals is noticed. The striking feature is the sharp decrease in the quantity of methyl ketones and similar increase in saturated aldehydes during storage of *burfi*. The build up of saturated aldehydes during storage is as expected,[1] but disappearance of methyl ketones is contrary to the published literature and they have been shown to accumulate in stored dairy products.[2] Reduction of methyl ketones to secondary alcohols is known to take place in blue cheese,[10] but this is as a result of microbial activity. During storage of *burfi* no fungus growth was observed and hence disappearance of methyl ketones cannot be attributed to microbial activity. It is likely that the simple mechanism of oxidation of ketones to α-ketohydroperoxides which then decompose to aldehydes and acids[11] as shown below is operative in lowering of methyl ketones during storage of *burfi*. The increase in saturated aldehydes in stored *burfi* also may be at the expense of methyl ketones according to the following reaction.

$$R_1-\underset{\underset{O}{\|}}{C}-CH_2-R_2 \rightarrow R_1-\underset{\underset{O}{\|}}{C}-\underset{\underset{OOH}{|}}{CH}-R_2 \rightarrow$$

$$\rightarrow R_1-\underset{\underset{O}{\|}}{C}-OH + R_2-C{<}^{H}_{O}$$

Carbonyl identity: The pattern of methyl ketones isolated from *khoa*, fresh and stored *burfi*, is shown in Fig. 1. At least 8 spots could be noticed on the chromato plate. Though no spot was identified, the first

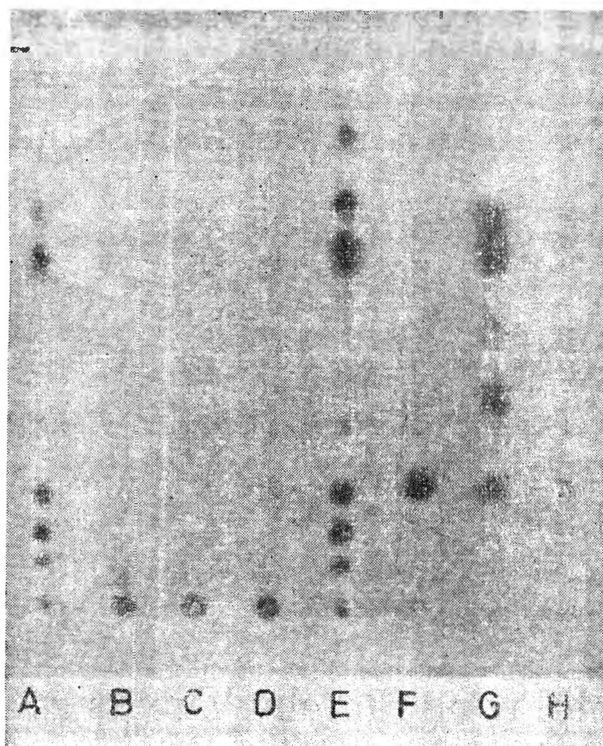

Fig. 1. Thin layer chromatoplate of 2, 4-dinitrophenyl hydrazones of saturated aldehydes and methyl ketones from *khoa*, fresh and stored *burfi*. A and E, n-saturated aldehyde standards C_3, C_4, C_6, C_9, C_{10}, C_{11}, C_{12}. B, C and D, saturated aldehydes from *khoa*, fresh *burfi* and stored *burfi*, respectively. F, G and H, methyl ketones from *khoa*, fresh *burfi* and stored *burfi* respectively.

spot from the base was similar in its mobility to acetone. Comparison of the chromatoplate with others in the published literature suggests that the methyl ketones in *burfi* range upto C_{10} chain length. The important point, however, is the qualitative difference in the methyl ketones between *khoa* and fresh *burfi* the former having only one dense spot representing a lower methyl ketone while the latter has a uniform distribution of the methyl ketones. There does not

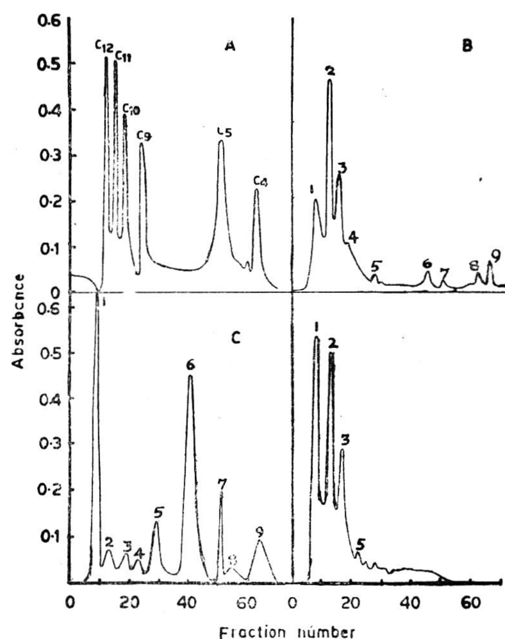

Fig. 2. Partition chromatograms of the saturated aldehydes from *khoa* (B), fresh *burfi* (C) and stored *burfi* (D); A, n-saturated aldehyde standards. Peak identity: 1-unidentified fast moving fraction. 2-C_{12}, 3-C_{10}, 4-C_9, 5–C_8, 6-C_6, 7-C_5, 8-unidentified, 9-C_4.

appear to be much difference between the pattern of methyl ketones from fresh and stored *burfi*.

The saturated aldehydes could not be separated as distinctly as the methyl ketones and hence were taken on the acetonitrile-hexane partition column and the elution peaks are given in Fig. 2. An unidentified peak (No. 1) was observed in all the three samples of *khoa*, fresh and stored *burfi*. The aldehydes patterns show that the higher aldehydes C_{12} and C_{11} (peaks 2 & 3) increase during preparation of *burfi* while exactly the reverse trend is noticed for the lower aldehydes.

Odour evaluation: The odour description of the regenerated carbonyls, given in Table 3, reveals the

TABLE 3. ODOUR DESCRIPTION OF THE REGENERATED CARBONYLS ISOLATED FROM *khoa* AND FRESH AND STORED *burfi*

Carbonyl	*Khoa*	*Burfi* (fresh)	*Burfi* (stored)
Methyl ketones	Fruity, minty, sweet smell eucalyptus oil, fresh grass	Estery, fruity, pleasant sweet smell	Fruity
Saturated aldehydes	Burnt rubber, boiled rubber, terpentine like	Boiled rubber	Painty, corky
Enals	Oily, stale oil, oxidized oil, characteristic smell of stored *burfi*	Cardboardy, tallowy, corky	Cardboardy, tallowy
Di-enals	...	Fruity	...
Ketoglycerides	Burnt machine oil like, coal tar like, paint like	Burnt machine oil like, burnt paint like	Burnt machine oil like
Total monocarbonyls	Not characteristic	Not characteristic	Not characteristic

importance of methyl ketones in *burfi* aroma. Similarly the dienals cannot be discounted though present only in traces, since they are known to impart characteristic aroma at ppb concentrations. The increase in saturated aldehydes and enals during storage of *burfi* is probably the cause of off flavour as in other milk products. The regeneration of the total monocarbonyls did not give the full aroma in either of the three samples though they were reminiscent of the product. This suggests that the carbonyls retained on the defatting column also have a role to play in the flavour of *burfi*.

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Glutamic Acid Fermentation Employing Starchy Tubers as Raw Material

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The possibility of using starchy tubers (potato, sweet potato and tapioca) as raw materials in glutamic acid fermentation by *Micrococcus glutamicus* is shown. Preliminary work was done in the case of potato which, after enzyme hydrolysis and removal of excess biotin enabled accumulation of extracellular glutamic acid. A simple technique to remove excess biotin by adsorption on activated charcoal is described for the first time, which was necessitated because of the ineffectiveness of the additions of penicillin, isobutyl, isopropyl alcohols and sodium oleate in this system. In a medium containing potato hydrolysate (5 per cent total soluble solids), 0.3 per cent urea, 0.2 per cent peptone, 0.2 per cent meat extract, 0.2 per cent K_2HPO_4, 0.2 per cent KH_2PO_4, a yield of 10.75 mg per ml of glutamic acid was obtained after 72 hr in agitated culture (vol. 200 ml) at room temperature (28-30°C).

Since its isolation as the key flavour component of sea tangle by Ikeda in 1909[1] monosodium glutamate (MSG) has achieved considerable world-wide popularity as a flavour potentiator for meat products. Around 250 million pounds of MSG are annually produced, the bulk of which is by fermentation, mainly in Japan and the USA.

In view of the potential use of MSG in this country as a food additive and the lack of technical know-how, the present studies were carried out to explore the possibility of evolving a microbial process for glutamate production employing starchy tubers as the raw material. Nearly 7.5 million tonnes of potato, sweet potato and tapioca (together) are produced in India annually, a considerable quantity of which is utilised by the starch industry[2,3]. Preliminary studies have shown that media prepared out of these tubers were able to support luxuriant growth of the glutamate excreting *Micrococcus glutamicus*, and that considerable amount of glutamate accumulated when the biotin content of the medium was adjusted to the optimum level by removal of the excess by adsorption on activated charcoal.

Materials and Methods

Organism: Micrococcus glutamicus ATCC 13032 culture was maintained on nutrient agar slants at 4°C with fortnightly transfers.

Preparation of the fermentation medium: Separate media were prepared from potato, sweet potato and

tapioca. In order to render the tubers suitable for microbial growth the suitability of both the acid and enzyme hydrolysis method was tested.

Enzyme hydrolysis: The tubers were washed and cooked to gelatinise starch by steaming for 25-30 min, peeled and mashed using a meat homogeniser. To the mash, half the weight of distilled water was added, and fungal-amyloglucosidase enzyme preparation was added. The mixture was incubated at 55°C for 24 hr. The hydrolysed material was pressed out in a cheese cloth.

Acid hydrolysis: The pH of the mashed tuber was adjusted to a value of 2.0 with hydrochloric acid and this material was autoclaved at 15 psi for 30 min. The hydrolysate was pressed out as before.

Fermentation: Prior to fermentation, the hydrolysed tuber was diluted to contain 5 per cent total soluble solids pH adjusted to 7.2 with dilute NaOH solution and autoclaved at 10 psi for 15 min. To 50 ml of the diluted hydrolysate taken in a 250 ml capacity conical flask one ml of inoculum containing approximately 10^8 cells per ml of an overnight culture was added. Incubation was done at 30°C in a rotary flask shaker for 72 hr, after which the cells were removed by centrifugation and glutamic acid in the culture fluid was estimated by paper chromatography according to the method of Krishnamurthy and Swaminathan[4] and by microbiological assay employing *Leuconostoc mesenteroides*[5].

Cell growth was measured turbidimetrically in a Klett-Summerson photoelectric colorimeter using a filter No. 66 after suitable dilutions and expressed as klett units. pH measurements were made in Elico model pH-meter. Biotin was estimated by microbiological assay using *Lactobacillus arabinosus*[6].

Total soluble solids was determined in a 0-50 per cent range sugar refractometer, and the reducing sugars by the method of Shaffer and Hartman[6].

Results

Hydrolysis of tuber starch: The enyme hydrolysis of tuber was preferred because the product obtained in this case was less brownish in colour and the growth of the organism in it was better than that in the acid hydrolysed sample. α-amyloglucosidase was tested at various levels and it was found that addition of 3 ml of enzyme preparation (containing 200 units of enzyme per ml) per 100 g of mashed tuber was adequate to bring about hydrolysis of starch in the tuber in 24 hr. The presence of starch was tested with iodine solution.

Despite considerable cell growth in both the enzyme and acid hydrolysed tubers, extracellular glutamate could not be detected.

Removal of excess biotin: By microbiological assay it was found that the tubers contain considerable amount of biotin which in the final fermentation medium would be so much in excess as to inhibit glutamate excretion by the organism. On fresh weight basis, potato, sweet potato and tapioca were found to contain respectively 0.015, 0.021 and 0.032 μg biotin/g of tuber. The biotin content of several samples of hydrolysed tuber after dilution to contain 5 per cent total soluble solids was found to be in the range of 4-10 μg. It had been shown earlier by Tauro *et al*[7]., that in glucose containing synthetic medium 1.0 μg of biotin per litre of fermentation broth was optimum for glutamate excretion by *M. glutamicus*. In order to counteract the inhibitory effect of excess biotin on glutamate production, penicillin at levels of 20 to 200 units/ml, isobutyl, isopropyl alcohols at 1.0 to 1.25 per cent or sodium oleate at 50 to 100 mg/ml were added to the tuber hydrolysate prior to fermentation. Except variations in the amount of cell growth observed no glutamate production during fermentation could be detected.

In attempts to remove excess biotin from the tuber hydrolysate, its treatment with activated charcoal was found to be quite effective. Conditions for removal of excess biotin from potato hydrolysate in relation to maximum yield of glutamate in fermentation was standardised as follows: Activated charcoal was added at 1.0 per cent level to diluted potato hydrolysate (5 per cent total soluble solids) at pH 4.0, adjusted with HCl. The mixture was shaken in a rotary shaker at room temperature for 30 min after which charcoal was removed by filtration.

In order to test the effect of charcoal treatment on glutamate production and compare it with other conditions of the presence of excess biotin, fermentation was carried out using the following media: (*i*) Untreated potato hydrolysate, (*ii*) potato hydrolysate treated with charcoal, (*iii*) charcoal treated hydrolysate with added biotin at 30 μg and (*iv*) charcoal treated hydrolysate to which was added ammonia-ethanol eluate of the used charcoal at one ml per 50 ml of the medium. After the fermentation, cell density and extra-cellular glutamate were determined. The data are given in Table 1. It was seen that only in the case of charcoal treated hydrolysate was there an accumulation of extracellular glutamate. In the cases of the other 3 media viz., the untreated hydrolysate and the treated samples of hydrolysate containing either the added biotin or the eluate from used charcoal, glutamate production could not be seen although the cell growth in these media was nearly 8 times more

than that in the charcoal treated hydrolysate. In the charcoal treated tuber hydrolysate the amount of biotin was in the range of 0.5 to 1.0 μg.

The influence of nitrogen source on glutamate yield: Five nitrogenous substances as indicated in Table 2 were tested for their suitability to promote glutamate production in the potato hydrolysate medium diluted to contain 5 per cent total soluble solids. The nitrogenous compounds were added in quantities which gave almost the same amount of nitrogen in each case. It was seen that urea added at a concentration of 0.3 per cent has served as the best nitrogen source with a yield of 6.2 mg/ml glutamate. The nitrates had no beneficial effect.

The influence of concentration of potato hydrolysate and urea on glutamate yield: The potato hydrolysate was diluted to contain four levels of total soluble solids, viz., 5.0, 7.5, 10.0 and 15.0 per cent and urea was added in concentrations proportional to the total soluble solids in the hydrolysate as indicated in Table 3. After the fermentation, the culture fluids were analysed for extracellular glutamate and reducing sugar. It could be seen from data in Table 3 that although potato hydrolysate of 7.5 per cent soluble solids with 0.45 per cent urea gave a maximum yield of 7.5 mg/ml glutamate, maximum efficiency of conversion of utilised sugar to glutamate was observed only with 5 per cent soluble solids and 0.3 per cent urea.

The influence of various growth substances on glutamate yield: To the basal fermentation medium containing potato hydrolysate with 5 per cent total soluble solids and 0.3 per cent urea peptone, meat extract and yeast extract were added at various levels as indicated in Table 4, and fermentation carried out. It was seen that addition of 0.2 per cent peptone and 0.2 per cent meat extract together to the medium gave rise to the maximum production of 10.5 mg/ml glutamate. Presence of yeast extract in the medium was found to be inhibitory to glutamate production, the inhibition being proportional to the concentration of yeast extract.

Time course of fermentation: Fermentation was carried out by using 200 ml of the medium taken in 1 litre conical flasks. The medium used was of the following composition:

Potato hydrolysate (10.0% solids) ml	100.0
Urea%	0.3
Peptone%	0.2
Meat extract%	0.2
KH_2PO_4%	0.2
K_2HPO_4%	0.2

The phosphate salts were added for the purpose of buffering. Fermentation was carried out at 30°C in rotary shaker. At periodic intervals extracellular glutamate, cell density, and pH of the fermentation broth were determined. The results are depicted in Fig. 1. It could be seen that after an initial rise during the 3rd and 6th hr of fermentation the pH value dropped to 5.3 at the 24th hr. Extracellular glutamate was detectable only after 12 hr of fermentation and attained a steady state by the 48th hr.

TABLE 1. EFFECT OF REMOVAL OF EXCESS BIOTIN ON CELL GROWTH AND GLUTAMATE YIELD

Fermentation medium	Cell growth (Klett units)	Extracellular glutamate (mg/ml)
Potato hydrolysate	195	0
Charcoal treated potato hydrolysate	25	1.7
,, + 30 μg/1 biotin	202	0
,, + 1 ml charcoal eluate	197	0

TABLE 2. EFFECT OF DIFFERENT NITROGEN SOURCES ON GLUTAMATE YIELD

N-Source	N-content %	Amount of N-source added %	Extracellular glutamate (mg/ml)*
Control	...	...	1.5
$(NH_4)_2SO_4$	21.20	0.66	5.2
NH_4Cl	26.17	0.53	5.6
KNO_3	13.86	1.08	1.5
$NaNO_3$	16.47	0.85	1.4
Urea	46.70	0.30	6.2

* Values are average of triplicates

TABLE 3. EFFECT OF CONCENTRATION OF POTATO HYDROLYSATE AND UREA ON GLUTAMATE YIELD

TSS of potato hydrolysate %	Urea g %	Extracellular glutamate (mg/ml)*	Residual sugar reducing g %
5.0	0.30	6.3	0.8
7.5	0.45	7.5	1.8
10.0	0.60	5.0	2.4
15.0	0.90	5.0	4.6

* Values are average of triplicates
TSS: Total Soluble Solids

TABLE 4. EFFECT OF GROWTH FACTORS ON GLUTAMATE YIELD

Peptone	Growth factors (g %)		Extracellular glutamate (mg/ml)*
	Meat extract	Yeast extract	
nil	0.2	0.01	7.90
0.1	0.2	0.01	8.50
0.2	0.2	0.01	10.50
0.3	0.2	0.01	10.00
0.6	0.2	0.01	10.20
0.2	nil	0.01	8.00
0.2	0.1	0.01	9.00
0.2	0.2	0.01	10.20
0.2	0.3	0.01	10.00
0.2	0.6	0.01	10.00
0.2	0.2	nil	10.50
0.2	0.2	0.01	9.67
0.2	0.2	0.02	9.08
0.2	0.2	0.04	4.73
0.2	0.2	0.06	2.12
0.2	0.2	0.12	0.36

* Values are average of triplicates

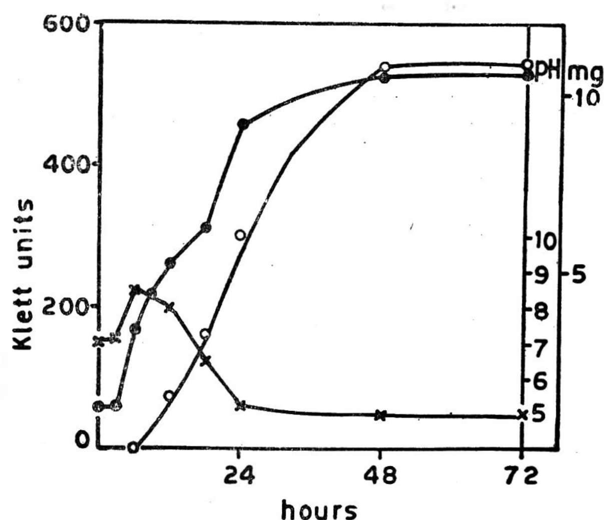

Fig. 1. Effect of fermentation period (hr) on cell growth of *Micrococcus glutamicus* (closed circles), pH of fermentation broth (crosses) and extracellular glutamic acid (open circles).

After 72 hr the glutamate yield was 10.7 mg/ml. The cell growth after an initial lag of 3 hr showed a steady increase in rate and appeared to attain a steady state by the 48th hr.

Discussion

The regulatory role of biotin on glutamate excretion by microorganisms producing this amino acid is fairly well known.[8,9] Increased production is brought about by sub-optimal levels of biotin in the medium. In commercial practice, it is imperative that a readily available and inexpensive natural substrate is employed as carbohydrate source, and usually these contain excess biotin. Several workers have shown that the inhibitory effect of excess biotin can be overcome by the addition of Penicillin,[10] aliphatic C_3-C_5 alcohols[11], sodium oleate[12], etc., which enhanced permeability of glutamate. In molasses for example, Shio *et al.*,[13] showed that the effect of excess biotin can be counteracted by the addition of penicillin or fatty acid derivative leading to glutamate excretion by *Brevibacterium flavum.* Likewise in *Microbacterium flavum* and *Micrococcus glutamicus* ATCC 13058[14] systems the penicillin addition was helpful. The reason for the ineffectiveness of penicillin addition in the present case was probably due to the resistance of *M. glutamicus* ATCC 13032 to the antibiotic (revealed in a followup study).[15] The beneficial role of the additions of isobutyl and isopropyl alcohols in glutamate production by *Brevibacterium saccharolyticum* No. 7636 in a biotin rich medium was demonstrated by Kono *et al.*[11] Addition of sodium oleate was effective in the case of *Microbacterium ammoniaphilum.*[12] Since none of these additions were useful in the present case removal of excess biotin by treatment with activated charcoal was attempted and found to be effective. Charcoal can adsorb many other substances besides biotin, such as other vitamins, nucleotides, pigments, etc., but here it could be shown that it was the removal of biotin which was responsible for bringing about glutamate accumulation (Table 1).

The results have indicated the possibility of utilising starchy tubers as a raw material for glutamate fermentations, and can probably provide a model for any system which employs biotin-rich growth substrate and a penicillin resistant, glutamate excreting microorganism. The large scale trials with aids for aeration, agitation and pH control (the time-course study has suggested a possible controlling role of pH) would help in the assessment of the economic feasibility of the process.

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Biochemical Characteristics of Different Varieties of Punjab Wheat (*Triticum aestivum*)

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The investigation reports variations in biochemical constituents of seven varieties of Punjab wheat, which include two indigenous varieties (*C-281, C-306*) and five Mexican hybrid (*S-307, S-331, Lermarajo, S-308* and *K-227*) varieties. *C-306* variety has the lowest protein content, *C-281* and *K-227* have lower starch, total lipid and phospholipid contents; whereas crude fibre was lowest in *S-331* and highest in *C-281*. Protein content was similar in all the varieties except *C-306*. Choline containing phospholipids form a good proportion of the total phospholipids in all the samples; *S-308, C-306* and *K-227* have a relatively higher proportion of these phospholipids. Phosphatidyl ethanolamine was also present in good amounts in all samples except *S-308* which had negligible amounts. Differences were noted in the contents of other phospholipids. Starch from indigenous varieties had lower proportion of amylose than those of the Mexican hybrid varieties and there were differences in the amylose content of starch of various Mexican hybrid varieties. The number of glucose residues per non-reducing end group of amylopectin was higher in starch from indigenous variety *C-306* whereas this number was lower and similar in other six varieties.

The role of different flour constituents in determining the wheat flour quality has been the subject of many investigations. Wheat gluten in a unique way influences the viscoelastic, rheological and bread baking properties of wheat[1-3]. Differences in starch component to some extent determine the rheological and gas holding properties of bread[3,4]. Characteristics of wheat starch as well as the relative proportion of amylose and amylopectin fractions also determines their preferential use in certain foods and other industries and the amylopectin fraction has been implicated in changes associated with staling phenomenon in food stuffs[5,6]. Further it has been reported that the starch from the durum varieties of wheat have higher amylose content compared to starch from other varieties[7] and such differences in amylose content may account for the differences in the quality of wheat. Amylose content of rice starch has been reported to be related to the characteristics of rice grains and their cooking quality[8,9]. There are several reports on the lipids of wheat[10-12] and the role of lipids in baking technology is well recognised. High quality hard red winter wheat has been reported to contain substantially more free lipids and higher ratio of free to total lipids than the poor bread making wheat[13] and visual differences on the chromatogram were seen in the individual phospholipid components from different varieties of wheat. Further, quality and quantity and classes of lipid in wheat flour may also determine the storage quality of wheat and its products. The investigation reported herein gives the differences in the composition of seven different varieties of Punjab wheat as well as the individual phospholipid components of their lipids, and also the difference in characteristics of starches separated from these varieties of wheat. Such studies may be helpful in correlating the quality and other characteristics of various varieties of wheat with the variation in biochemical parameters.

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Materials and Methods

The investigation includes seven different varieties of freshly harvested Punjab wheat received through the courtesy of the Farm Superintendent, Agricultural University, Hissar. The growth and other characteristics of these varieties of wheat are described earlier[14] and are mentioned in Table 1. The wheat samples were ground to pass through a 40-mesh seive and representative samples were then weighed out for analysis.

Protein content of the ground wheat samples was estimated by the Kjeldahl method. Starch was estimated by the procedure of Hassid and Naufeld[15]. Method recommended by AOAC[16] was followed for the estimation of crude fibre. The total lipid present in dried wheat flour samples were extracted by using a mixture of chloroform and methanol (2:1) as the solvent, washed, and the extract made up to a known volume. The extract was used for the determination of the total lipids, for the estimation of phospholipids[17] and for the thinlayer chromatography of phospholipids by the method of Stahl[18]. Quantitative analysis of the individual phospholipid components identified was by estimating their phosphorus content by Merrinettis' modification[19] of Bartlett's method[20].

The iodine binding capacities of purified starches obtained from various wheat samples were determined by the iodine-potentiometric titration method of Bates *et al.*,[21] as modified by Lansky *et al.*[22] The details of the methods, and determination of amylose content have been described earlier[23]. The number of glucose residues per non-reducing end group in the amylopectin fraction of different starches was determined by periodate oxidation employing essentially the conditions and concentrations of Potter and Hassid[24].

Results and Discussion

The protein, starch, crude fibre, total lipids and phospholipid content of different varieties of wheat are shown in Table 1. The indigenous variety *C-306* has the lowest content of protein, whereas other six varieties do not differ much in their protein contents. Starch content is lower in indigenous variety *C-281* and Mexican hybrid *Kalyan Sona* (*K-227*). An indirect relationship between starch and protein contents of hard and soft varieties of *Azerbaidzhan* wheat has been reported.[25] Similar relationship is observed in the two indigenous varieties, *C-281* and *C-306*, but no such relationship exists in five hybrid varieties of Mexican wheat.

Crude fibre represents the amount of indigestible matter and has an important bearing on the nutritive quality of the wheat. Indigenous variety *C-281* and the two Mexican hybrid varieties i.e., *Chhotilerma* (*S-307*) and *Lerma Rajo* have relatively large proportion of crude fibre.

Total lipid content as well as phospholipid contents also showed marked variations among the different varieties. Chung *et al.*,[26] observed that profound changes in rheological properties and bread-making characteristics of wheat flour were accompanied by a small decrease in the ratio of non-polar to polar lipids and by an increase in the phosphorus content of the polar lipids. Further it has been reported that high quality wheat contained substantially more free lipids (petroleum ether-extractable) and had higher ratio of free to total lipids than the poor bread making wheats[13]. The present data indicate that the ratio of phospholipids to total lipids also varies a lot in these wheat samples and these differences may cause the differences in the bread-making[26] and other qualities of flour prepared from these varieties of wheat.

Characteristics of starch separated from these different varieties of wheat also show marked differences (Table 2). Iodine-binding capacity or amylose contents of starch from the two indigenous varieties of wheat (*C-281*), (*C-306*) are lower than those of the Mexican hybrid varieties. Among the five Mexican hybrid varieties the starch from *Kalyan Sona* (*K-227*) has the lowest amylose content and this variety amongst the five has the highest yield potential. An inverse relationship between the amylose content of the starch and yield potential of particular variety of wheat in indigenous and Mexican hybrid varieties is supported but it needs to be further studied. The amylopectin

TABLE 1. PROXIMATE COMPOSITION* OF DIFFERENT VARIETIES OF WHEAT ON DRY MATTER BASIS

Variety	Code No.	Protein %	Starch %	Crude fibre %	Total lipids %	Phospho-lipids %	Phospho-lipids as % of total lipids
...	*C-281*	14.6	58.7	2.19	0.99	0.09	9.1
...	*C-305*	12.8	68.8	1.50	1.20	0.06	5.1
Chhoti lerma	*S-307*	14.5	67.7	2.09	1.76	0.13	7.2
Safaid lerma	*S-331*	15.0	67.1	0.60	1.27	0.11	8.4
Lerma Rajo	...	14.8	63.5	2.11	1.63	0.11	6.5
Sonalika	*S-308*	14.6	68.6	1.66	1.33	0.09	6.4
Kalyan Sona	*K-227*	15.0	58.2	1.62	0.69	0.09	12.7

* Dry weight basis

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TABLE 2. CHARACTERISTICS OF STARCHES FROM DIFFERENT VARIETIES OF WHEAT

Variety	Code No.	Iodine binding capacity %	Amylose content %	No. of glucose residues*
...	*C-281*	2.56	12.1	27
...	*C-306*	2.20	10.4	33
Chhoti lerma	*S-307*	3.92	18.5	27
Safaid lerma	*S-331*	3.71	17.5	26
Lerma Rajo	...	3.43	16.2	28
Sonalika	*S-308*	3.24	15.4	28
Kalyan Sona	*K-227*	3.16	14.9	27

* Av. number of glucose residues per non-reducing end group in amylopectin.

fraction of starch from indigenous variety *C-306* has the highest number of glucose residues per non-reducing end group, whereas this number was lower and similar in other six varieties.

The impor:ance of change in the ratio of polar/nonpolar lipids as well as the change in phosphorus content in the polar lipids of wheat flour in the baking technology[26] made it desirable to study the differences in the individual phospholipid components present in different varieties of wheat. A detailed analysis of the lipids in a single sample of flour has been reported[27] and Chiu and Pomeranz[13] noted visual differences on the chromatogram in the individual polar lipid components in hard red spring and hard red winter wheats. The present data indicate large quantitative differences in the relative proportion of individual phospholipid components present in different varieties of wheat studied (Table 3).

Among the two indigenous varieties, *C-281* has higher relative proportion of lysolecithin and phosphatidyl ethanolamine whereas *C-306* has markedly higher proportion of lecithin and there are minor differences in the proportions of other phospholipids.

Among the Mexican hybrid varieties *S-307* and *S-331* have larger proportions of phosphatidyl ethanolamine whereas *S-308* has negligible amounts. Lysolecithin concentration is highest in *Sonalika* (*S-308*) and lecithin concentration is highest in *Kalyan Sona* (*K-227*). A trend pointing toward a relationship between the relative percentage of the choline containing phospholipids with the yield potential in the two indigenous varieties as well as in the five Mexican hybrid varieties is indicated but this suggestive relationship needs to be confirmed and verified.

TABLE 3. INDIVIDUAL COMPONENTS OF PHOSPHOLIPIDS* IN DIFFERENT VARIETIES OF WHEAT

Variety	Code No.	P.S.	Lyso lec.	Lec.	Unidentified spot I	Unidentified spot PE	Unidentified spot II
...	*C-281*	1.22	13.9	26.8	15.3	34.2	8.55
...	*C-306*	1.96	traces	54.8	15.7	13.7	13.72
Chhoti lerma	*S-307*	11.90	7.15	28.5	19.1	33.5	traces
Safaid lerma	*S-331*	5.34	7.26	40.8	18.5	28.1	,,
Lerma Rajo	...	14.72	7.14	24.4	10.5	16.1	27.1
Sonalika	*S-308*	traces	39.60	45.8	4.7	traces	9.6
Kalyan Sona	*K-227*	,,	22.30	55.2	2.5	20.0	traces

* The units are in relative percentage of individual phospholipids which is calculated on the basis of total recovered lipid phosphorus. The recovery of lipid phosphorus varied from 89 to 106%. The values reported here are average of two determinations.

PS= Phosphatidyl serine; Lysolec.=Lysolecithin; Lec= Lecithin; PE=Phosphatidyl ethanolamine.

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Electron Microscopic Observations on the Casein Micelles of Buffalo Milk: A Preliminary Study*

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The electron microscopic patterns of casein micelles of buffalo and cow milk and the changes in buffalo casein micelles on treatment with urea, 2-mercaptoethanol and rennet, have been studied. Buffalo and cow casein micelles were seen as aggregates of spheroidal granules and the aggregation was found to be more in buffalo micelles. The size of the particles are bigger in buffalo micelles. The diameter of the particles were in the range of 80 to 250 nm and 50-200 nm for buffalo and cow casein micelles, respectively. On treatment with urea, aggregation of particles was noted and in higher urea concentration the micelles look partially dissolved. There was slight deformation in shape in the 2-mercaptoethanol treated samples. In rennet treated samples, the micelle aggregates dissolved partially indicating partial proteolysis of the protein.

Electron microscopy is an indispensable tool in studying particle size of protein molecules. Several workers have studied the shape and size distribution of casein micelles in cow milk using this technique. Nitschmann[1] reported such data on the micelles of skim milk. Shimmin and Hill[2] made a study of the internal structure of casein micelles using very thin sections cut from micelles embedded in araldite. The internal structure of casein micelles from bovine milk has been reinvestigated by Rose and Colvin[3]. Carroll *et al*[4]. studied the casein micelle size using glutaraldehyde as a fixative for electron microscopic study.

However, a similar study with the casein micelles of buffalo milk is lacking. The observed difference in the properties of casein micelles of buffalo and cow milk[5] demands examination of their particle size. The present paper reports some preliminary data of such an endeavour. Changes in the buffalo casein micelles on treatment with dissociating agents are also delineated.

Materials and Methods

The casein micelles were collected from freshly drawn cow and buffalo milk from Institute herd after skimming by ultracentrifugation in a Beckman model L-preparative ultra-centrifuge at 105,000 g for 30 min. The settled micelles were collected and dispersed in phosphate buffer, pH 7, so that their concentration was similar to that in milk. For the observation of the size and shape of particles, the samples were fixed with 2 per cent glutaraldehyde solution for 30 min[4]. The fixed samples were diluted 20 times with the buffer and mounted on copper grids coated with formvar film with a sterilized platinum loop and dried in air. The samples were then shadow-caseted with gold palladium (60:40) at a 3:1 angle (18.5°).

For the study of changes in the buffalo casein micelles on treatment with dissociating agents, the following experiments were conducted (*i*) 4.0 M and 6.6

*N.D.R.I. Publications No. 72-69.

M urea treatment, (*ii*) 5 per cent and 10 per cent 2—mercaptoethanol (2 MCE) treatment, (*iii*) 30 and 60 minutes rennet action. To 5 ml casein micelle sample either 1 ml of 4 M/6.6 M urea or 5—10 per cent 2 MCE solution was added. For rennet treatment, 0.2 ml of rennet solution (Hansen rennet, 50 mg/ml) was added to 5 ml of the sample and after 30 or 60 min interval, the reaction mixture was fixed with 2 per cent glutaraldehyde solution for 30 min. After fixation, the samples were diluted 20 times with the buffer and further processed as described above. The grids were examined under electron microscope (Philips Model E.M. 100). The final magnification of the figures are ×20,000.

Results and Discussion

Particle size of casein micelles of buffalo and cow milk: The electron microscopic patterns of the casein micelles of buffalo and cow milk are presented in Fig. 1 and Fig. 2 respectively. Both buffalo and cow casein micelles appeared as aggregates of spheroidal granules. Calapai[6] also observed that bovine casein micelles were aggregates of spheroidal granules arrange in a spherical symmetry. The size of the particles were bigger in the case of buffalo micelles and so aggregation was greater. The diameter of the buffalo casein micellar particles was in the range of 80 to 250 nm, with most of them ranging between 110 nm to 160 nm. The corresponding values for cow micelles were 50 to 200 nm and 70 to 110 nm, respectively. Nitschmann[1] found that most micelles had a diameter of 80 to 100 nm in the case of bovine casein micelles. Hostettler and Imhof[7] also reported a diameter of 50-100 nm for bovine casein particles. Calapaj[6] found that the most frequent micelle diameter was 95 nm in cow's milk. Carroll *et al*[4]., observed a range of size from 500 to 2500°A (50-250 nm) in case of cow micelles. Our observations of 50 to 200 nm diameter for cow casein micelles agree with these observations. In certain cases some electron dense particles were observed in the micelles. These are probably granules of colloidal calcium phosphate as suggested by Rose and Colvin[3].

Changes in the micelle size on treatment dissociating agents: Changes in the buffalo casein micelle on treatment with 4 M and 6.6 M urea are depicted in Fig. 3 and 4, respectively. Aggregation of particles was enhanced in higher concentration of urea. On 6.6 M urea treatment, the micelle samples appeared to be partially dissolved.

The electron microphotographs of buffalo casein micelles treated with 5 and 10 per cent 2-MCE are shown in Fig. 5 and 6, respectively. No aggregation was observed. Slight deformation in shape at 10 per cent 2-MCE level could be observed.

The changes in buffalo casein micelle on treatment with rennet for 30 and 60 min are shown in Fig. 7 and 8. Aggregation of micelle particles are evident in these samples. Hostettler and Imhof[8] found that the electron microscopic picture of rennet-treated milk showed aggregated casein with certain regularity. The shape is mostly spherical. In the case of 30 min rennet treatment some portion of the micelle[8] dissolved which when dried over the grid made the particles electron dense. This suggested partial proteolysis of the micelles. On 60 min rennet action, most of the particles were deformed and the micelle aggregates became more electron dense indicating more proteolysis of the particles.

Acknowledgment

The authors are indebted to Dr D. Sundaresan, Director, National Dairy Research Institute, Karnal for his kind interest and encouragement. Grateful thanks are also due to Dr C. Dakshinamurthy of the Indian Agricultural Research Institute, New Delhi for providing electron microscope facilities and to Dr S. Nandi, Cancer Research Genetics Laboratory, University of California, Berkeley, U.S.A., for the generous supply of glutaraldehyde solutions.

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PLATE 1

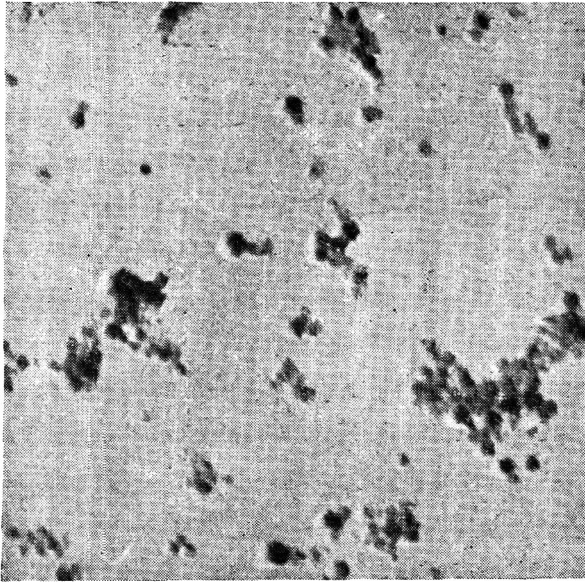

Fig. 1. Buffalo milk casein : 2% glutaraldehyde fixed; × 20,000.

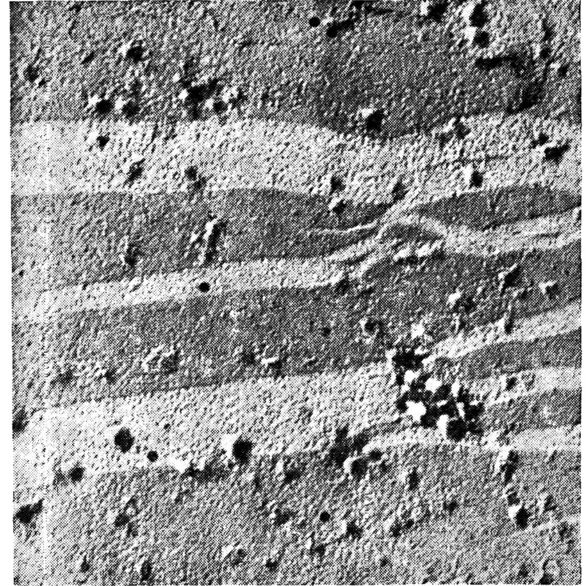

Fig. 2. Cow milk casein : 2% glutaraldehyde fixed ; × 20,000. The diameter of the dark polyestyrene globule is 88 mμ.

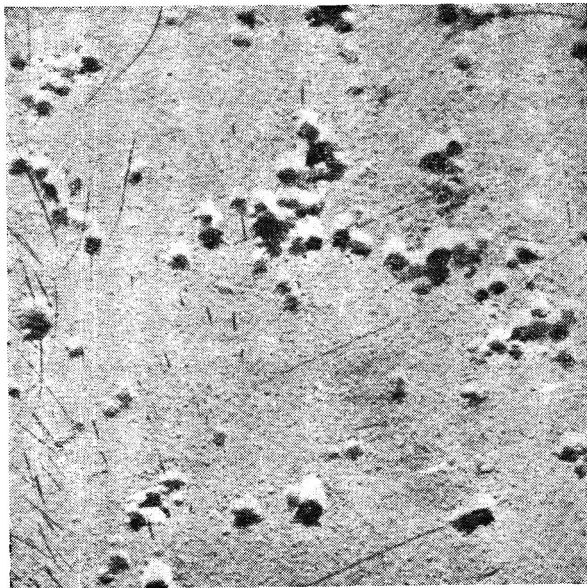

Fig. 3. Buffalo milk casein : treated with 4 M urea ; 2 % glutaraldehyde fixed ; × 20,000.

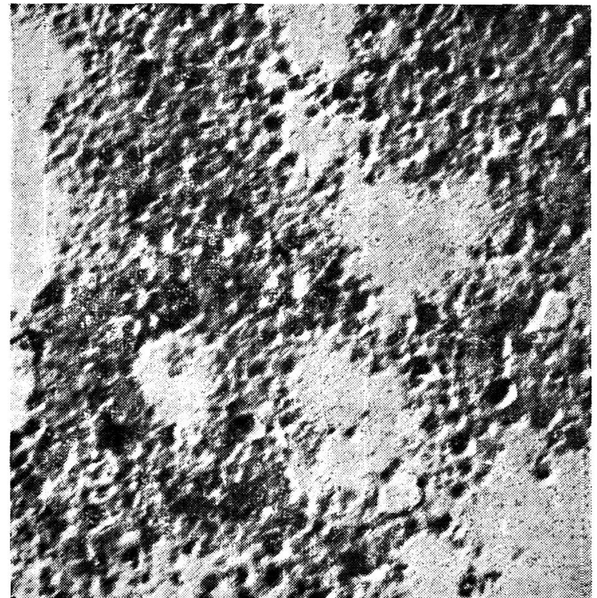

Fig. 4. Buffalo milk casein : treated with 6.6 M urea ; 2 % glutaraldehyde fixed ; × 20,000.

PLATE 2

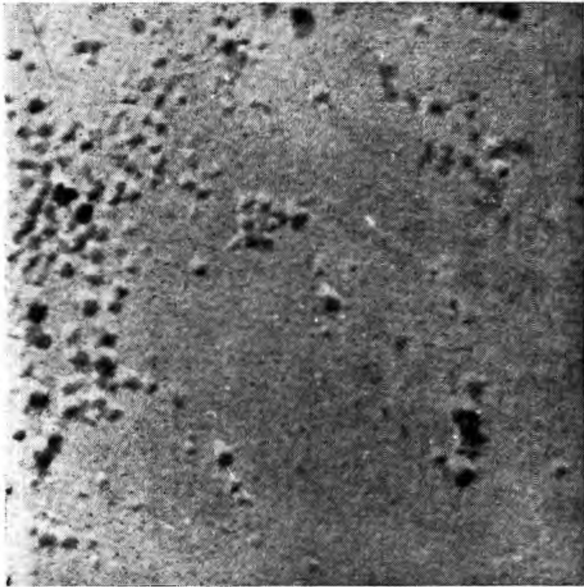

Fig. 5. Buffalo milk casein: treated with 5% 2-mercaptoethanol; 2% glutaraldehyde fixed; × 20,000.

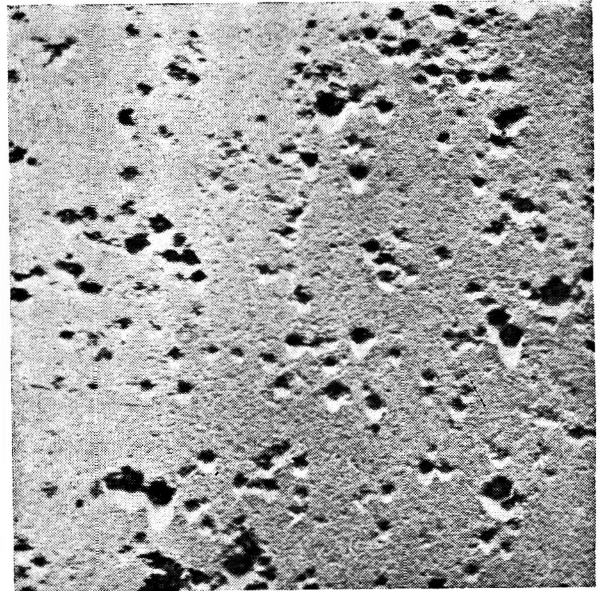

Fig. 6. Buffalo milk casein: treated with 10% 2-mercaptoethanol; 2% glutaraldehyde fixed; ×20,000.

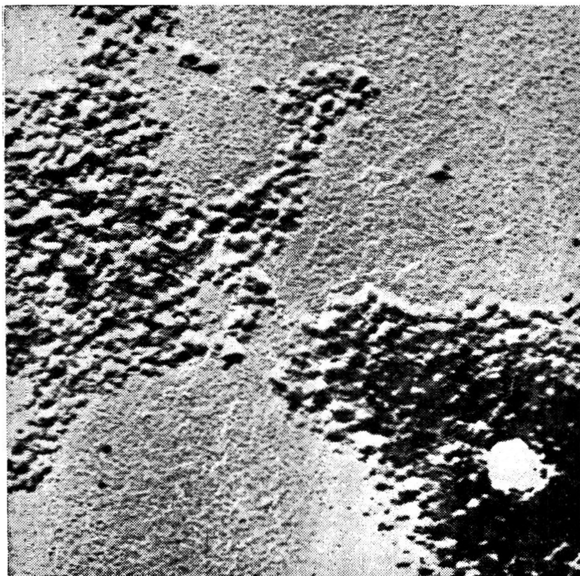

Fig. 7. Buffalo milk casein : 30 minute rennet treated; 2% glutaraldehyde fixed; ×20,000.

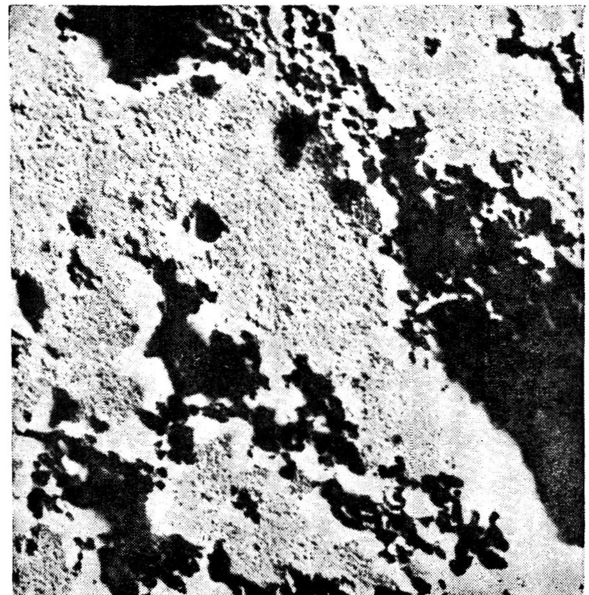

Fig. 8. Buffalo milk casein: 60 minutes rennet treated; 2% glutaraldehyde fixed; × 20,000.

Comparative Studies on Some Chemical Aspects of White and Red Globe Onions

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Manuscript Received: 15 May 1973

Comparative studies on the composition of volatile odorous compounds of fresh white and red globe onion by gas liquid chromatography, and on diethyl ether extract of both varieties of onions by thin layer chromatography, ultra violet and infra-red spectroscopy, showed that, besides variation in amount of the volatile components, there was no radical change in the composition of the volatiles between the two varieties of onion and that identical nature of compounds in both the extracts were present. The primary distinction between white and red globe onion was the presence of anthocyanin pigment in red variety.

Knowledge about the onion flavour compounds and methods for their determination has become increasingly important for the evaluation of quality of onion and onion products. Amongst the several varieties of onions commonly called red and white globe onions are most widely cultivated and frequently consumed; however, a comparative study with respect to some of their chemical parameters is lacking.

Recent works[1-3] on odoriferous constituents of both varieties of onions revealed that they consist principally of various sulphur containing compounds which are developed through an enzymatic degradation process with alkyl-l-cysteine sulfoxides as precursors[4-6]. Since enzymatic action caused after tissue injury is solely responsible for the liberation of flavour substances from onion, it might be expected that the nature and composition of these compounds might vary depending upon varietal differences and degree of freshness of onion. Bandyopadhyay *et al.*,[7] had observed a considerable change in head space gas composition of fresh and stored onion of the red variety.

The colour of red onions is attributed to the presence of anthocyanin pigments[8]. Besides other flavonoids[9] a new type of nitrogenous pink pigment was observed by Joslyn *et al.*[10], during dehydration of both white and red globe onion. *In vitro* studies by Lukes[11] and Shannon *et al.*,[12] revealed that the pinking of onions is due to an initial enzymatic reaction with unknown precursors producing an ether soluble colourless compound named as 'colour developer' which subsequently undergoes chemical reaction with naturally occurring amino acids and carbonyls resulting water soluble pink coloured pigment. However, the nature and composition of both colour developer and pink pigment are not well understood.

The present investigation deals with the analysis of volatile odorous compounds and ether soluble components of white and red globe onions.

Materials and Methods

Red and white globe onions (Nasik, Maharashtra) stored for about one month after harvest were procured from a local market and used for subsequent analysis.

Head space gas analysis: Volatile components of both onion varieties trapped as head space gas were analysed by gas liquid chromatography (GLC) under identical conditions. Cut pieces of onions, 200 g were placed in a 250 ml conical flask. The flask was fitted with an adaptor having a side tube with a stopcock and a small aperture at the top. The aperture was closed with the help of self sealing silicone elastomer. The flask was then kept at -35°C for 1 hr and then rapidly put under vacuum (20 in Hg) for a short time. Thereafter, when the flask reached at room temperature, it was kept in a oven at 50°C for 2 hr. Vapour (10 ml) was then removed from the head space and injected directly in the column of a gas chromatograph (BARC Model) equipped with a flame ionisation detector. The column was packed with 10 per cent carbowax 20 M supported on acid washed chromosorb W (60-80 mesh). The carrier gas was nitrogen with a flow rate of 25 ml/min. The oven was equilibrated at 70°C. Identification of the components in the head space gas was followed by comparison of their retention times with that of authentic reference samples.

Onion extract: Ether soluble components of both varieties of onions were obtained by macerating the crushed onion with sufficient volume of peroxide-free diethyl ether in a Waring blender. A filtrate consisting of aqueous and ether layer was obtained after squeezing the pulpy mass with the help of four-fold mull cloth. The residue was similarly treated twice with diethyl ether. The aqueous layer was drawn off and re-extracted with an equal volume of diethyl ether. The ether layer was collected after centrifuging the whole mass, washed with distilled water, dried over anhydrous sodium sulphate and filtered. Finally the product (onion extract) obtained after removal of ether in a flash evaporator at 30°C was quantitised and analysed by thin layer chromatography (TLC).

Analysis of onion extract: For TLC silica gel plates were prepared according to the method described elsewhere[7]. 200 μg of each extract in benzene solution was spotted on silica gel plate and the plate was developed with petroleum ether (60-80°C b.p.)-diethyl ether-acetic acid (80:20:1). The chromatogram was made visible by spraying the plate with 50 per cent sulphuric acid followed by charring at 140°C for a short time. In another set of experiment, each extract was similarly chromatographed on silica gel plate and the plate after development was sprayed with glycine-formaldehyde reagent (9ml of 0.1 M glycine and 1 ml of 3×10^{-4}M formaldehyde) prepared according to the method of Shannon *et al.*,[12] and the pink spots were noted after heating the plate at 100°C for 1 hr.

Preparative TLC separation of the components of each ether extract of onions was done on silica gel plate using the same solvent system as above. The fractions thus separated in bands were located by exposing the plate in iodine vapour and subsequently recovered after eluting with chloroform according to the method described by Bandyopadhyay *et al*[7]. Infrared spectrum (IR) of each fraction from each extract was obtained in Perkin—Elmer Infracord spectrophotometer model 137B using sodium chloride optics.

Ultraviolet and visible spectra of ether extract of both white and red onions was obtained in Beckman DB spectrophotometer.

Pigment content: Anthocyanin content of red onions was determined spectrophotometrically by the method of Fuleki *et al.*[13] Known quantity of onions were cut and soaked in 1.5 N HCl-ethanol (15:85) overnight and then blended with same solvent. The macerate was filtered through mull cloth and made to volume in 500 ml flask with the same solvent while maintaining the pH at 1. Optical density was measured at 535 mμ in Beckman DB spectrophotometer.

Both the pink coloured juices of white and red globe onions obtained after ageing the respective juices overnight at room temperature were subjected to spectrophotometric analysis and the absorption maximum in the visible range was determined[11].

Results and Discussion

Fig. 1 represents the head space gas analysis of fresh red and white globe onions by GLC. By comparing the retention time of standard samples, injected separately as well as internally, the presence of acetaldehyde, ethyl methyl ketone, ethyl alcohol, n-propyl alcohol, propionaldehyde, di-n-propyl disulfide and propenyl propyl disulfide in volatiles of both white and red globe onions have been detected. Amongst the sulphur compounds the major pungent flavour substances are known to be di-n-propyl disulfide[3] and propenyl propyl disulfide[14], while the lachrymatory factor, recently characterised as thiopropanal S-oxide[15], which spontaneously occur when onion is cut, decomposes quickly to propinoaldehyde[6]. Fig. 1 shows that although there is no radical change in the chromatographic picture of volatile components of both varieties of onion, the variation in amount of volatile constituents in the two varieties of onion is noticeable. Thus, the volatiles of white globe onion contain predominent amount of ethyl methyl ketone than red one, while the major pungent flavour substances are found to be equally present in both the varieties. The yield of onion extract from both varieties are found to be same and that amounts to 0.1 per cent of the wet onion. Both these extracts have strong odour.

TLC separation of diethyl ether extract of both white and red globe onions is shown in Fig. 2. In both cases seven distinct spots having identical Rf values including those at the origin and at the solvent front are obtained. Rf values of the respective spots are 0.0, 0.11, 0.21, 0.33, 0.42, 0.86 and 0.96. Identical Rf values indicate that the components present in both the extracts are presumed to be identical in nature and in behaviour. There is also no noticeable variation in amount of separated components of the respective extract as reflected in the intensity of the colour of spots after charring with sulfuric acid. Further, TLC spots of both the extracts having Rf values of 0.11, 0.21 and 0.33 equally respond to glycine-formaldehyde reagent forming pink colour, although the appearance of pink colour in respective spots seems to vary with time; for example, the spot having Rf value of 0.11 turns intense pink colour within 15 min while those of 0.21 and 0.33 become less intense pink colour after 1 hr of heating at 100°C. These compounds may be considered as colour

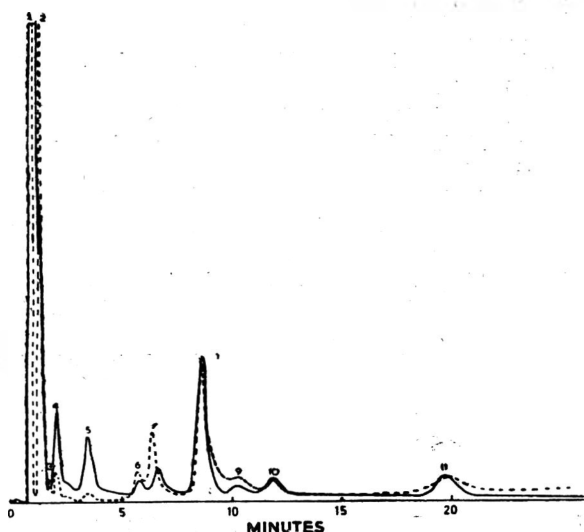

Fig. 1. Gas chromatogram of volatile components of onion from the head space. (— — — —), Fresh white Globe onion, (————); fresh Red Globe onion. 1. acetaldehyde; 2. ethyl methyl ketone; 3. ethyl alcohol, 4. n-propyl alcohol; 5. propionaldehyde; 6 and 7. unidentified; 8. di-n-propyl disulfide; 9. unidentified; 10. propenyl propyl disulfide; 11. unidentified.

developer[12]. Shannon *et al.*,[12] attempted to separate the colour developer present in ether extract of onion by paper chromatography using various polar and non-polar solvent system and obtained a single spot in every case, which indicated that the colour developer piesent in ether extract was believed to be a single component. The present TLC method of separation of ether extract of onions, however, shows that at least three such components are present in both varieties of onions. One interesting observation has been made on TLC plate was that the spots responsible for pink colour formation with glycine formaldehyde reagent turn pink to violet colour by exposing the plate in air overnight without pre-treatment with the spraying reagent. This might be attributed to the oxidative changes of the colour developers.

A comparative spectrophotometric study of diethyl ether extract of both white and red globe onion indicate no difference in spectral characteristics between the two in both visible and UV range; also nature of IR spectra of individual components of both the extracts isolated from preparative TLC plate are identical.

White globe onion differs from red one due to the presence of anthocyanin pigment in the latter variety. Total anthocyanin content of freshly harvested red globe onion was found to be 14.6 mg per 100 g of wet

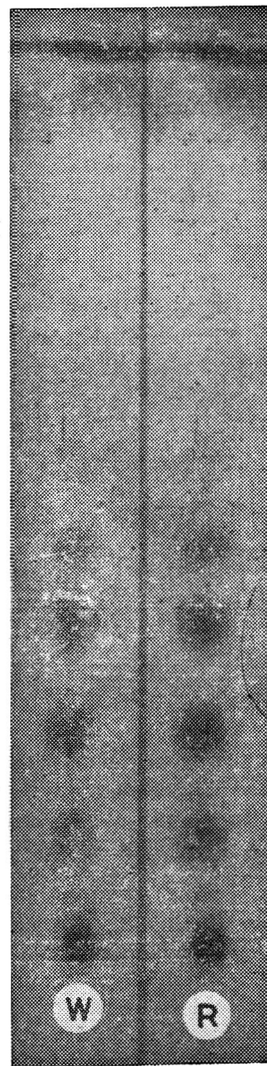

Fig. 2. TLC separation of ether extract of whtie (W) and red (R) globe onions on a silica gel plate using petroleum ether-diethyl ether-acetic acid (80: 20: 1) as developing solvent. The chromatogram was detected by spraying the plate with 50 per cent sulphuric acid followed by charring.

onion. However, the juice from both varieties of onions turns pink in colour during ageing. Pink coloured juice of white onion shows an absorption maximum at 520 mμ, and while that of red onion shows two maxima at 520 mμ and 535 mμ, the latter is due to anthocyanin pigment.

Acknowledgement

The authors wish to thank Dr A. Banerjee for synthesizing sulphur compounds and to Dr A. Sreenivasan for his helpful suggestions and criticism.

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Enzymic Browning of Whole Wheat Meal Flour: Cysteine Inhibition of Tyrosinase*

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Oxygen uptake experiments with tyrosinase from wheat, tyrosine, DOPA and chlorogenic acid in the presence and absence of cysteine were performed to determine the possible sites of cysteine inhibition of enzymatic browning of the dough prepared form the whole wheat meal flour. The presence of cysteine with tyrosine resulted in an induction period before the oxidation of tyrosine began. The rate of oxidation was decreased with successive increase in the concentration of cysteine. Low concentrations of cysteine did not inhibit the oxidation of DOPA significantly, however, at higher concentrations, the oxidation was inhibited. Cysteine did not inhibit the oxidation of chlorogenic acid. Oxygen uptake with chlorogenic acid plus cysteine was higher than in the absence of cysteine. Cysteine concentrations which effectively inhibited tyrosine oxidation did not inhibit oxidation in the presence of tyrosine plus chlorogenic acid. The rate of oxidation of DOPA plus chlorogenic acid was higher in comparison to DOPA and chlorogenic acid alone. The initial site of cysteine inhibition of enzymatic browning caused by tyrosine oxidation appears to occur at the oxidation of tyrosine to DOPA. Further, it seems to combine with quinones produced and checks their further oxidation.

Introduction of dwarf Mexican germ plasm under the high yielding varieties programme[1], responsible for 'green revolution' in this country, has resulted in the availability of wheat grains of inferior quality from the view point of *chapati*-making[2]. Amongst the various attributes, the one which pertains to *chapati*-making is that the dough prepared from the whole meal flour of these varieties darkens on standing[3] and subsequently gives brown *chapatties*[4]. Besides the seed coat colour, the enzyme tyrosinase (polyphenol oxidase) mainly localized in the bran[3] has been shown to be directly related to dough darkening[4]. The reaction involves enzymatic oxidation of phenols and some amino acids like tyrosine to quinones which are subsequently converted into complex products by polymerization and interaction with proteins[5].

Different characteristics of the tyrosinase of wheat grains; factors responsible for the extensive browning of dough and the detailed substrate specificity and inhibition of this enzyme in relation to browning have already been discussed in our earlier communications.[6-8] The present investigation demonstrates the possible mechanism of cysteine inhibition of enzymatic browning of whole wheat meal dough.

Materials and Methods

Kalyan 227 variety of Mexican wheat commonly grown in this country was selected for the present investigation and was obtained from the Plant Breeding Department of this University.

Enzyme preparation: The enzyme was isolated

*Part of the thesis submitted to Haryana Agricultural University, Hissar, in partial fulfilment of the requirements for the M.Sc. degree by the junior author.

and partially purified by following the procedures described in our earlier communications.[6,7]

Measurement of enzyme activity: The enzyme activity was measured by the manometric determination of the rate of oxygen consumption.

The following stock solutions were used: 1 mg/ml L-tyrosine, D,L-DOPA and chlorogenic acid; 1×10^{-1} M cysteine (free base); 20 per cent KOH (0.2 ml) for CO_2 absorption. Tyrosine was put in the side arm unless stated otherwise. The pre-incubation time for the cysteine and the enzyme in the main compartment was approximately 30 min, and the tyrosine was tipped in from the side arm. Oxygen uptake was determined in a Warburg apparatus set at 37°C and 120 oscillations per min.

Results and Discussion

Enzymatic oxidation of tyrosine in presence and absence of cysteine is shown in Fig. 1. The presence of cysteine resulted in an induction period before oxidation of the tyrosine began. Following the induction period, the oxygen uptake essentially paralleled the curve where no cysteine was present. There was corresponding decrease in the rate of oxidation with successive increase in the concentration of cysteine. With 0.4 and 0.6 ml cysteine (10^{-1} M), tyrosine oxidation was inhibited for over 150 and 180 min respectively. Fig. 1 also shows the effect of incubating the enzyme with cysteine. Practically no difference was observed when tyrosine was taken in the compartment or in the side-arm of the vessel during the equilibration period.

Low concentrations of cysteine (0.2 ml) did not inhibit the oxidation of DOPA significantly (Fig. 2), however, with higher concentrations (0.4 and 0.6 ml), the oxidation of DOPA was inhibited. Since cysteine will form addition products with oxidized DOPA, higher concentrations of these addition products may result in enzyme inhibition.

It appears from these experiments that the initial effect of the cysteine is to inhibit the oxidation of tyrosine to DOPA. Oxidation of tyrosine to DOPA is, therefore, more sensitive to cysteine inhibition than the oxidation of DOPA to DOPA-quinone. If the initial inhibition was due to the reaction of cysteine with a quinone, then oxygen uptake should have occurred with no induction period. This would be expected because oxygen is required for both the oxidation of tyrosine and DOPA before the first possible reactive quinone is formed. Addition products formed by reaction of cysteine and oxidized DOPA may result in inhibition at a later stage due to competitive mechanism.

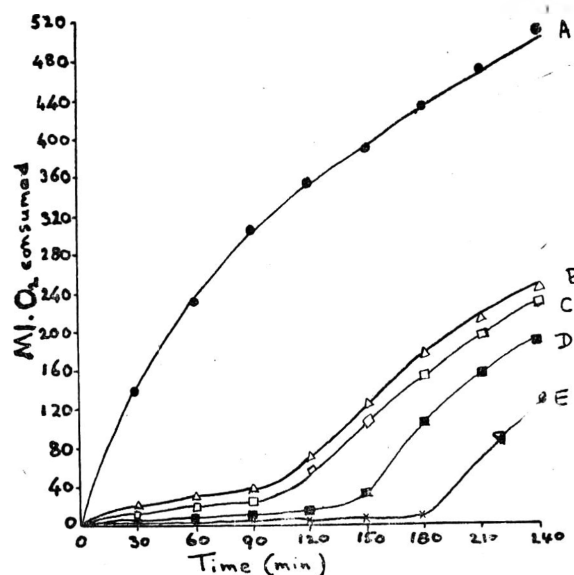

Fig. 1. Cysteine inhibition of the enzymatic oxidation of tyrosine. The reaction mixture contained: 1.0 ml enzyme preparation; 1.0 ml (1 mg/ml) tyrosine; 0.0, 0.2, 0.4 and 0.6 ml 10^{-1}M cysteine; phosphate buffer (pH 6.1) to a final reaction volume of 3.0 ml. A-Tyrosine; B-Tyrosine +0.2 ml cysteine; C-Tyrosine +0.2 ml cysteine (Enzyme in side arm); D-Tyrosine+0.4 ml cysteine; E-Tyrosine + 0.6 ml cysteine.

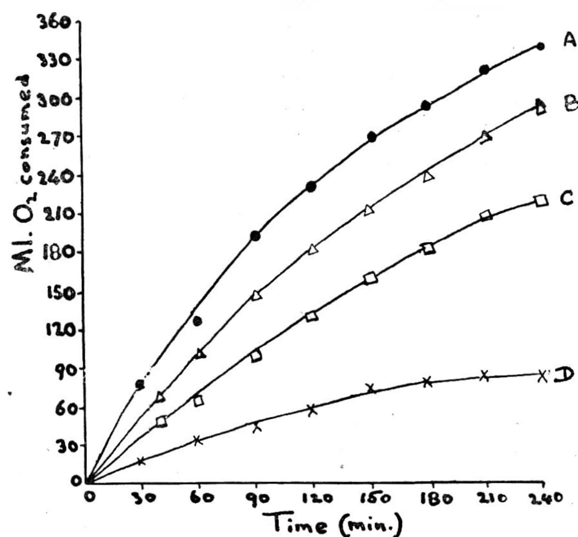

Fig. 2. Cysteine inhibition of the enzymatic oxidation of DOPA. The reaction mixture contained: 1.0 ml enzyme preparation; 1.0 ml. (1 mg/ml) DOPA; 0.0, 0.2, 0.4 and 1.0 ml 10^{-1}M cysteine; phosphate buffer (pH 6.1) to a final reaction volume of 3.0 ml A-DOPA; B-DOPA+0.2 ml cysteine; C-DOPA+0.4 ml cysteine; D-DOPA+1.0 ml cysteine.

Fig. 3 shows that the oxidation of chlorogenic acid was not inhibited at all by 0.2 and 0.4 ml (10^{-1}M) cysteine used in these experiments. On the other hand, the rate of oxidation was somewhat increased in

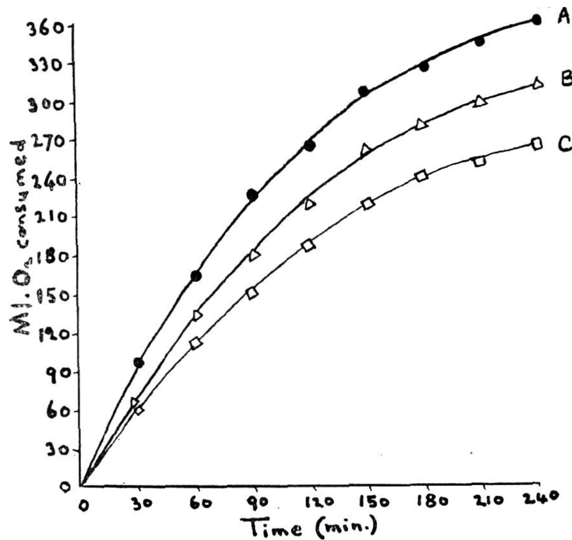

FIG. 3. Cysteine inhibition of the enzymatic oxidation of chlorogenic acid.
The reaction mixture contained: 1.0 ml enzyme preparation; 1.0 ml (1 mg/ml) chlorogenic acid; 0.0, 0.2, 0.4 ml 10^{-1}M cysteine; phosphate buffer (pH 6.1) to a final reaction volume of 3.0 ml. A-Chlorogenic acid+0.4 ml cysteine; B-Chlorogenic acid+0.2 ml cysteine; C-Chlorogenic acid.

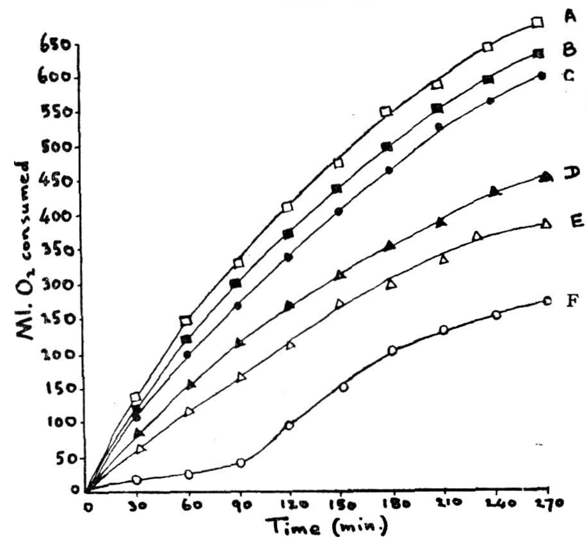

FIG. 4. Interaction of tyrosine, chlorogenic acid, cysteine in enzymatic browning.
A-Tyrosine + chlorogenic acid+0.2 ml cysteine; B-Tyrosine +chlorogenic acid; C-Tyrosine; D-Chlorogenic acid+0.2 ml cysteine; E-Chlorogenic acid; F-Tyrosine+0.2 ml cysteine.

the presence of cysteine. There are several possible explanations for this. The cysteine may act as a reducing agent on the quinone to give significantly higher oxygen uptake than with chlorogenic acid alone. The rate of reaction inactivation may also be prevented or slowed down by cysteine. Relative rates of quinone reduction and the formation of inactive products from the addition of cysteine to the quinone may also affect the total oxygen uptake. The reaction between cysteine and oxidized chlorogenic acid does not give colourless products but forms a light yellow-tan pigment. This further supports Henze's[9] and Muneta and Walradt[10] evidences that cysteine inhibits enzymatic browning by combining with the quinone. However, enzymatic oxidation can occur with little pigment formation because of the formation of sulfhydryl addition products. This further supports the idea that cysteine inhibits enzymatic browning caused by the oxidation of O-hydroxyphenols by combining with the quinones which are formed, rather than by inhibiting the initial oxidation. However, cysteine can also inhibit enzymatic darkening by inhibiting the oxidation of tyrosine to DOPA.

Since chlorogenic acid, tyrosine and DOPA are considered to be the possible substrates most commonly involved in this type of browning,[5,7,10] interactions between these compounds and cysteine were also studied (Fig. 4 and 5). Oxygen uptake was not inhibited when chlorogenic acid, tyrosine and cysteine

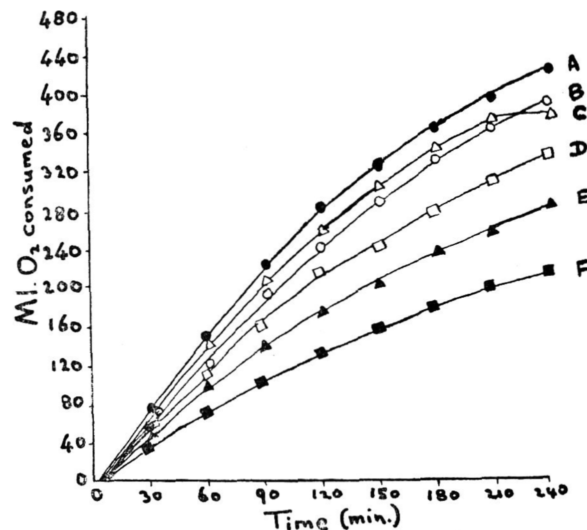

FIG. 5. Interaction of DOPA, chlorogenic acid, cysteine in enzymatic browning.
A-DOPA + chlorogenic acid; B-DOPA; C-DOPA+chlorogenic acid+0.4 ml cysteine; D-Chlorogenic acid+0.4 ml cysteine; E-Chlorogenic acid; F-DOPA+0.4 ml cysteine.

together were used (Fig. 4). The oxygen uptake rather started immediately upon mixing substrates and enzyme with immediate appearance of the yellow colour of the oxidized chlorogenic acid. Tyrosine and chlorogenic acid together consumed more oxygen than tyrosine or chlorogenic acid alone. This could be expected as both the substrates are readily oxidized by the enzyme. The explanation for no induction period

of tyrosine oxidation in presence of chlorogenic acid may be that the cysteine inhibitory levels are reduced by chlorogenic acid to such a degree that the oxidation of tryosine could proceed rapidly. The oxidized chlorogenic acid might have lowered the cysteine concentration by reacting with it to form either addition products[9] or oxidized cysteine compounds[11]. Chlorogenic acid itself may have some catalytic effect on tyrosine oxidation since simple O-dihydroxyphenols have catalytic effect on tyrosine oxidation.[10] Hence due to above said reasons, cysteine concentration which effectively inhibited tyrosine oxidation did not inhibit it in the presence of chlorogenic acid. Mapson *et al.*[12] also showed that the rate of pigment formation from tyrosine oxidation is increased in the presence of chlorogenic acid.

Fig. 5 shows the enzymatic oxidation of DOPA and chlorogenic acid alone and in combination, in presence and absence of cysteine. The rate of oxidation of DOPA plus chlorogenic acid in absence of cysteine was much more than that of DOPA and chlorogenic acid alone. This is again because of the fact that both the substrates are readily oxidized by the enzyme. Cysteine did not inhibit the oxidation of DOPA plus chlorogenic acid to any significant extent. This is again probably because of the reasons already explained.

Acknowledgement

The authors are thankful to Dr B. M. Lal, Professor of Biochemistry for providing the necessary facilities and Sh. D. R. Vasudeva for providing the samples of the wheat.

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Utilisation of Musk Melon *(Cucumis Melo* L*)*, Variety *Hara Madhu*

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Hara Madhu **variety of musk melon can be successfully utilized in the form of canned slices and cubes in syrup. Organoleptic evaluation has shown that the consumer's acceptance of canned fruit is very favourable and is quite promising. The product could stand very well for two months without impairment of quality. The fruit was also successfully utilized for the preparation of jam; the consumer acceptance was found very favourable. The product had retained its colour and was found excellent in flavour, consistency and overall acceptability.**

Musk melon is a commercial fruit of considerable importance in Punjab, Mysore, Andhra Pradesh and Maharashtra. A number of workers[1-4] carried out studies on the quality evaluation of musk melon varieties.

Siddappa and Bhatia[5] worked on canning aspects of musk melon along with other fruits. Since this fruit has a pH of 5.0 to 5.21 which is higher than the recommended 4 to 5, attempts were made to can it in acidified syrup as well as with other fruits like mango, pine-apple and grapes having a lower pH.

Rao *et al.*[6] did the evaluation of nine musk melon varieties of Andhra Pradesh for canning. They also studied the effect of calcium treatment for the canning purposes. It was noticed that calcium failed to bring about any significant improvement in the texture of canned melon cubes. On the other hand, it imparted a saline taste to the product and natural flavour and aroma were also impaired to some extent.

Except for these preliminary trails no systematic work has been done in India on the canning of musk melon. Suitability of *Hara Madhu* for canning has not been tried earlier. It was, therefore, felt worthwhile to carry out these investigations.

Hara Madhu is an important late maturing variety of musk melon. The Fruit is quite large and is very sweet, having 13-18 per cent soluble solids. The flesh is thick, green and juicy and gives an excellent flavour. *Hara Madhu* is available comparatively at cheap price and is quite popular in this region. It has a very attractive flavour and colour, and was, therefore, selected for jam making.

Materials and Methods

1. *Canning of musk melon:* Fresh, medium sized, musk melon of optimum maturity, procured from the fields of Department of Horticulture, of the University were used. Fruits were hand peeled, seeds and soft portions around the seed cavity were removed and fruits were cut into cubes and filled in plain A 2½ size cans. In another lot the cans were filled with slices, covered with syrup (40°Brix), exhausted for about 5-7 min. in boiling water, sealed and processed for 10 min. in boiling water. The following treatments were given to the contents:

(*i*) Slices were covered with syrup containing 0.30 per cent citric acid. (*ii*) Slices were covered with syrup containing 0.4 per cent citric acid. (*iii*) Cubes were covered with syrup containing 0.3 per cent citric acid. (*iv*) Cubes were covered with syrup containing 0.4 per cent citric acid.

Cubes and slices were dipped in 0.15 per cent solution of calcium chloride for 15 min. at room temperature. The treated slices and cubes were processed in cans as in the control. Other treatments were the same as in the control.

Calcium chloride was added at the rate of 0.04 per cent of the syrup to the can.

These cans were stored for two months at room temperature and the samples were subjected to cut out analysis. The over all quality test was done by a panel of judges. The judges were asked to evaluate the coded samples as very good (4) good (3) fair

(2) poor (1) or unacceptable (0). They were also asked to evaluate the same overall quality test proforma, the comments as just good, lacks crispness, too soft, lacks colour or any other defects.

The canned slices of the fruits were further subjected to the following tests.

(*a*) *Organoleptic evaluation:* A sensory evaluation of the canned product was done each month for a period of two months. A panel of 6-8 judges was selected for the evaluation and an evaluation chart was used for the purpose. The results were statistically analysed by an overall quality test.

(*b*) The canned product was analysed for net weight, drained weight, internal appearance of can in terms of corrosiveness, number of slices and cubes in a can, pH, acidity as citric acid and overall quality by standard methods.

2. *Preparation of Jam:* Fresh ripe musk melons were procured as stated earlier.

In selecting the fruit special attention was given to the characters like freshness, size of the fruit, freedom from blemishes, molds, diseases, malformation and insect damaged portions, smooth skin, free from wrinkles and molds, high soluble solids content, and good flavour. These factors were kept in view because the variety of fruit, its maturity and locality in which it is grown, have a marked effect on flavour and keeping quality of products. The fruits were prepared as for canning. The fruit pieces were heated for 5 min at a temperature of 190°C. This method inactivates enzymes like pectinases and improves the flavour and consistency of the pulp. The yield of juice is also higher. The heated fruit was then passed through a screw type juice extractor and the pulp was obtained. This pulp was used for the preparation of jam. Recipe recommended for other jams by Lal *et al.*,[7] was modified and used.

Recipe

1. Fruit 1 kg.
2. Sugar 1 kg.
3. Pectin (200 grade) 5.5 gm.
4. Acidity 0.5 per cent of final weight of jam.

Jam was prepared and packed in glass jars by the usual methods.

Sensory evaluation: To study the consumer's acceptance of the experimental product and to judge its degree of acceptability in comparison with commercial jams (Tabls 3 and 4), sensory evaluation was conducted by a panel of 8 judges. The judges were asked to grade the samples on the basis of flavour, colour, consistency and overall acceptability by the ranking methods of preference, using an evaluation chart.

As the jam is generally taken with bread and butter, it was considered necessary to evaluate it as such and that spread on bread with butter. The results were analysed statistically by the rank-sum method of Kramer[8].

Results and Discussion

Canning of slices and cubes: Results of the cut-out examination of a typical pack after a storage period of one to two months at room temperature are presented in Table 1 and 2. The original texture of fruit cubes and slices were well retained. As was expected, there was a decrease in °Brix of syrup and increase in per

TABLE 1. CUT-OUT EXAMINATION OF CANNED SLICES

Particulars	After one month						After two months					
	Control		$CaCl_2$ added		Slices dipped in $CaCl_2$		Control		$CaCl_2$ added		Slices dipped in $CaCl_2$	
Syrup acidity % (initial)	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4
Drained wt. %	43.1	53.2	53.1	53.2	53.2	53.0	53.8	53.8	53.8	54.0	53.9	54.0
Internal appearance of can	nc	mc	vc	vc	mc	vc	mc	mc	vc	vc	mc	vc
°Brix	30.0	30.0	31.5	33.0	33.0	33.0	29.0	29.0	30.0	32.0	32.5	32.0
pH of syrup	3.5	3.0	3.5	3.0	3.5	3.0	4.5	4.0	4.5	4.0	4.5	4.0
Acidity as citric %	0.28	0.32	0.28	0.32	0.28	0.33	0.25	0.27	0.27	0.30	0.27	0.30
Overall quality (5)	3.6	3.6	3.3	3.2	3.6	3.1	3.4	2.5	3.3	2.4	2.5	2.8

nc=not corrosive mc=mild corrosive vc=very corrosive

External appearance of cans before opening was sound and normal in all the cases. Vacuum found in all cases was 5" mercury and head space was 0.5 cm. Appearance of the product on opening was attractive.

TABLE 2. CUT-OUT EXAMINATION OF CANNED SLICES CUT INTO CUBES

Particulars	After one month						After two months					
	Control		$CaCl_2$ added		Dipped in $CaCl_2$		Control		$CaCl_2$ added		Dipped in $CaCl_2$	
Syrup acidity% (initial)	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4
Drained wt. %	53.3	54.4	53.3	53.1	53.0	53.0	53.9	54.9	53.9	53.7	53.8	53.9
Internal appearance of can	mc	mc	mc	mc	mc	mc	mc	mc	me	mc	mc	mc
Cut out °Brix	28.0	28.5	29.0	29.5	31.0	32.5	25.5	25.5	25.0	25.0	29.0	30.0
pH of syrup	3.5	3.0	3.5	3.0	3.5	3.0	4.5	4.0	4.5	4.0	4.5	4.0
Acidity as citric %	0.28	0.30	0.27	0.31	0.29	0.34	0.24	0.26	0.24	0.27	0.28	0.32
Overall quality (5)	3.5	3.5	3.1	3.3	3.4	3.3	3.3	2.8	3.3	3.4	2.8	3.8

nc=not corrosive mc=mild corrosive vc=very corrosive

External appearance of cans before opening was sound and normal in all the cases. Vacuum found in all cases was 5" mercury and head space was 0.5 cm. Appearance of the product on opening was attractive.

cent of drained weight with storage. This is due to the lower Brix of musk melon. Similarly there was decrease in the acidity of the syrup resulting in an increase in the pH during the storage.

Calcium chloride treatment at the two levels used failed to bring about any significant improvement in the texture of the canned fruit possibly due to the low pectin content. On the other hand it imparted an astringent taste to the product. The natural flavour and aroma of the product were also affected to some extent. The overall quality of canned fruit was better in the cases of slices which were dipped in calcium chloride slution. There was no corrosion in cans in the experiment tried by Bhatia *et al.*,[4] but in present studies corrosion was found in many cases. This may be due to low vacuum or poor quality of the tin plate used for cans in the present investigation. In spite of lower vacuum (i.e., 5" of mercury) in cans, the product stood well. On the whole canned musk melon is an excellent product having good appearance, texture and characteristic pleasant flavour.

Jam: Based on organoleptic evaluation of the jam and the results of the statistical analysis summarised in Tables 3 and 4, it was found that *Hara-Madhu* jam was rated superior to other jams in flavour, consistency and overall acceptability. As regards colour (green) it was rated at par with others. Commercial jams (except plum jam) were not significantly different in any of the quality factors.

Results were very similar when evaluation was repeated using bread with butter. Musk melon jam proved significantly superior in flavour, consistency and overall acceptability by the judges.

TABLE 3. SENSORY EVALUATION OF JAM (WITHOUT BREAD AND BUTTER)

Jam samples	Colour	Flavour	Consistency	Overall acceptability
Mixed fruit	22	28	22	24
Strawberry	44	40	37	44
Mango	29	34	34	28
Musk melon	28	16*	15*	15*
Apple	30	33	36	27
Pineapple	20	22	38	32
Plum	51†	51†	42	50†

* Superior; † Highly significant; poor

Rank total required for significance at 5% level (PO, 05)=18.46

Rank total required for significance at 1% level (PO, 01)=16.48

TABLE 4. SENSORY EVALUATION OF JAM (WITH BREAD AND BUTTER)

Jam samples	Rank sums			
Fruit Base	Colour	Flavour	Consistency	Overall acceptability
Mixed fruit	19	34	22	27
Strawberry	43	36	38	38
Mango	32	29	34	25
Musk melon	29	17*	16*	20
Apple	27	33	36	25
Pineapple	21	22	37	36
Plum	53†	49†	41	53†

* Superior † Highly significant; poor

Rank total required for significance at 5% level (PO, 0.05)=18.46

Rank total required for significance at 1% level (PO, 0.01)=16.48

Acknowledgement

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Chemical Composition of Raw and Roasted Fenugreek Seeds

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Twenty four samples of raw fenugreek seeds were analysed for their chemical composition. The seeds were roasted at 150°, 175° and 200°C and were analysed for their chemical composition. Light roasted (150°C) seeds were found superior to the medium (175°C) and dark roasted (200°C) seeds with regard to flavour. Fenugreek seeds were found to be a rich source of protein.

Fenugreek (*Trigonellafoenum-graecum* L.) is used as a spice, as a vegetable and to a small extent for medicinal purposes. India produces about 20,000 tonnes of fenugreek seeds, grown in about 40,000 hectares, out of which nearly 1500 tonnes are exported, earning foreign exchange to the tune of 2 million rupees annually[1]. It is essential to carry out scientific and technological research on this very important spice of our country. As a spice, fenugreek adds nutritive value to foods as well as flavour. Fenugreek is used in maple and rum flavours. The seeds are usually roasted before they are used in the preparation of curry powder. The work reported here was undertaken at this Institute to ascertain the chemical composition of both raw and roasted fenugreek seeds.

Material and Methods

Raw seeds: Fenugreek seeds obtained from Agricultural College, Coimbatore, Directorate of Arecanut and Spices, Calicut and Mysore market were analysed for moisture, ash, petroleum ether extract, alcohol extract, dichloroethane extract, water extract, total nitrogen and crude protein, total and free sugars, according to AOAC methods[2].

Roasted seeds: Fenugreek seeds obtained from Mysore market were roasted (5-10 min) in an electric roaster at different finishing temperatures viz., light roast at 150°C, medium at 175°C and dark roast at 200°C. The roasted seeds were analysed for moisture, colour (reflectance value), ash, solvent extract, nitrogen, crude protein, total and free sugars, as per AOAC methods[2].

Free-amino acids: The number of free-amino acids present in both raw and roasted seeds were detected using descending paper chromatographic technique[3]. The amino acids were extracted from the powdered seeds (50g) using 150 ml. of 80 per cent ethanol and this was evaporated on a water bath and the residue was finally taken up in 10 per cent isopropanol (5 ml) for spotting. The extracts were spotted (2λ) on Whatman No. 3 paper and the chromatogram was developed in solvent butanol, acetic acid and water (6:1:2) for 16 hr. The air dried paper was sprayed with 0.25 per cent ninhydrin in acetone and dried in an oven at 60°C for 10 min.

Free-sugars: Free-sugars of both unroasted and roasted seeds were detected by using descending paper chromatography[4]. The powdered seeds (50 g.) were extracted with 500 ml. of 50 per cent ethanol and this was evaporated on water bath. The residue was taken up in pyridine (5 ml) and spotted (2λ) on Whatman No. 1 and developed in butanol, acetic acid and water (6:1:2) for 16 hours. The air dried paper was sprayed with modified benzidine reagent and then the paper was heated in an oven at 110°C for 10 minutes. The

free reducing sugars appeared on the paper as brown and yellow spots. The free non-reducing sugars were detected by exposing the air dried chromatogram in an iodine chamber. The sugars were seen as red-brown spots.

VRS of fenugreek seeds: The volatile reducing substances of both raw and roasted fenugreek seeds, after powdering, were estimated by the method described by Farber[5].

Results and Discussion

Raw seeds: The results of analysis of raw seeds of fenugreek are given in Table 1. The range of values (as per cent) varies as follows: moisture, 7-10; total ash, 3.35—6.80; acid insoluble ash, 0.18-2.32; petroleum ether extract, 5.16-8.24; alcohol extract, 16.55-24.83; hot water extract, 29.00-39.67; total nitrogen, 4.43-6.17; crude protein, 27.68-38.56. Alcohol is able to extract about twice the quantity of oleoresin as compared to petroleum ether. The results show that fenugreek seeds are good source of protein.

Roasted seeds: It can be seen from Table 2 that the loss on roasting goes on increasing as the temperature increases but the colour value (per cent of reflectance) goes down. An important observation is that light roasted seeds yield highest solvents extract and the dark roasted seeds yield lowest solvents extract as compared to the light and medium roasted seeds. The capacity to extract oleoresin increases in the order petroleum ether < dichloroethane < alcohol. No appreciable loss in the total nitrogen and crude protein was observed in the roasted seeds, but the quantity of total and free-sugars was found to decrease considerably as the temperature increased.

It is interesting to note that the raw seeds are very poor in VRS and the roasting increases the VRS of the seeds very much. Medium roasted seeds were found to contain maximum VRS and dark roasting is not desirable from VRS point of view.

The paper chromatogram of free sugars show that there are five reducing and three non-reducing sugars present in the raw seeds, but only three free-reducing sugars and three non-reducing sugars present in the roasted seeds. The quantity of the free-sugars present in the roasted seeds depends on the temperature of roasting.

Eight free-amino acids were found present in the raw seeds of fenugreek and they decreased on roasting. No amino acid was found present in considerable quantity in the dark roasted seeds. Light roasted seeds were found to contain more number of amino acids as compared to the medium roasted seeds.

TABLE 1. ANALYSIS OF FENUGREEK SEEDS (ALL VALUES ARE ON MOISTURE-FREE BASIS)

Sample No.	Moisture %	Total ash %	Acid insoluble ash%	Petroleum ether extract%	Alcohol extract%	Hot water extract%	Total nitrogen%	Crude protein% (N×6.25)
1	7.0	5.30	0.68	6.23	22.08	31.72	4.60	28.75
2	9.0	4.72	0.92	6.33	18.17	30.49	4.88	30.50
3	8.0	5.32	1.28	6.29	20.60	34.50	5.17	32.31
4	9.0	6.42	1.96	8.24	24.83	35.16	6.02	37.62
5	9.0	4.68	1.21	5.68	16.90	30.49	4.70	29.37
6	9.0	4.68	1.38	5.71	16.90	33.29	4.79	29.93
7	10.0	4.50	0.64	6.05	17.94	32.20	4.66	28.12
8	8.0	5.04	0.65	7.58	22.97	37.70	6.00	37.50
9	9.0	4.20	0.51	5.41	18.60	29.80	4.43	27.68
10	7.0	6.66	2.32	7.38	22.66	35.75	5.70	35.62
11	8.0	4.64	0.52	5.16	22.44	34.50	4.65	28.06
12	9.0	4.06	0.76	6.26	18.80	33.30	4.79	29.93
13	8.0	4.57	0.65	5.40	22.22	34.50	4.43	27.68
14	9.0	4.49	1.10	5.30	16.75	30.21	4.70	29.37
15	9.0	6.10	1.47	7.41	23.84	38.73	5.75	35.93
16	9.0	6.21	1.67	7.39	20.84	40.27	5.80	36.41
17	8.0	6.80	1.93	6.00	16.90	31.40	4.52	28.25
18	9.0	5.40	0.85	6.79	23.63	36.42	5.58	34.87
19	9.0	3.73	0.76	6.07	19.75	31.30	4.48	27.90
20	8.0	4.46	0.21	6.75	21.03	39.67	5.82	36.37
21	11.0	4.28	0.74	5.23	17.50	34.55	5.03	31.43
22	10.0	5.00	0.50	7.55	24.50	38.72	6.17	38.56
23	8.0	3.35	0.18	5.31	23.05	33.40	4.60	28.75
24	10.0	4.17	0.20	5.60	16.55	29.00	4.66	28.12
Minimum	7.0	3.35	0.18	5.16	16.55	29.00	4.43	27.68
Average	8.7	4.94	0.96	6.29	20.35	34.04	5.08	31.62
Maximum	11.0	6.80	2.32	8.24	24.83	39.67	6.17	38.56

TABLE 2. CHEMICAL COMPOSITION OF RAW AND ROASTED FENUGREEK SEEDS (VALUES ON MOISTURE-FREE BASIS)

Sl. No.	Constituents	Raw seeds	Light roasted (150°C)	Medium roasted (175°C)	Dark roasted (200°C)
1.	Loss on roasting%	...	5.00	7.00	10.25
2.	Colour value (Tristimulus green)	23.00	20.00	13.00	8.00
3.	Aroma and taste	...	...	...	...
4.	Moisture%	10.00	4.50	3.50	2.00
5.	Total ash%	3.59	3.23	3.29	3.32
6.	Acid insoluble ash%	0.26	0.24	0.25	0.28
7.	Petroleum ether extract%	5.63	5.78	5.68	4.69
8.	Dichloroethane extract%	8.02	8.04	7.84	7.71
9.	Hot alcohol extract%	17.59	17.83	15.42	15.18
10.	Cold alcohol extract %	13.19	12.96	10.62	7.53
11.	Hot water extract%	31.62	29.32	29.80	30.23
12.	Cold water extract%	28.89	30.10	29.27	28.44
13.	Total nitrogen%	4.48	4.43	4.43	4.42
14.	Crude protein (6.25×N)%	28.00	27.70	27.70	27.60
15.	Total sugars%	37.50	35.69	35.20	32.40
16.	Free sugars%	00.19	Tr.	Tr.	Tr.
17.	V.R.S. (μeq $KMnO_4$/g)	77.70	136.10	196.90	102.10
18.	No. of amino acids present	8.00	7.00	6.00	Traces
19.	No. of free sugars	8.00	6.00	6.00	6.00

The colour of the powder of the raw, light medium and dark roasted seeds powder was yellow, light brown and dark brown- respectively. The aroma and taste of raw seeds was raw, where as that of light and medium roasted was aromatic and good, that of dark roasted was not good.

In general, it can be concluded that light roasting is preferable to the other roastings with regard to flavour and nutritive values. Raw seeds of fenugreek are very poor in flavour as shown by VRS values and roasting is very essential to get good flavour. It is necessary to carry out further research work on the details of the changes taking places in the chemical composition of the roasted seeds of fenugreek.

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Protein-Enriched Milk Biscuits*

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With the object of evaluating several formulations for milk-biscuits, the characteristics of several formulations have been studied.

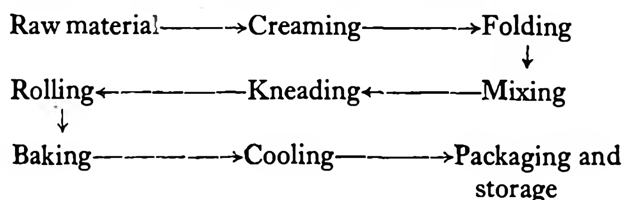
Chapman *et al.*,[1] reported the production of New Zealand milk biscuits using vacuum ovens. Chapman[2] suggested a pilot plant for the manufacture of 454 kg (1000 lb) of New Zealand milk biscuits per day. He also showed that these biscuits can be stored for six months in sealed plastic pouches protected from light. Townsend and Buchanan[3] described the use of lactose-free milk solids in Australian milk biscuits; they used co-precipitated lactose-free calcium caseinate and cheese as major source of milk proteins and milk solids. They claimed that it could be produced using conventional biscuit-making ovens and that these high-protein milk biscuits were acceptable after 42 weeks storage at 21-27°C (70-80°F). Buchanan and Townsend[4] observed that Australian milk biscuits represent a convenient means of supplementing the diet of any section of a community suffering from deficiencies in vitamins or minerals. Henderson and Buchanan[5] developed 'Home-baked high protein milk biscuits'. Henderson and Buchanan[6] developed a premix of the milk biscuits for sale in consumer packs. Chapman[7] reported a new process for manufacturing New Zealand milk biscuits. Prabhakaran and Srinivasan[8] reported the production of high protein biscuits using dairy by-products.

The work reported here was designed to develop a milk biscuit which can help in meeting the protein deficiencies of children in particular and other vulnerable sections of the community.

For the production of the reported product, no new equipment or alterations are required and it can even be produced with the facilities available for baking in the homes or small bakeries. In other type of biscuits, viz., the Australian lactose-free milk-biscuits, co-precipitated calcium caseinate is used as the major source of milk proteins, which is not available in India. Further, the addition of eggs is also essential. In order to overcome all these difficulties, the present investigation was taken up.

Materials and Methods

The protein-enriched milk biscuits were prepared according to the following flow-diagram:



* N.D.R.I. Publications. No. 72-116.

Selection of raw material: Good quality raw materials were selected for the manufacture of protein-enriched milk-biscuits. After preliminary trials, the final trials were conducted with the ingredients in the following proportion:

Wheat flour (maida) (g)	100
Sodium caseinate (g)	50
Castor sugar (g)	100
Butter (unsalted) (g)	120
Skim milk powder (g)	10
Baking powder (g)	2
Water (ml)	20

Creaming: Accurately weighed quantitites of butter, sugar and skim milk powder were taken and rubbed together to a creamy consistency.

Folding: Then wheat flour, sodium caseinate and baking powder were sifted together and added to the above mixture and folded in by hand till crumb-texture was reached.

Mixing: The measured quantity of water was added to the above mixture and mixed well.

Kneading: The above mixture was kneaded to a smooth homogenous mass.

Rolling: The dough so obtained was rolled out into thin sheets of 3-5 mm thickness by means of a wooden roller. The sheets were cut into desired shape pieces by means of a biscuit-cutter mould.

Baking: The cut pieces were placed over wire gauze trays of the oven in single layers and baked at 190±5°C for 25-30 min.

Cooling: The well-baked biscuits were removed from the trays and cooled to room temperature.

Packaging and Storage: The cooled biscuits were packed in two ways: (*a*) put in sealed polythene bag and (*b*) wrapped in thin packing paper. The storage studies were carried out at 37±1°C.

Analytical: The raw materials used and finished biscuits obtained were analysed as follows:

Wheat flour was analysed for flavour, moisture, ash and organoleptic qualities[9], fat[10], and total protein[11]. Flavour, fat, moisture, ash and organoliptic qualities of sodium caseinate were also determined according to IS specification[12]. The unsalted butter was analysed for flavour, organoleptic qualities, fat and moisture[13]. Biscuits were analysed for flavour, body, texture, colour, appearance, organoleptic acceptability, fat, carbohydrate[10], protein[11], moisture and ash[14].

Results and Discussion

Six preliminary production trials were conducted and the average results have been presented in Table 1. Table 2 shows average gross chemical composition of sodium-caseinate enriched milk-biscuits obtained in final trials compared with those of commercial samples. Fig. 1 shows graphically the changes in moisture content of caseinate-biscuits during storage.

Table 1 shows that the optimum temperature-time combination of baking caseinate-biscuits was found to be 190±5°C for 25-30 min. Further, as sodium caseinate content was reduced with respect to wheat-maida, the gummy feel in mouth and bitter after-taste was also reduced correspondingly. Addition of sodium

TABLE 1. PRODUCTION DATA FOR SODIUM CASEINATE-ENRICHED MILK BISCUITS
(Preliminary trials—Average of six)
For each trial: Wheat maida, 100 g; Castor sugar, 100 g.

Baking conditions		Raw material used					Physical properties				Remarks
Temp. (°C)	Time (min.)	S.C. (g)	C.B. (g)	B.P. (g)	S.M.P. (g)	Water (ml.)	Colour and appearance	Flavour	Body and texture	Acceptability	
180±5	40	100	200	7	...	80	F	F	Crisp and soft	F	*
180±5	40	80	150	5	...	60	F	F	,,	F	†
190±5	30-35	60	120	2	...	50	G	G	Crisp and uniform	G	‡
190±5	25-30	50	120	2	10	20	VG	VG	,,	VG	§
190±5	25-30	50	120	2	20	20	VG	VG	,,	VG	$

S.C.=Sodium caseinate
C.B.=Cooking butter
B.P.=Baking powder
S.M.P.=Skim milk powder

F=Fair
G=Good
VG=Very Good

* Gummy feel in mouth and bitter after taste
† Slight feel in mouth and bitter after taste
‡ Very slight in mouth and bitter after taste and colour improved
§ Gumminess and bitter after taste absent
$ Colour and appearance greatly improved; formula used for final trials

caseinate and wheat-maida in the ratio of 1:2 gave best results. Variation of fat content (cooking butter) affected the flavour, body and texture, and acceptability of the final product, a ratio of 1.2:1 of cooking butter: wheat maida gave optimum results. From the same Table it is also apparent that addition of 10-20 per cent skim milk powder improved the colour and appearance of the finished biscuits.

In Table 2 it will be observed that the average moisture content of caseinate biscuits was 2.55 per cent, which is within the ISI specifications[14] of 6 per cent moisture. The New Zealand whole milk biscuits and Australian milk biscuits contain 3 per cent moisture as reported by Bolin and Davis.[15] The average protein content of caseinate biscuits was 16.56 per cent, which is higher than in commercial samples manufactured in India.

Fig. 1 shows that moisture content of caseinate biscuits increased significantly in paper packing, but slightly in polythene-packaging, during the period under study.

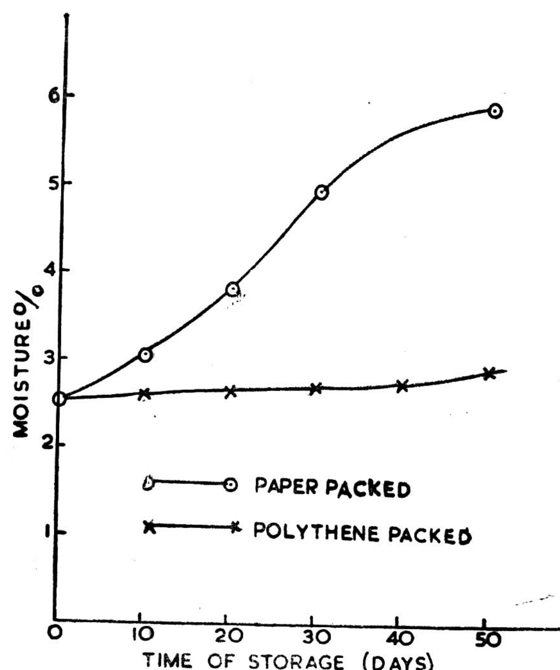


Fig. 1. Changes in Moisture Content of Sodium Caseinate-Enriched Milk Biscuits During Storage at 37 ±1°C

Acknowledgement

The authors are highly indebted to Shri M. R. Srinivasan, Head of the Division of Dairy Technology, for the help and guidance in the above work. Thanks are also due to Dr Sundaresan, Director for his keen interest. One of the authors (R. K. Bassi) is thankful to ICAR for providing a Junior Fellowship.

TABLE 2. GROSS CHEMICAL COMPOSITION OF SODIUM CASEINATE ENRICHED MILK BISCUITS COMPARED WITH COMMERCIAL SAMPLES

(Final trials—Average of six)

Type of biscuit	Moisture %	Protein %	Fat %	Ash %	Carbohydrate % (by diff)
Sodium caseinate*	2.6	16.6	28.6	0.83	57.4
Commercial samples I†	7.3	14.4	13.0	1.83	63.5
,, II†	7.7	15.6	14.0	3.40	60.0
,, III	4.3	8.1	15.2	0.77	71.7
,, IV	3.6	5.6	18.1	1.03	71.7
,, V	2.7	6.3	18.2	1.02	71.8

* Production formula given in Table 1
† High-protein biscuits (India)

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Beta-amylase Activity in Germinated Bajra and Barley Varieties

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Chemical composition of 3 varieties each of bajra and barley grown commercially in Haryana and Punjab is reported. β-amylase activity during germination was also studied in all the six varieties. Comparison was made to find out the suitability of bajra for malt production. Starch content among bajra varieties ranged from 56.42 to 61.91 per cent while it ranged from 67.13 to 68.09 per cent in various barley varieties. β-amylase activity increased progressively with germination upto 30 hours and then decreased upto 72 hours in case of bajra varieties while it continuously increased upto 72 hours in case of barley varieties. The rate of increase in enzyme activity, however, was slower in the first 24 hr of germination in the case of barley.

Rapid developments in the field of agriculture have given rise to high yielding varieties which in turn have led to a significant surplus of certain grains like bajra. The total production of bajra in India has increased from 3.6 million tonnes in 1960-63 to 8 mil tonnes in 1970-71[1]. To meet the increasing demand for malt it is necessary to seek new raw material for malt production. The possibility of using bajra as raw material for malt production comes from the fact that ragi (*Eleusine corcana*), a similar millet, is being used for malt production.

The two main factors essential for a certain source to be good for malt production are (*i*) high starch content and (*ii*) high β-amylase activity during germination. The present investigation reports a comparative study of bajra and barley varieties with respect to the two important characteristics for the suitability of malt making.

Materials and Methods

The three varieties each of bajra viz., *HB-1*, *HB-3* and *HB-4* and of barley viz., *BG-1*, *C-138* and *C-164* were procured from the Department of Plant Breeding, Haryana Agricultural University, Hissar.

Chemical composition was determined by AOAC methods[2]. Crude protein (N×6.25) was determined by micro-kjeldahl method[3].

Germination Studies: Weighed amounts of properly washed and dried seeds of different varieties were soaked in distilled water for one hour. After that these were germinated in petri dishes having wet filter paper (Whatman No. 1). The germination of bajra seeds were carried out at 35°C in an incubator, while that of barley at 25°C in a refrigerated incubator. Samples were taken every 6 hourly interval upto 72 hours during the germination.

Extraction of enzyme was done according to the method of Nason[4] with a few modifications. The germinated seeds were ground in cold 0.016 M acetate buffer (pH 4.8) in a mortar and pestle in ice bath. The suspension was then centrifuged at 0° for 15 min at 10,000 rpm in a refrigerated centrifuge (Janetzki, K-24). The supernatant was made up to 25 ml. with cold buffer. The method of Noelting and Bernfeld[5] was adopted for enzyme activity determination. Activity was expressed as mg of maltose formed per ml. enzyme extract at 37°C in 3 min.

Results and Discussion

The proximate analysis of three varieties each of bajra and of barley are presented in Table 1. The data showed that the major components varied little in the varieties of the two cereals. There was, however, a difference in the starch content of the two cereals, it being slightly lower in the case of bajra varieties as compared to barley varieties. The two crops also differed from each other for ash, crude fibre, crude protein and ether extract, the former two were higher in barley while latter two components were comparatively high in bajra.

Mylolytic activity was seen during germination of these varieties of bajra and barley. The results are presented in Fig. 1 and Fig. 2. An important observation was that β-amylase activity in bajra varieties reached a maximum value after 30 hr of germination and at 72 hr was almost equal to that at zero time (Fig. 1). In the case of bajra there was a sudden increase in enzyme activity from 6 to 12 hr, there-

TABLE 1. CHEMICAL COMPOSITION OF BAJRA AND BARLEY VARIETIES

Crop	Varieties	Moisture (%)	Ash (%)	Crude fibre (%)	Crude protein (N×6.25) (%)	Ether extract (%)	Nitrogen free ex-tract (By dif.) (%)	Starch (By acid hydrolysis) (%)
	HB-1	10.1	1.57	1.04	12.9	5.39	69.0	56.4
Bajra	*HB-3*	10.8	1.40	1.13	12.4	5.47	69.6	61.9
	HB-4	10.2	1.53	1.18	13.0	4.78	69.2	58.7
	BG-1	10.2	2.83	4.06	8.5	2.04	72.4	67.1
Barley	*C-138*	10.8	2.10	3.62	9.0	2.35	72.1	67.4
	C-164	10.9	2.05	2.71	8.6	2.15	73.6	68.1

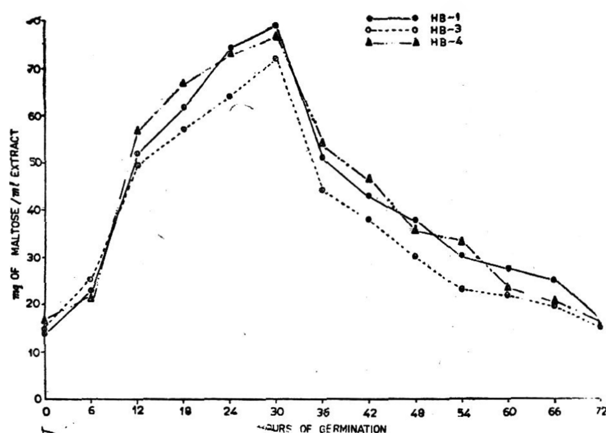

Fig. 1. Enzyme activity at different periods of germination of Bajra varieties.

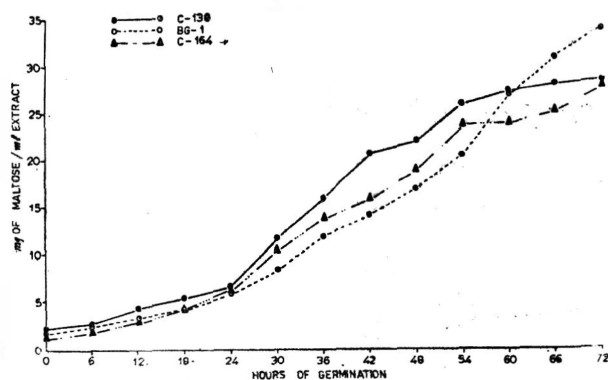

Fig. 2. Enzyme activity at different periods of germination of Barley varieties

after there was an almost constant and steady increase upto 30 hr. Subsequently there was again a sudden fall in the enzyme activity up to 36 hr and then comparatively a lower rate of decrease in activity upto 72 hr till it approximated that at zero period.

The enzyme activity in barley varieties showed a small increase upto 24 hr of germination (Fig. 2) following this it showed a significant increase. The activity gradually increased until 72 hr of germination. The fact that amylase activity increased rather slowly upto 24 hr may in part be due to the slow process of germination. It was seen that in the case of bajra varieties sprouting began after 7 or 8 hr of germination while in the case of barley varieties it occurred after about 24 hr.

Similar behaviour of beta-amylase in other crops has been noted. Oparin and Kaden[6] observed in wheat seeds that beta-amylase activity increases in the first 6 days of sprouting and then it falls off. Roca and Ondarza[7] obtained similar results in corn with dextrinogenic and saccharifying amylases increasing progressively during germination. Proskuriakov and Nikiforoveskaia[8] and Patel and Patel[9] reported germination studies in wheat and groundnut respectively. They found that activity of beta-amylase increases with time and then starts falling off. Saharan and Wagle[10] reported that in wheat β-amylase reaches to a maximum value at 96 or 120 hr of germination depending upon variety and after 192 hr it drops down to nearly that at zero time.

When enzyme activity of individual variety among each crop was compared, it was observed that *HB-3* amongst the three bajra varieties, had lowest activity at every stage except zero and 6 hours while *HB-1* had lowest at zero hour but highest at 24, 30, 60, 66 and 72 hr and at other stages it had medium value. *HB-4* had fluctuating values for the enzyme activity. Amongst the barley varieties, *C-138* had highest value upto 60 hours of germination and medium at 66 and 72 hr. On the other hand *BG-1*, which had medium values upto 18 hr, had lowest value starting from 18 hours onwards upto 54 hr and highest at 66 and 72 hr of germination. In case of *C-164* it was lowest upto 18 hr, then medium upto 54 hr and again lowest at the end.

When the relation for starch content with enzyme activity was seen, it was found that in none of the varieties the enzyme activity was in any way related to starch content. Similarly, there was no correlation between enzyme activity and crude protein content of grains.

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ANNOUNCEMENT

In order to do justice to our contributors who have sustained this Journal by sending papers of high Technological and Scientific content for the last 10 years, we are planning to bring out the Journal from 1974 onwards as a bimonthly publication. Volume Eleven and the subsequent volumes will have six numbers as against four at present. It is hoped that the present time gap between the receipt of manuscripts and their subsequent publication may be considerably reduced. The increase in the frequency of publication is bound to entail additional expenditure. Moreover, the costs of paper and printing have been increasing at an alarming rate lately. Under the circumstances we have to increase the present rate of subscription. All our subscribers may kindly note the revised new rates which will be effective from the year 1974.

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IMPORTANCE OF VOLATILE OIL AND COLD WATER EXTRACT ESTIMATION IN ANALYSIS OF CUMIN (JIRA)

Determination of volatile oil and water extract is recommended to prevent admixture of exhausted spice in cumin.

In more recent times, essential oils instead of the spices are being used for flavouring purposes. Federal Food and Drug Administration[1] has defined spices as 'aromatic vegetable substances ... from them no portion of any volatile oil or other flavouring principle has been removed'.

With the exception of exhausted spices, the presence of most foreign ingredients is best detected by the use of the microscope, although chemical analysis furnishes valuable corroborative evidence. But the difficulty arises in case of exhausted spices, as obviously no characteristic difference in structure can be found in such cases.

This investigation is based on the same lines as that for coriander[2]. Thirty samples of cumin were collected from the local market, crushed and analysed as per the Prevention of Food Adulteration Rules 1955[3]; volatile oil and cold water extract were also estimated. After the volatile oil extraction, the residue was brought to its original state and the experiments repeated on the exhausted stuff.

Another set of experiments was conducted to see the effect of hot water extraction upon the whole seed as it is a common practice to serve *Jira Pani*, in restaurants.

The volatile oil determination was done by B. P. method[4] and cold water extract by ISI method[5]. The results are tabulated in Table 1.

TABLE 1. Results of Analysis of Thirty Samples of Cumin (Jira)

	Moisture %	Total ash %	Ash insoluble in dil. HCl%	Volatile oil %	Cold water extract %
Max.	10.0	7.6	0.8	3.6	20.4
Min.	6.0	6.9	0.2	2.5	14.9
Av.	7.7	7.3	0.4	3.1	17.5

Table 2 gives the frequency distribution. The idea for such classification is to ascertain which of the ranges are covered by the largest number of samples and to suggest these for fixing a revised standard.

The upper limit of 9.5 per cent for total ash fixed in the PFA Rules[3] appears to be rather high. Results of analysis show that it rarely reaches 8.0 per cent and nearly two third of our results fall below 7.5 per cent. Although many other countries have followed the same standard, in our opinion the higher limit for total ash of Indian cumin may reasonably be fixed at 8.5 per cent and in no case it should be above 9.0 per cent. The upper limit for ash insoluble in dilute HCl may also be fixed at 1.25 per cent as none of our results exceed even 1.0 per cent. Regarding volatile oil, we recommend 2.5 per cent as the minimum value as more than 90 per cent of our results are well above that and none falls below. In support of our findings we can also cite the reference of IPC[6] and Thorpe[7]. Considering the frequency distribution, a lower limit of 14.0 per cent can be safely recommended for the minimum standard of cold water extract.

Analysis of the exhausted stuff shows that though the powders are devoid of any volatile oil and whole seeds contain only traces (ranging from 0.15 to 1.2, showing a loss of 64 to 95 per cent of the actual values), the total ash and ash insoluble in dil HCl are within the prescribed limits[3]. Results of cold water extract also show a steep declination, in cases of powder they are only 3.2 to 4.6 (showing a loss of 69 to 82 per cent) whereas in case of whole seeds they are only 4.6 to 5.6 (the loss being 69 to 74 per cent). It can be easily observed from our findings that though the exhausted stuffs (both powder and whole) contain much less cold water extract and volatile oil content (in case of powder the latter is totally absent) yet according to P.F.A. standards[3] we are forced to declare those inferior stuffs as genuine. In suggesting therefore suitable standards for cumin, the figures for volatile oil and cold water extract should be taken into consideration as otherwise it is practically impossible for the public analysts to cope with the unscrupulous vendors.

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TABLE 2. SPREAD OF VALUES

Moisture %	Total ash %	Ash insoluble in dil. HCl %	Volatile oil %	Cold water extract %
6.0 (4)	6.9— 7.0 (4)	0.2 (4)	2.5 (2)	14.9 (2)
7.0— 8.0 (18)	7.1— 7.3 (16)	0.3— 0.4 (20)	2.8— 2.9 (4)	15.9— 16.2 (4)
8.5— 9.0 (6)	7.4— 7.6 (10)	0.5— 0.8 (6)	3.0— 3.3 (18)	16.5— 17.7 (6)
10.0 (2)			3.4— 3.6 (6)	18.6— 19.1 (4)
				19.8— 20.4 (4)

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EFFECT OF HOMOGENIZATION AND PASTEURIZATION ON GERBER FAT TEST OF BUFFALO MILK

Investigation was carried out to find out the effect of homogenization and pasteurization on Gerber fat test of buffalo milk. The sequence adopted for homogenization was before and after pasteurization. Pasteurization was by Holder and HTST methods under laboratory conditions.

The demand for homogenized and pasteurized milk has considerably increased in the leading dairy countries of the world, owing to the various beneficial effects attributed to homogenization[1]. In India too it is expected that in the near future homogenization of market milk may be adopted on commercial scale. At present homogenization is adopted mostly in recombined milk preparation and ice-cream industry. Legal limits are in force in terms of fat and solids not fat of milk in various states of India[2]. For determination of fat Gerber test is commonly adopted.

Trout and Lucas[3] compared the results of Gerber fat test of homogenized and unhomogenized 24 cow milk samples and reported that 12 were the same, 5 of the homogenized were lower, ranging from 0.02-0.07 per cent and 7 were higher, ranging from 0.02-0.08 per cent. Burr observed that the Gerber test gave variable results of homogenized cow milk depending on the time of centrifuging. Likewise, a number of workers[1] reported lower fat test by Babcock method on homogenization of cow milk as the fat cannot be completely released with the conventional process.

Buffalo milk constitutes a major portion of market milk in India. The average fat content is 6.5 per cent, with fat globule size of 5.01 microns and the number of fat globules is 3.2 million/mm^3. The corresponding figures for cow milk are 4.5 per cent, 3.85 microns and 2.96 million/mm^3 respectively[4].

From the above observations it seemed that the conventional Gerber fat test on homogenized and pasteurised buffalo milk may not agree with that of raw milk and hence this study was carried out.

Bulk buffalo milk from the experimental dairy of National Dairy Research Institute, Karnal standardised to 6 per cent fat was used in the experiments.

Holder pasteurization: Standardised milk was held in waterbath at about 80°C so as to raise the temperature of milk to 63°C in about 5 min. Later, the milk was held in another water bath maintained at 63°C for a period of 30 minutes. *HTST pasteurization:* Standardised milk was held in a water bath maintained at 80°C such that milk was allowed to come to a temperature of 73°C within 15 min and maintained at this temperature for 15 sec. Milk was homogenized at 60-63°C with Rannie piston type homogenizer, the pressures for the first and second stage being 180 kg/cm^2 and 40 kg/cm^2 respectively. The sequence of homogenization was as follows (*i*) Homogenization after pasteurization. (*ii*) Homogenization prior to pasteurization. All samples were cooled and maintained at 4-6°C for a minimum period of 3 hr prior to analysis.

The fat content of milk samples were determined according to Gerber method[5]. Thirteen replicates of each of the above treatments were statistically analysed by analysis of variance method.

TABLE 1. THE FAT PERCENTAGE (GERBER METHOD) UNDER DIFFERENT METHODS OF PASTEURIZATION, STAGES OF HOMOGENIZATION AND SEQUENCE OF HOMOGENIZATION. 13 REPLICATES

Per cent Fat

Sequence of homoge-nization	Pasteurization		Homogenization			
			Holder paste-urization		H.T.S.T. paste-urization	
	Holder	H.T.-S.T.	Single stage	Two stage	Single stage	Two stage
H.A.P.	6.03	6.01	5.95	5.92	5.97	5.92
H.P.P.	6.02	6.02	5.96	5.92	5.95	5.91
Average	6.03	6.02	5.96	5.92	5.96	5.92
Per cent devi-ation						
Overall	+0.50	+0.33	—0.67	—1.33	—0.67	—1.33
H.A.P.	+0.50	+0.16	—0.83	—1.33	—0.50	—1.33
H.P.P.	+0.33	+0.33	—0.67	—1.33	—0.83	—1.50

Fat content of raw milk: 6.0%;
Critical differences: 0.04
H.A.P. = Homogenization after pasteurization
H.P.P. = Homogenization prior to pasteurization

From the statistical analysis of the data (Table 1) it is seen that pasteurization has insignificant influence on Gerber fat test. An overall higher fat tests of 0.50 and 0.33 were observed for Holder and HTST pasteurized milk respectively on percentage basis over the raw milk fat test.

Homogenized and pasteurized milk samples in both the sequence of homogenization showed lower fat tests than the raw milk on percentage basis over the raw milk fat test. The average values for single and two stage homogenized holder pasteurized milk fat tests were lower by 0.83 and 1.33 respectively in case of homogenization after pasteurization while the corresponding values were 0.67 and 1.33 when homogenization was adopted prior to pasteurization.

Likewise, the average values for single and two stage HTST pasteurized milk showed lower Gerber fat test of 0.50 and 1.33 respectively over raw milk fat test on percentage basis when homogenization was done after pasteurization; while these values were 0.83 and 1.50 respectively when milk was homogenized prior to pasteurization. This indicated that although the sequence of homogenization was not significant enough in lowering the fat test, homogenization prior to pasteurisation showed overall lower fat values (1.08) compared to those of samples homogenized after pasteurization (0.997) over raw milk fat test on percentage basis. The two stage homogenization resulted in an overall lowering of that fat test (1.33 per cent) than single stage homogenization (0.67 per cent).

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National Dairy Research Institute, Karnal — C. PRASAD, C. A. MULAY

22 December 1972

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AN IMPROVED METHOD FOR DETERMINING SOLUBILITY OF EGG POWDER

Solubility of egg powder is widely used as an index of quality. The Indian Standards Institution method for estimation of solubility using water for extraction did not give consistent results. An improved method using solvent mixture, salt solution and water, was found to give consistent and comparable results.

The solubility of egg powder has been widely used to correlate the results obtained with its functional properties and as an index of quality. A number of procedures[1-3] have been recommended for estimating solubility of whole egg powders. Hawthorne[2] considered the chemical method as the best but as it was too time consuming, a refractometric method which could correlate best with the chemical method was devised by Haenni. This was subsequently modified by White and Grant[5]. The Indian Standards Institution[6] has also laid down the method of determining the solubility of egg powder.

During the course of studies on storage stability of foam mat dried egg powder, the solubility, when determined by the ISI method[6], was found to decrease on storage after 3 and 6 months but increased after nine months, which was contrary to the findings of earlier workers[7-9].

Employing ISI method[6] for estimating solubility of egg powder freshly obtained from Accelerated Freeze Drying Unit, Delhi, the results varied from 35.9 to 51.3 per cent in eight estimations whereas the solubility

was 97 per cent by Haenni's method. The dry sediment obtained in estimations of solubility by ISI method[6] was extracted with petroleum ether and the extracts after removing the solvent in four cases were found to vary from 23.7 to 27.3 per cent when calculated on the weight of the original sample taken.

Difficulty was also experienced to carry out the estimation as per the method without disturbing the sediment as laid down therein.

Due to variations and difficulties encountered in obtaining reproducible and correlative solubility index data by the ISI method[6], work was initiated to develop an improved method for estimation of solubility of egg powder.

Petroleum ether and benzene mixture 80:20 is added to 1 g sample of egg powder in the centrifuge tube kept half immersed in water at 55-60°C, mixed well with a glass rod and centrifuged for 15 min at 3500 rpm. The supernatant liquid is decanted off and the extraction with the solvent mixture repeated once more. The contents of the centrifuge tube are dried thereafter at ambient room temperature. Sodium chloride solution 5 per cent (w/v), is then added to the sediment inside the tube and mixed well for about 15 min. It is centrifuged as before and the supernatant liquid is decanted off. Finally warm distilled water at 55 to 60°C is added to the contents and mixed thoroughly with a glass rod. It is centrifuged again and the supernatant liquid is decanted off. The sediment in the tube is dried at 105±2°C to a constant weight and the solubility percentage calculated.

Different types of samples prepared from three types of egg powder were first packed in cellophane 300 grade and then in paper/foil/polyethylene laminate, the plain can and 0.04 mm thickness laminate were used only for packing under nitrogen as this laminate has been found impermeable to gas by Ghosh *et al*[10]. The quantity of egg powder packed was 30 g. in each case.

Egg powder tablet was made by compressing the egg powder at ambient temperature into blocks of 4 cm×4 cm size at a pressure of 400 psi/10 sec using a 12 ton Carver laboratory press and an iron mould in case of AFD egg powder and foam-mat dried egg powder. However, to produce a satisfactory cohesive block from spray dried egg powder, a minimum pressure of 1500 psi/10 sec was necessary.

The solubility of AFD egg powder was estimated by the improved method and by Haenni's method. The solubilities were 98.1 and 97 per cent respectively. The estimations were done in duplicate and triplicate. Using only petroleum ether instead of the mixture of solvents the figure for solubility was found to be 82.2 per cent and the sediment showed presence of some fatty matter.

TABLE 1. PERCENTAGE SOLUBILITY OF EGG POWDER AS DETERMINED BY DIFFERENT METHODS

Particulars of egg powder	Storage Conditions		Solubility		
	Temp. °C	Period (months)	ISI method	Improved method	Haenni's method*
AFD egg powder tablet	37	8	28.2, 31.8	70.2, 69.8	68.5
AFD egg powder tablet	20-30	8	45.5, 37.8	74.6, 74.8	75.9
AFD egg powder under N_2	20-30	8	51.2, 52.4	78.2, 77.4	...
FMD egg powder tablet	37	9	42.7, 35.9	82.0, 82.1	75.9
FMD egg powder in tin under N_2	37	9	31.9, 36.3	80.9, 79.9	78.6
AFD egg powder tablet	37	10	... ...	69.8, 68.9	68.5
AFD egg powder tablet	20-30	10	... ...	73.4, 74.1	75.9
AFD egg powder under N_2	20-30	10	... ...	76.0, 75.7	81.0
FMD egg powder tablet	37	12	... ...	80.6, 80.5	73.1
FMD egg powder in tin under N_2	37	12	... ...	77.9, 76.9	73.1
AFD egg powder tablet	37	12	... ...	66.5, 66.5	63.4
AFD egg powder tablet	20-30	12	... ...	73.7, 73.0	73.1
AFD egg powder under N_2	20-30	12	... ...	74.2, 74.5	78.5
SD egg powder	37	9	... ...	89.3, 89.6	88.7

AFD=Accelerated freeze dried; FMD=Foam mat dried; SD=Spray dried.

* Same values were obtained in these repetitions

Solubility data obtained on different types of samples from three types of egg powder stored for different periods when packed in flexible packs are enumerated in Table 1. It is observed that the ISI method[6] using water only gives inconsistent and low results. The improved method gives fairly reproducible results which are fairly comparable at ambient room temperature with those obtained by Haenni's method, being mostly within the limits of experimental error. At higher temperature (37°C) the figures obtained by the improved method are in some cases slightly higher than those obtained by Haenni's method.

It is concluded that there is no inconsistency in the results obtained by the improved method and the solubility figures on samples stored at ambient temperature are quite comparable with those obtained by Haenni's method which was considered as the best physical method by Hawthorne[2] for estimation of solubility of egg powder. The difficulty experienced

in solubility estimation without disturbing the sediment as laid down in IS specification[6] has been eliminated in the improved method as the fat is removed by the solvent mixture.

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RANDHUNI (*Apium Graveolens* L.) AND ITS QUALITY STANDARDS

Standards based on ash, volatile oil ether extract etc., are necessary to check sophistication of this spice.

Randhuni or Indian celery, the dried ripe fruits of *Apium graveolens* L., is widely used as a spice in this country. The ridged fruit consists of an ovate, dark brown cremocarp, often being found separated when the spice is purchased. The seeds of *randhuni* are highly valued as a condiment and for medicine, either directly or in the form of an extract.

Due to its small size and dark colour it is liable to sophistication in different ways. Admixture with extraneous sandy matter and foreign seeds of similar appearance are most common. Suitable standards are therefore desirable to ensure its genuineness, and we are supplying some data to help in framing such standards for purity.

Before the main analysis a cursory examination is made for the presence of waste materials such as excess stalk, foreign seeds, etc[1]. Whole spices are then ground thoroughly before the analysis is commenced. The carbon tetrachloride test for extraneous matter should invariably be carried out on the powdered sample. It has been observed on a number of occasions that such extraneous impurities may firmly adhere to the surface of the whole sample and will not settle down and thereby escape detection. Moisture is determined by distillation from toluene, and total ash and ash insoluble in dil. HCl are determined by the conventional methods. Direct estimation of volatile oil is done by B.P. method[2], whereas non-volatile ether extract and cold water soluble extract are done by ISI[3] methods. The results are given in Tables 1 and 2.

The limits of the foreign organic matter, foreign seeds and volatile oil of randhuni are similar to that of celery[4]. Steam distillation yields a slightly pale yellow thin oil ranging from 1.5 to 3.0 per cent. A minute fraction is soluble in water resulting in slight turbidity, but as the figures corraborate with earlier publications[5,6], it seems to be negligible. For whole seeds moisture content is not so much important. But our main problem is with the results of total ash, ash insoluble in dilute HCl and non-volatile ether extract. As regards the former two, four of our findings are unusually high, because of the sandy matter they contain. For that reason, they should be neglected. Even so, ash insoluble in dilute HCl exceeds the limit for celery[4]. The non-volatile ether extract of *randhuni* is also much lower to that of celery[7]. Even our maximum does not reach the limit for celery. Presumably, this may be due to local variation. The cold water soluble extract, in conjunction with volatile oil and non-volatile ether extract, will check any admixture with exhausted or inferior stuff.

Though the number of samples analysed is not much and to draw a conclusion on the basis of these results alone is not very wise, yet in our opinion the data obtained will be helpful for further studies in fixing the specification for this spice.

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TABLE 1. ANALYSIS OF RANDHUNI FOR DIFFERENT CONSTITUENTS

	Moisture %	Total ash %	Ash insoluble in dil. HCl %	Volatile oil (v/w) %	Non-volatile ether extractive %	Cold water extract %
Max.	11.0	11.0	4.0	3.0	14.2	12.6
Min.	5.0	6.9	0.5	1.5	5.8	5.9
Av.	7.8	8.8	2.5	2.4	9.4	8.4

TABLE 2. SPREAD OF VALUES

Moisture %	Total ash %	Ash insoluble in dil. HCl %	Volatile oil (v/w) %	Non-volatile ether extract %	Cold water soluble extract %
10.0—11.0 (2)	10.5—11.0 (4)	3.6—4.0 (4)	3.0 (2)	11.9—14.2 (4)	12.6 (2)
8.0— 9.0 (15)	9.3 —10.0 (5)	3.0 (6)	2.7—2.8 (7)	10.5—10.9 (4)	9.7—10.4 (3)
6.0— 7.0 (7)	8.4 — 9.1 (6)	2.1—2.8 (7)	2.5 (6)	9.1—10.0 (7)	8.5—9.4 (5)
	7.6 — 8.3 (7)	1.3—1.8 (6)	2.0—2.3 (7)	8.0—8.6 (6)	7.5—8.1 (11)
5.0 (1)	6.9 — 7.3 (3)	0.5—0.9 (2)	1.5 (3)	5.3—7.6 (4)	5.9—6.6 (4)

• Bracketed figures show the number of samples,

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VALINE PRODUCTION BY AN ACHROMOGENIC VARIANT OF *MICROCOCCUS GLUTAMICUS*

A promising source of microbiol valine is discussed.

A number of applications for L-valine in the food[1-6] and pharmaceutical industries[7, 8] are known. Several authors have studied the production of valine by microbial fermentation[9-11]. Tauro *et al.*,[10] isolated an *Aerobacter* sp. from coconut neera capable of excreting 4.0 mg per ml valine under the optimum conditions. A strain of *Aerobacter aerogenes* excreting 1.73 mg per ml. valine was reported by Mandel and Majumdar[11]. During our attempts to obtain amino acid excreting mutants of *Micrococcus glutamicus* for industrial fermentation, a considerable number of auxotrophic mutants were found to excrete lysine and valine[12]. Among the non-auxotrophic mutants (colour and colony variants) which were also tested, an achromogenic variant obtained after treatment with ethyl methane sulfonate[12] was found to excrete considerable amounts of L-valine. The colonies of the parent organism were of pale straw yellow colour while those of the variant were devoid of the pigment and appeared whitish. A prototrophic variant having different amino acid excretion pattern is rather a rare observation and it was therefore of interest to test the potency of this variant to excrete valine under optimum levels of the constituents of the fermentation medium.

Fermentation was carried out by inoculating 50 ml. of fermentation broth in 250 ml conical flask with 5 ml of an overnight culture containing about 10^7 cells per ml and incubating at 30°C in a rotary shaker for 72 hr. Extracellular valine in the culture fluid was estimated by microbiological assay using *Streptococcus faecalis*[13]. The basal fermentation medium was of the following composition: glucose, 5%; ammonium

sulphate, 1.0%; KH_2PO_4, 0.05%; yeast extract, 0.01%; meat extract, 0.2%; peptone, 0.2%; $MgSO_4.7H_2O$, 0.05; $CaCO_3$, 1.0 per cent. In the basal medium the concentrations of glucose, nitrogenous compound, peptone, meat extract and yeast extract were varied individually each time in order to standardise their respective concentrations in the fermentation medium for maximum yield of valine. For selecting a suitable nitrogen source, ammonium sulphate in the medium was substituted with other nitrogenous compounds (Table 2).

Cell density was measured turbidimetrically in a Klett-Summerson photoelectric colorimeter using a filter No. 66 after suitable dilution and expressed as Klett units.

The yield of valine and cell growth with various concentrations of glucose in the fermentation medium are shown in Table 1. A maximum yield of 2.2 mg/ml of valine was obtained with 2.5 per cent glucose in the medium. At higher concentrations of glucose, the cell density and valine yield were both lowered.

With 2.5 per cent glucose in the medium, various nitrogenous substances as shown in Table 2 were added to the fermentation medium at levels corresponding to the same amount of nitrogen in each case. In the cases of 3 nitrogenous compounds, viz., KNO_3, $NaNO_3$, and $(NH_4)_2HPO_4$, no extracellular valine could be detected and the cell growth was also considerably low. Ammonium chloride was found to be the most valuable nitrogen source (Table 2), a concentration of 1.5 per cent yielding a maximum of 2.9 mg/ml of valine (Table 3).

The effects of addition of peptone, yeast extract and meat extract on valine yield and cell density can be seen from data summarised in Table 4. The best yield of valine, 4.5 mg per ml was obtained with 0.25 per cent peptone, 0.05 per cent yeast extract and 0.5 per cent meat extract in the medium. It is evident that the mutant is capable of excreting considerable amount of L-valine in a medium containing glucose 2.5; NH_4Cl, 1.5; KH_2PO_4, 0.05; K_2HPO_4, 0.05 per cent; yeast extract 0.05 per cent; meat extract, 0.5 per cent; peptone, 0.25 per cent; $MgSO_4.7H_2O$, 0.05 per cent and $CaCO_3$, 1.0 per cent.

It has been a common observation that auxotrophic mutants of bacteria give not only better yields but also produce amino acids which the parental strain is incapable of producing. Nakayama[9] and his group have isolated valine excreting, iso-leucineless and leucineless mutants of the glutamate excreting parent *M. glutamicus*. In the present case, however, since no apparent metabolic block is involved in the change

TABLE 1. EFFECT OF GLUCOSE CONCENTRATION ON CELL DENSITY AND EXTRACELLULAR VALINE*

D-glucose g%	Cell density (Klett units)	Extracellular valine (mg/ml)
0	100	0.1
1.0	620	1.3
2.5	1310	2.2
5.0	760	1.6
7.5	540	1.2
10.0	340	1.3

* Values are average of triplicates

TABLE 2. EFFECT OF NITROGEN SOURCE ON CELL DENSITY AND EXTRA-CELLULAR VALINE*

Nitrogen compound	N %	Concentration %	Cell density (Klett units)	Extra-cellular valine (mg/ml)
Control	...	...	490	...
NH_4Cl	26.16	1.42	1010	2.2
NH_4NO_3	34.90	1.06	1040	1.6
$(NH_4)_2SO_4$	21.20	1.76	1330	1.5
KNO_3	13.86	2.69	510	...
$NaNO_3$	16.47	2.26	520	...
$(NH_4)_2HPO_4$	12.17	3.06	270	...
Ammonium tartrate	15.25	2.45	1780	...
Urea	46.70	0.80	1550	0.3

* Values are average of triplicates

TABLE 3. EFFECT OF DIFFERENT CONCENTRATIONS OF AMMONIUM CHLORIDE ON CELL DENSITY AND EXTRACELLULAR VALINE*

NH_4Cl (g%)	Cell density (Klett units)	Extracellular valine (mg/ml)
Control	620	...
0.50	1520	1.9
0.75	1800	2.3
1.00	2080	2.6
1.50	2320	2.9
1.75	2200	2.7
2.00	2100	2.6

* Values are average of triplicates

TABLE 4. EFFECT OF DIFFERENT CONCENTRATIONS OF PEPTONE YEAST EXTRACT AND MEAT EXTRACT ON CELL DENSITY AND EXTRACELLULAR VALINE*

Concentrations of growth factors (g%)			Cell density (Klett units)	Extracellular valine (mg/ml)
Peptone	Yeast extract	Meat extract		
nil	0.01	0.2	405	0.8
0.10	0.01	0.2	1050	1.5
0.20	0.01	0.2	1320	1.9
0.25	0.01	0.2	1430	2.3
0.50	0.01	0.2	1660	1.8
1.00	0.01	0.2	1640	0.9
0.25	nil	0.2	1300	2.8
0.25	0.01	0.2	1540	3.3
0.25	0.02	0.2	1690	3.6
0.25	0.05	0.2	1920	4.1
0.25	0.10	0.2	1750	3.7
0.25	0 05	nil	1210	2.7
0.25	0.05	0.1	1810	4.0
0.25	0.05	0.2	1810	4.0
0.25	0.05	0.5	2000	4.5
0.25	0.05	1.0	1500	2.2

* Values are average of triplicates

over from glutamate excretion to valine excretion, it is possible that the achromogenic culture is a variant with altered regulation of valine biosynthesis. Further studies could be made to test this and it may be worthwhile to assess the industrial utilization of this culture for large scale production of L-valine.

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A SINGLE-STEP EXTRACTION AND CLEAN-UP METHOD FOR ESTIMATION OF CARBARYL IN FRUITS AND VEGETABLES

Carbaryl is extracted from the substrate with methylene chloride and the extract is passed through 'Florisil' column activated at 130°C for 24 hr. Insecticide is hydrolysed by methanolic alkali and the intensity of the colour developed by adding p-nitrobenzene diazonium fluoborate, is measured at 470 mu.

The method of Johnson[1] for estimation of carbaryl, adopted as official, requires a special equipment, which is not easily available for evaporating methylene chloride, a solvent. So Benson and Finocchiaro[2] modified this method and recommended a steam bath for evaporation. In both these methods coagulating solution is added during clean-up to remove the interfering substances. This step is followed by many steps like filtration through hyflosupercel and then partitioning with methylene chloride which are time consuming.

Therefore, a study was undertaken to find out any other suitable method which will be a quick one and at the same time will not reduce the recovery. The method presented here is a modification of the method given by Whitehurst *et al*[3].

Take 25 g substrate and cut it into pieces. Blend the substance along with 150 ml of distilled methylene chloride for 4 min at high speed. Filter the extract through funnel fitted with permanent filter of one porosity. Pass the extract through 'Florisil' (100-200 mesh size) column activated at 130°C for 24 hr (when the inactivated 'Florisil' advocated by Whitehurst[3] was used, it was found that the blank values were undesirably more, thereby reducing the sensitivity of the method. So an activated 'Florisil' was used in the present method). Give two washings with 10 ml portions of methylene chloride. To the cleaned extract add 1 drop of diethylene glycol as keeper. Evaporate the solvent by keeping the beaker below windy fan to one or two drops. To the residue left in the beaker add 2.5 ml. of 0.5 N KOH in methanol and allow the reaction to take place for 5 min. Then add 18 ml of glacial acetic acid and 1 ml of freshly prepared dye (p-nitrobenzene diazonium fluoborate -5 mg. in 10 ml of methanol). Immediately measure the absorbance at 470 mμ against substrate blank.

This method worked well with 9 substrates viz., okra, brinjal, cabbage, cauliflower, tomato, guava, potato, apple and peach but failed in case of maize, orange, spinach and leaves of okra. Results of analysis of the samples of different substrates fortified with different concentrations, are presented in Table 1.

The sensitivity of the method was calculated by the method given by Bates[4] which was found to be 0.03 ppm (based on the blank values of all the substrates). Though this method is quicker one as compared to earlier methods, it is not suitable for highly coloured substrates like maize, spinach, orange etc.

Preparation of standard curve: Take 0, 10, 20, 30, 40, 50, 60 and 70 μg of carbaryl in 150 ml methylene chloride and pass it through 'Florisil'. The remaining procedure is same as described earlier. The standard curve was prepared by plotting absorbance against amount of carbaryl (Fig. 1).

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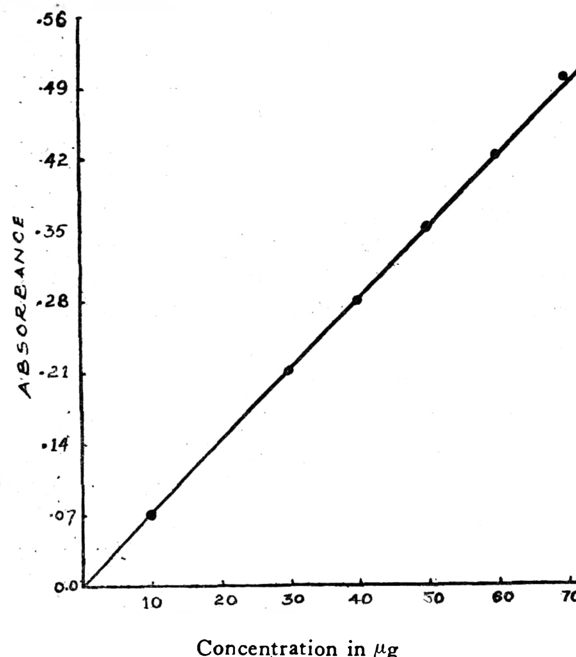
TABLE 1. RECOVERIES OF CARBARYL FROM FORTIFIED SAMPLES OF DIFFERENT SUBSTRATES

Substrate	Carbaryl added (ppm)	Carbaryl recovered (ppm)	Recovery %	Mean recovery %
	0.4	0.379	94.67	
Okra	0.8	0.743	92.85	91.73
	1.6	1.403	87.67	
	0.4	0.379	94.67	
Brinjal	0.8	0.715	89.35	91.38
	1.6	1.443	90.12	
	0.4	0.351	87.67	
Cabbage	0.8	0.715	89.35	88.23
	1.6	1.403	87.67	
Cauliflower	0.8	0.688	39.95	
	1.6	1.403	87.67	86.81
Tomato	0.8	0.715	89.35	
	1.6	1.387	86.70	88.02
Guava	0.8	0.743	92.85	
	1.6	1.403	87.67	90.26
Potato	0.8	0.698	85.95	
	1.6	1.387	86.70	81.32
Apple	0.8	0.743	92.85	
	1.6	1.403	87.67	90.26
Peach	0.4	0.3507	37.67	
	0.8	0.7128	89.35	88.51

All the experiments were repeated twice only



Concentration in μg
FIG. 1. Standard Curve for Carbaryl

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QUALITATIVE STUDIES ON IDENTIFICATION OF PULSES

Identification of different pulses by chromatography of phenolic constituents present seems feasible.

Pulses form an essential constituent in human diet in this country. On account of greater demand for pulses and their products, there is scope for adulteration of costlier pulses such as red gram and Bengal gram with *kesari dal* (*lathyrus sativus*), which has been prohibited under the Prevention of Food Adulteration Act[1].

Pulses have been analysed for various chemical constituents from time to time[2]. These analysed constituents which are common to all the pulses vary only in their proportion but they do not give any idea as to the characteristic of each pulse, so that it can be identified individually. Only in the case of *lathyrus sativus*, the presence of the toxic amino acid β-N-oxalyl-α-β diamino propionic acid has been reported[3] and a method has been devised for characterising the pulse[4].

Methods at our disposal for the identification of pulses are the physical and microscopic examination which have their own limitations[5]. Therefore it was contemplated to find out characteristic chemical constituents in each pulse so that the findings could primarily be applied for the identification of pulses individually or in mixture particularly when they are in powdered form. A study of phenolic compounds in various pulses was therefore undertaken and paper chromatographic technique was used for this study.

Local varieties of ten pulses namely, Bengal gram dhal (*Cicer arietinum*), green gram (*Phaseolus aureus*), Black gram (*Phaseolus mungo*), *masur dal* (*Lens esculents*), *tur dhal* (*Cajanus cajan*), dry pea (*Pisum sativum*), Cow pea (*Vigna catjang*), field bean (*Dollichos lablab*), horse gram (*Dolichos biflorus*) and soya bean (*Glycine max*) were purchased from Governmental Agencies, freed from foreign matter and used. *Kesari dal* (*Lathyrus sativus*) was procured from the Department of Agriculture in Mysore, Bangalore.

The chromatographic pattern of phenolic compounds in the ethanol extracts of the powdered and defatted pulses were obtained using the method reported by the authors[6]. The chromatograms were observed in day light and under ultra-violet light before and after treating with chromogenic reagents used to locate phenolic compounds[7].

The chromatographic patterns of phenolic compounds varied with different pulses. Individual pulses contained at least one characteristic phenolic compound, which was not found in other pulses. The Rf values and characteristic colours with several chromogenic reagents is presented in Table 1.

This chromatographic data can be used in the identification of pulses individually or in mixtures as in the case of detection of adulteration with *Kesari dal*[8] (Fig. 1).

TABLE 1. CHARACTERISTIC OF PHENOLICS SPOTS IN PULSES ON PAPER CHROMATOGRAMS DEVELOPED WITH BUTANOL : ACETIC ACID : WATER (4 : 1 : 5) SOLVENT SYSTEM

Pulse	Rf of phenolic spot	Before treatment		Exposure to HCl fumes		Exposure to NH_3 fumes		Sodium carbonate (5%) spray		Aluminium trichloride (5%) spray	
		Day Light	U.V. Light	Day Light	U.V. Light	Day Light	U.V. Light	Day Light	U.V. Light	Day Light	U.V. Light
Kesari dal	0.50	...	P	...	...	...	Y	...	Y	...	YG
Bengal gram	0.44	...	...	...	...	...	...	...	...	...	Int Bl
Green gram	0.15	...	Y	...	...	...	...	...	Sky Bl	...	...
Black gram	0.42	...	L.Bl.	...	...	...	...	...	YG	...	...
Masur dal	0.38	...	Deep P	...	LY	Y	Y	Y	YG	YG	Canary Y
Tur dal	0.31	...	L.Bl	...	...	...	...	...	...	...	L YG
Pea, dry	0.83	...	...	...	...	...	Deep Bl	...	Br Bl	...	...
Cow pea	0.46	...	P	...	...	...	...	LP	Deep Bl	Y	YG
Field bean *dal*	0.62	...	BlG	...	...	...	...	...	Int Bl	...	...
Horse gram	0.92	...	Bl	...	BlG	...	Int BlG	...	Int Bl G	...	...
Soyabean	0.11	...	BrBl	...	...	...	Int Bl	...	...	...	...

P=Pink; Y=Yellow; G=Green; Bl=Blue; L=Light; Int=Intense; Br=Bright

The presence of a characteristic phenolic compound in each pulse is further supported by the reports of Braverman that chromatographic pattern of these compounds are characteristic of individual species of plants and are definitely controlled by specific genes present in these plants[9]. Further studies on the chemical characterization of the phenolic compounds in the pulses are in progress.

Acknowledgement

The authors are grateful to Dr T. L. Puttaswamy, Deputy Director (Laboratories), Public Health Insti-

tute, Bangalore for his encouragement and to Shri P. N. Prasad for the help extended in getting the photographs.

Food and Water Analysis Laboratory,
Bangalore
8 *January 1973*

C. P. HARTMAN
N. G. DIVAKAR
U. N. NAGARAJA RAO

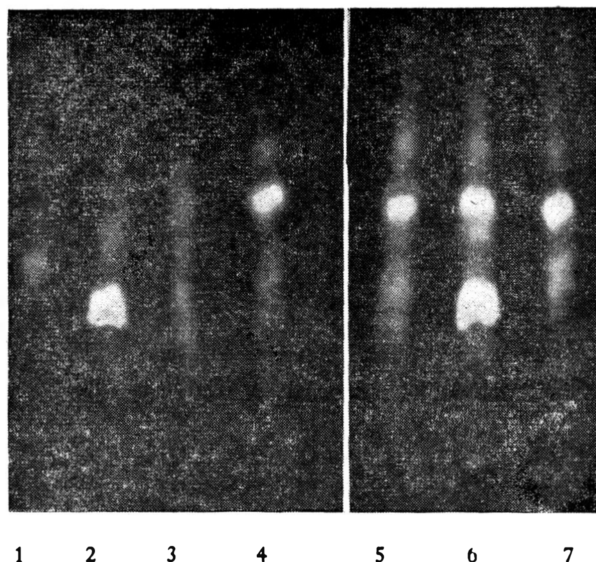

FIG. 1. Chromatographic pattern with aluminium trichloride spray and ultra-violet observation of dhals of (1) Red gram (2) Masur (3) Bengal gram (4) *Kesari* (5) Red gram with *Kesari* (6) *Masur* with *Kesari* and (7) Bengal gram with *Kesari*.

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A SIMPLE INEXPENSIVE DEVICE FOR DETECTING AND ESTIMATING CRACKS IN INTACT PADDY GRAINS

Based on the principle of partial illumination through narrow slit transmitting light from below, a unit has been developed to detect and count the cracks in intact paddy grains. A counter unit for holding 100 grains and examining them for cracks has been prepared and found suitable in processing work.

In an investigation to study the effect of steam treatment and adverse weather conditions on the milling quality of paddy the need was felt for a simple and rapid method for estimating the proportion of cracked grains in a large number of paddy samples. Hand dehusking was tedious while the use of soft X-rays[1-3] was expensive for routine work. The possibility of developing a rapid, simple and inexpensive method was therefore investigated.

Henderson[3] had observed that partial illumination reveals cracks in brown rice grain. Stermer[4] had also developed a technique in which the brown rice grains were made to pass in a line over polarized light and the cracked grains could be identified. Kunze *et al*[5]., have also indicated the possibility of a simple unit for detecting cracks in brown rice. A pointed light was moved under the rice grain and the cracks were visible. Realising that the paddy grain with intact husk may need more illumination than dehusked grain and keeping the need for simplicity and rapidity of technique in view, the following method was standardized after much exploratory work.

Direct lighting of paddy from below through a small round or oblong slit illuminating only part of the grain could detect the cracks in intact paddy (Fig. 1). In order to assess the percentage of cracked grains a plate with indentations for holding 100 grains was prepared and a cut was made in the indentation for admitting the light from below and illuminating the grain. The grains were laid flat on the slit leaving no side space for leakage of light.

Illumination could be suitably provided from an assembly of 12 volts, 15 W bulbs arranged in a line

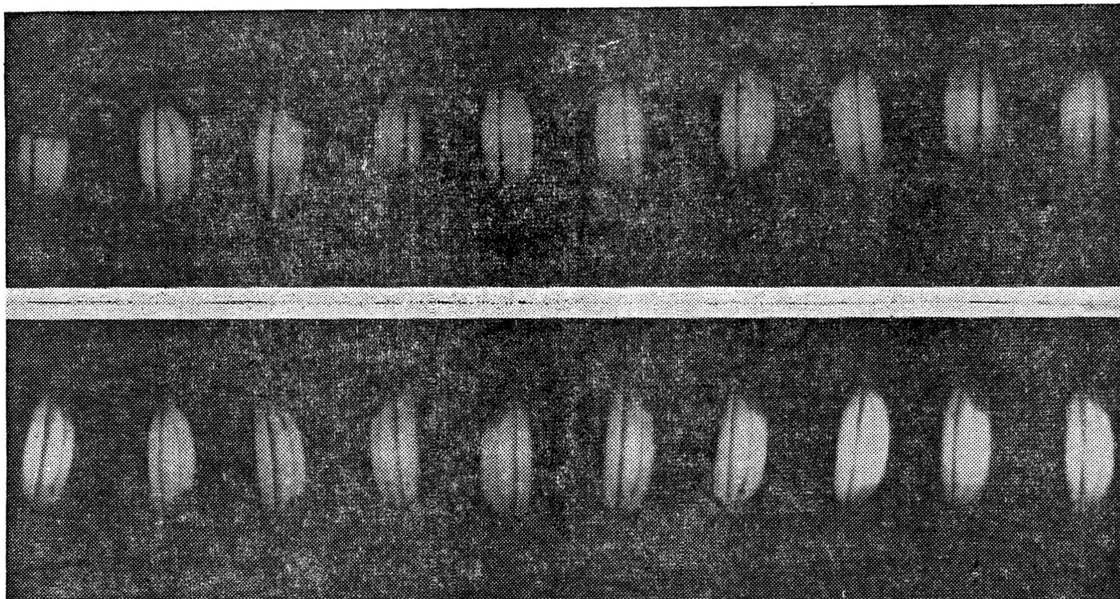

Fig. 1. Photograph of cracked (top) and normal (bottom) grains, illuminated from below through a slit.

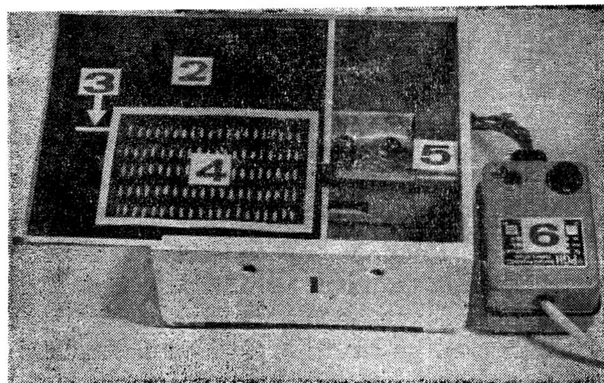

Fig. 2. Paddy crack detector assembly. (1) wooden box, (2) box cover partly opened to expose illuminating bulb, (3) line of horizontal slit, (4) counter plate with grains mounted, (5) bulb assembly fitted below the cover plate, and (6) transformer.

below the slit and connected to a suitable transformer. Ordinary bulbs (60-100 w) were also suitable but these caused heating of the counting plate. Varieties with highly coloured or thick husk required stronger illumination than normal varieties. Even bright sunlight reflected from a mirror could be used as a source of illumination and the cracks detected. A cover box for protecting the counter plate against unwanted light increases ease of detection.

Slight movement of the grain was found helpful to arrive at the most advantageous position for easy detection of cracks. Instead of moving each grain or moving the source of light it was found convenient if a row of grains in the counter was held against a narrow horizontal slit of 2 mm wide admitting the light from below the counter plate. Sliding of the plate at right angles to the horizontal slit facilitated easier and surer detection of cracks. Counting of cracks in 100 grains mounted on the counter can be completed within 2-3 min. Such an assembly has been fabricated and shown in Figure 2. The unit is inexpensive and can be fabricated and assembled at a cost of about Rs. 100. With experience even abdominal white, internal chalkiness and other defects in paddy can be detected. The plate could be made of metal, plastic or wood all coated with black paint.

The usefulness of the above unit in processing study has been established. The progressive formation of cracks during mechanical drying or as a result of overmaturity of paddy grains has been demonstrated using the detector counter (Table 1).

Acknowledgement

The authors wish to thank Dr H. A. B. Parpia for many helpful suggestions in the development of the counter unit. Thanks are also due to Mr K. V. Achutha Rao for photographic help.

Central Food Technological Research Institute, Mysore
17 August 1973

T. Srinivas
H. S. R. Desikachar

TABLE 1. ESTIMATE OF CRACKED GRAINS IN TWO VARIETIES OF PADDY USING THE COUNTER PLATE BEFORE AND AFTER DEHUSKING

	S-1092			*Suma*			*Suma*				
	Control	Sun dried		Control	Oven dried with hot air		Paddy harvested at different moisture contents				
		4½ hr	7½ hr		3 hr	6 hr					
Moisture%	10.1	6	5.7	13	7	5	22.6	20	18.5	17	15
Cracked grains in paddy%	2	20	26	10	10	14	10	13	16	28	36
Cracked grains in dehusked rice%	4	20	28	10	12	14	10	13	16	29	36

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Prepared Snack Foods: by M. GUTCHO, Noyes Data Corporation, Park Ridge, New Jersey, U.S.A., 1973, pp. 289. Price $ 36.

The book gives detailed descriptive information based on U.S. patents since 1961 relating to prepared snack and convenience foods with the intention of enabling the reader to solve his processing problems in the area of 'crisps' like potato chips, cornchips, popcorn, expanded products, nuts and nut products, french fried potatoes, onion rings and apple crisps.

Some of the improved techniques employed in preparing various types of snack foods and discussed in the patents are (1) hot oil cooking followed by radiant heat or microwave heating (2) coating with amylose starch (3) improving flavour by addition of methionine to cooking oil (4) use of selectively hydrogenated vegetable seed oil for frying (5) preparation of chips from dough base containing fresh potato puree or dried potato products with additives like amylopectin, egg albumin, stearylmonoglyceridyl citrate, gluten, starches, yeast etc. (6) imparting fried taste and appearance to baked food stuffs, (7) preparation of potato coated snacks from cereals like corn, wheat, rice and barley (8) giving rolled, sheeted, wavy or bent configurations to products (9) preparation of expanded products like high protein snack, low colorie salted snack, onion flavoured snack, and wafer from whole grain or legumes (10) puffing by vacuum, microwaves, oven baking, hot dry fluidizing gas or deep fat frying (11) partial defatting increasing shelf life and removing strong flavour components and blemishes from peanuts. (12) improvements in roasting, coating and colouring of nuts (13) preparation of synthetic nut meats using hydratable film forming substance, fused casein or soy bean fibres and puffed cereals impregnated with starch-fat slurry (14) improvements in the french fried potatoes processed from raw potatoes or potato dough base by modifying pretreatments or use of additives (15) preparation of a variety of snacks based on wheat, rye, limabeans, apples, bananas, onions and bacon flavour employing modified techniques.

Information given in the book and normally not covered in the journal literature, is commercially very useful and is likely to be overlooked by those who rely mainly on periodical journals. By highlighting the various technical possibilities and opening up profitable areas of research and development, the condensed information provided by the book is highly useful to research scientists in the field.

The table of contents has been presented in such a way that it serves as a subject index. Company, inventor and patent number indexes given at the end of the book help in providing easy access to the information contained in the book.

Like other books in the series of Food Technology reviews brought out by Noyes Data Corporation, the present volume has also been brought out with record speed for the benefit of potential users.

B. S. BHATIA

Consistency of food stuffs: By TOSHIMAN SONE, D. Reidel Publishing Company, Dordrecht, Holland, pp. 185. Price: f. 57.

In this era of novel foods, this book provides invaluable information on the texture of a wide range of food products. It consists of six chapters which are well arranged.

Chapter 1 deals briefly with food consistency. In Chapter 2, the objective methods of measurement of consistency have been well presented. Chapter 3 deals with the consistency of foods having different rheological properties. The author does full justice by bringing out clearly how polymer science, colloid science and rheology can be applied to deduce the structure of food, and explains their influence on food texture. In Chapter 4, a short but informative account of the important aspects of abnormal flow properties of foodstuffs is given. Later chapters deal briefly with the physiological aspects of food texture evaluation both by consumer and by instrument. The inter correlation between the two has been brought out with clarity. In addition, the author lays emphasis on the texture profile technique in evaluating textural properties and shows how much information can be obtained by using it.

The book is well produced, with selected bibliography and an extensive author and subject index. It is a useful up-to-date publication on all aspects of food consistency, which all Food Technologists will value.

K. G. RAGHUVEER

Livestock and Meat Marketing: By JOHN M. MCCOY, Avi Publishing Company, Inc., Westport, Connecticut, U.S.A., 1972, pp. 465. Price, $22-00.

The book deals with the production and distribution of livestock and meat. In the U.S.A. the quantity of meat, a highly perishable commodity, produced and distributed through a complex marketing system is a huge industry handling some 38 billion pounds of meat and involving 11.2 million people employed in the livestock and meat industry.

The presentation comprises 17 chapters dealing with the various aspects involved in the field. They are historical perspective, economic theory and principles of production, supply, marketing and consumption, wholesale and retail marketing, grading and regulatory/inspection measures, future market possibilities and international trade in livestock, meat and byproducts, marketing costs and competition from meat substitutes and synthetics.

The book as a whole presents the complexities in marketing such a variable commodity as livestock and meat in a convincing and lucid fashion. The aspects of particular interest especially in the context of the developing industry in this country are (*i*) Economic theory and principles—their relevance in marketing decision, (*ii*) Livestock production and supply characteristics, (*iii*) Meat packing and processing, (*iv*) Meat Marketing—wholesale and retail, (*v*) Grades and grading, (*vi*) Regulatory and Inspection measures, (*vii*) International Trade in meat and by products and (*viii*) Meat substitutes and synthetics.

At the end of each chapter, a bibliography listing various sources used in compiling the book are given. The book is a good addition to the library of individuals and institutions interested in meat science and technology.

B. R. BALIGA

Surface Active Chemicals: By H. E. GARRETT, Pergamon Press, Oxford, 1972, pp. 167. Price £3.

This book will serve as a good reference text book for those who require a thorough understanding of the basic, theoretical principles connected with surface phenomena.

The basic concepts are dealt with in detail and near ly a third of the book is devoted for this purpose.

Relevance of the basic principles to the industrial applications, like manufacture of (*a*) soap and detergents, (*b*) adhesives, both natural and synthetic, (*c*) flocculating agents and dispersing agents are discussed in separate chapters.

The basic concepts discussed in the first chapter are theoretical and very much advanced for a beginner in the subject.

The application aspects about the individual surface active chemicals form interesting reading and at the end of each chapter many reference works are listed.

B. S. RAMACHANDRA

Nutritious Foods for Everybody: Report of the Calcutta Workshop conducted by the Protein Foods Association of India during November 1972, pp. 127.

A marketing workshop on 'Nutritious Foods for Everybody' was organised by the Protein Foods Association of India at Calcutta on November 20-21, 1972, based on the findings of a comprehensive food habits survey in the Calcutta metropolitan area, carried out during 1969-70 by Hindustan Thompson Associates Ltd. The main objective was to evolve probable product profiles specially suited to provide better nutrition to the large masses of malnourished people. Four expert working groups in the areas of staples, snack foods, Beverages and Infant Foods were set up for this purpose and they were assigned the task of preparing working documents in the respective areas for discussion at the Workshop.

One of the main findings of the food habits survey is that calorie deficiency is the most pronounced in the diets of all age groups in Calcutta and protein is a less critical dietary deficiency. Only one-third of the population-both preschool children aged upto 4 years and adults-belonging to low income groups do not get the required quantity of protein from the foods they consume daily.

The report contains IX chapters, the first three covering the genesis and strategy of the Workshop, inauguration and the basis which includes a summary of the survey. Chapters IV-VII deal with the reports of the four working groups and discussions on them. The constraints in the manufacture and delivery of low cost nutritious foods are dealt with in the Concluding Panel Discussion (Chapter VIII). The last chapter gives some idea of the types of products that could be developed and used. The report will be of considerable value to all the agencies connected with the problem of food and nutrition, as it focuses attention on the many complex problems in the production and marketing of nutritious foods in a food-traditional country like India.

N. SUBRAMANIAN

Poultry Nutrition Feeding and Feed Processing: Short term Course: Booklet compiled by the Division of Poultry Research, Indian Veterinary Research Institute (ICAR) in collaboration with UNDP, 1973, pp. 150.

The booklet mentioned above is a collection of papers presented by scientists, representatives from field and government agencies working in the area of poultry nutrition, feeding and feed processing at a short term course, organised jointly by the Indian Veterinary Research Institute (ICAR) and UNDP, which was held at Indian Veterinary Research Institute, Izatnagar between 14th and 18th May 1973. It contains 29 valuable articles highlighting various aspects of poultry nutrition such as requirement of different nutrients for optimum production, effect of various methods of feeding schedule on production, utilisation of different industrial byproducts as poultry feed, compounding and processing of poultry feed and its quality control and the influence of genetic factors and infection on the nutritional requirement of poultry. The publication is a useful addition to our present knowledge of poultry science, especially in the field of poultry nutrition.

P. C. PANDA

Pesticide Residues in Food: Report of the 1972 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. *World Health Organization Technical Report Series*, 1973, No. 525; 47 pages. Price:50 p, $1.20, Sw. fr. 4.—. Available through WHO Regional Office, Indraprastha Estate, New Delhi 1.

The report of the 1972 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticides Residues contains a summary of the main conclusions and recommendations of the Meeting. Details of the evaluations, acceptable daily intakes (ADIs), and tolerances for pesticide residues in food are published in monographs that are issued by FAO and WHO as a separate document.

The term 'temporary ADI' is explained and the procedures to be adopted in cases where temporary ADIs have been established and where the required data are not forthcoming before the specified data are outlined.

Recent studies on the ophthalmological effects and potentiation of cholinesterase-inhibiting insecticides are discussed and further work is recommended. Results of studies carried out on the carcinogenicity of DDT are considered but no change is made in the conditional ADI already established for that compound. In discussing the use of organomercury compounds for seed dressings, the report recommends that further studies should be undertaken to develop suitable alternatives that would be less likely to produce poisoning incidents.

The report notes that, when considering the relative merits of different methods of residue analysis, preference should be given to multiresidue methods that have been subjected to collaborative check sample procedures. In cases where data indicate that there is little likelihood that residues would result in food commodities from approved use of a specific pesticide, residue limits 'at or about the limit of determination' are recommended. The difficulties of defining residues that occur in foods of animal origin are recognized and a classification of residues that occur in animal feeds is given.

In an attempt to solve certain problems that have arisen in the interpretation of tolerances, the basic principles used by the Joint Meeting in determining tolerance levels are summarized.

Following the pattern of previous years, the report also contains a summary of the evaluations of data for ADIs and for tolerances and practical residue limits made by the Meeting.

A list of items recommended for consideration at future Joint Meetings is included and the report is completed by annexes that provide (1) a revised version of the index to documentation and summary of recommendations concerning ADI's tolerances, practical residue limits, and guideline levels and (2) a list of further work or information required.

Symposium

A Symposium on 'The Contribution of Chemistry to Food Supplies' was held in Hamburg, during August 29-31, 1973. The Symposium was organised jointly by the Food Section of the International Union for Pure and Applied Chemistry and the International Union of Food Science and Technology. Papers on the subjects: Chemical Modification of Food, Contaminants in Food and Development and Evaluation of New Proteins, were read and discussed in the various sessions.

Conference on Animal Feeds of Tropical and Sub-Tropical Origin

An international conference an Animal Feeds of Tropical and Sub-Tropical Origin will be held in London from 1-5 April 1974. This conference is one of a series organised biennially by the Tropical Products Institute. The aim of the conference is to enable as wide a range of people as possible concerned with the production, marketing and utilisation of these materials to meet together, to discuss problems of common interest.

The conference will be restricted to concentrate feed materials, in relation both to the setting up of feed industries in developing countries and the production of feed components for the international trade. A provisional programme has been arranged in which papers on the quality, utilisation, marketing and other aspects of feed materials presently produced or which could be produced in tropical and sub-tropical countries will be given. Such materials include cereals, cassava, bananas, molasses, oilcakes, fishmeal, abattoir by-product meals, single cell protein and leaf meals. Some of the problems of manufacturing and marketing compounded animal feeds in developing countries will also be discussed.

ISI to hold Fifteenth Indian Standards Convention in Coimbatore

The fifteenth Indian Standards Convention of the Indian Standards Institution will be held at Coimbatore from 17 to 22 December, 1973. About 1,000 delegates representing industrial, business and commercial organizations; scientific, research and technical institutions; organized consumer and purchase bodies; and Central and State Governments are expected to participate in the Convention. A few delegates from the neighbouring countries are also expected to attend.

The Standards Convention will comprise two General and five Technical Sessions. The General Sessions will discuss traditional designs in textiles and standards in the development of handloom industry. The Technical Sessions will deal with importance of standardization and quality control in diverse fields, namely, textile machinery and components, motor and diesel pump sets for agriculture, book production, in-plant and inter-plant standardization and small-scale industries.

World Animal Review

World Animal Review is a new quarterly periodical published by the Food and Agriculture Organisation of the United Nations. There is at present no periodical which surveys, reviews and reports on the various aspects of animal production, animal health and animal products (meat and milk products, eggs, wool, etc.) with special reference to developments in these spheres in the developing countries. World Animal Review is written primarily for a technical audience, but also for the livestock policy maker and the decision taker in the developing countries and for the university teacher, research worker, and international expert. The articles will be illustrated and will normally be about 2,500 words in length. In addition there will be a few pages of news, notes, book notices, etc. Three separate editions are available in English, French and Spanish.

Congress of Agriculture and Food Industries

In response to the invitation of the Greek Government, the International Commission of Agricultural and Food Industries is organising, in collaboration with the Association of Greek Chemists, the 11th International Congress of Agricultural and Food Industries, which will be held at Athens from the 1st to 5th of April 1974. The programme of this meeting will be centred around the industries of special interest to Mediterranean countries: cereals, canning, olive oil, and fruit juice.

Registration and proposals to submit papers should reach the secretariate of the International Commission of Agricultural and Food Industries, 24 rue de Teheran, 75008-Paris, before 31st January 1974.

Automatic Milk Powder Filling Machine

The National Dairy Development Board and the Kaira District Cooperative Milk Producers Union have designed an automatic powder filling machine capable of filling 500-1000 grams of powder in containers. The speed is 40-45 tins per minute. The filling device is completely controlled by the application of electrical components. An air cooled clutch eliminates maintenance problems encountered in oil cooled clutches. The machine is so designed as to stop any powder from escaping . So, the operational area is dust free.

The machine costs only one fourth of the price of similar machines imported from abroad.

A New Volume of Coblentz Society Spectra

The Coblentz Society announces the publication of Volume 9 in its continuing collection of Evaluated Infrared Spectra. This volume will supplement the eight volumes of spectra which have previously been issued in this collection. Included are 1,000 high quality spectra which have been evaluated and selected by experienced spectroscopists to meet criteria established by the Coblentz Society. Previously published spectra are not included unless the new spectrum is more accurate, better resolved, has greater spectral range, or shows the sample in a different physical state.

New Fluorescence Spectra Published by Sadtler

Sadtler Research Laboratories, Inc., Philadelphia, announces the publication of two new continuing collections of Fluorescence Reference Spectra. The standard Fluorescence spectra collection contains a wide range of pure organic compounds of general interest. The Pharmaceutical collection contains spectra of substances such as drugs, medicinals and pharmaceutical preparations which are frequently encountered in medical research, pharmaceutical research and drug analysis. This collections are intended to provide the chemists with a large number of reliable fluorescence spectra which will be helpful in identifying unknown compounds.

Sadtler offers New Audio-Visual programs on infrared spectroscopy

Sadtler Research Laboratories, Inc. of Philadelphia, Pa., announces a new series of audio-visual programs on infrared spectroscopy. Technical consultants for this series were Professor Richard C. Lord, Spectroscopy Laboratory, M.I.T. and Professor Dana W. Mayo, Chairman, Department of Chemistry, Bowdoin College. The programs are designed to present a theoretical introduction to infrared spectroscopy.

The series consists of four programs entitled 'Basic Principles, Parts 1 and 2' and 'Quantitative Analysis, Parts 1 and 2'. Each program contains approximately 40 slides and an audio tape with approximately 25 minutes of narration. In addition, student note-books are available which review the information presented on the slides.

The first two programs introduce the basic theoretical principles of the single and double beam spectrophotometres including the electromagnetic spectrum, infrared spectra, molecular absorption, detection and measurement. The second two programs discuss the Beer-Lambert Law, the analysis of multicomponent systems, techniques for improving the accuracy of infrared quantitative analysis, and deviations from the Beer-Lambert Law.

Additional information about these programs is available from Sadtler Research Laboratories, Inc., 3316 Spring Garden Street, Philadelphia, Pa. 19104.

Sunflower Cultivation

Sunflower cultivation is being steadily extended in India to make up the shortage in edible oils which are used to manufacture Vanaspati.

The states of Andhra Pradesh, Tamil Nadu and Mysore have been growing sunflower for over a year now as a commercial crop. Its cultivation will be introduced in Maharashtra and Gujarat, where the seed will be distributed free in the form of minikits.

Sunflower seeds were originally imported from the USSR to manufacture oil and Vanaspati. Sunflower, which is not sensitive to sun rays, can be grown any time in the year to fit a number of multiple cropping patterns. Unlike groundnut, it can be grown in areas of marginal rainfall. Only 10 kgs. of sunflower seed is required for every hectare yielding one ton of seed. In comparison, groundnuts require 40 to 50 kgs of seed per hectare.

The Vanaspati Manufacturers' Association has agreed to lift the seed wherever and whenever it is grown.

Third National Symposium on Refrigeration and Air-Conditioning

The Third National Symposium on Refrigeration and Air-Conditioning is scheduled to be held at the Central Food Technological Research Institute, Mysore, in July 1974.

The symposium will discuss the latest researches and developments, techniques and designs in the field of refrigeration and air-conditioning. It will provide an opportunity to bring workers in the field

together on a common platform to discuss and exchange information on their problems, experience and contributions.

It is proposed to publish a report of the proceedings and recommendations of the Symposium soon after the conclusion of the Symposium.

Indian Standards Institution

Following Standard Specifications have been published:

Standard	Title	Price
IS: 920-1972	Common Salt and Cattle Licks for Animal Consumption (Revision)	Rs 5.00
IS:2397-1972	Wafers (Revision)	Rs 3.00
IS:6677-1972	Sardines (Sardinella sp) Canned in Brine and in their Juice	Rs 4.00
IS:1932-1972	Mustard and Rape Seed Oil-cake as Livestock Feed (Revision)	Rs 3.00
IS:6438-1972	Aluminium Phosphide Tablet Formulation	Rs 5.00
IS:2152-1972	Maize Gluten Feed (Revision)	Rs 3.00
IS:5701 (Part IV) 1972	Code for Breeding, Care, Management and Housing of Laboratory Animals	Rs 4.00
IS:6696-1972	Egg Washing Machines	Rs 2.50
IS:6671-1972	Germination Paper	Rs 2.50
IS:6705-1972	Sand used in Germination Tests	Rs 3.00
IS:1509-1972	Tapioca as Livestock Feed (Revision)	Rs 6.00
IS:2345-1972	Dried Prawns	Rs 5.00
IS:2126-1973	Phenyl Mercury Acetate, Technical	Rs 3.00
IS:6797-1972	Methyl Ester of Beta-APO-8'-Carotenoic Aicd	Rs 3.00
IS:6796-1972	Propyl Gallate, Food Grade	Rs 4.00
IS:6782-1972	Hog Gambrels	Rs 3.00
IS:6762-1972	Drinking Chocolate	Rs 5.00
IS:6692-1972	Method of Milk Recording of Cattle	Rs 5.50

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Eastern Regional Branch

The Association held its Annual General Meeting on 8th September 1973, in Calcutta. A talk on 'Problems of Malnutrition Amongst Indian Children' was delivered on the occasion by Dr A. K. Basu, Vice-chairman, State planning Board, Government of West Bengal.

All India Symposium on Development and Prospects of Spice Industry in India

The Association, in collaboration with the Central Food Technological Research Institute, Mysore, is planning to arrange a Symposium on Development and Prospects of Spice Industry in India, from 28th February to 2nd March 1974 at CFTRI, Mysore.

The main objective of the Symposium is to focus attention on the aspects covering: selection of suitable spice varieties for technological needs; their preservation and fumigation; spice processing; spice oils and oleoresins; spice blends and ground spices; spices in pickles, chutneys, sauces and preserves; methods of quality control and specifications; storage, packaging and marketing; requirements of equipment; spice flavours in foods; and research needs.

Invited lead papers from eminent specialists in the line along with accepted papers from various workers are proposed to be presented in suitable sessions. The main object is to bring together the industry, government agencies and research centres so as to evolve an integrated approach to the development of spice industry in the country. It is also proposed to arrange an exhibition of the different spices, spice products and processing equipment in the country.

A souvenir containing lead articles on various aspects of the Spice industry will be released on the occasion.

The discussions will be held in the following sessions:

1. Spice Production
2. Spice Processing and Packaging
3. Quality Control and Standards.
4. Spice Flavours and Products
5. Marketing of Spice and Spice Products
6. Future Needs of Spice Industry

(General Body Meeting of AFST will be held during the Symposium)

Hyderabad Centre

An *Ad hoc* Committee has been formed in the Sub-centre at Hyderabad, Secundrabad. The General meeting was held on 6th August 1973 and the following were elected as members of the Committee.

Dr M. M. Krishna, (*Convener*)
Sri G. V. Krishnamurthy
Sri P. V. Suryaprakasha Rao
Smt. Yamuna Ranga Rao
Sri P. Ravi
Sri H. D. Sharma

On this occasion, Dr B. L. Amla, Director, CFTRI, Mysore, addressed the members of the Sub-centre on the 'Outlook on future requirements of Food and Nutrition in our country'. This was followed by an interesting discussion.

Professor Stewart

Professor G. S. Stewart, President of IUFOST, visited CFTRI during 11th to 15th September, 1973. On 14th September, he addressed the joint AFST and CFTRI Technical Seminar on the subject 'International Food Science and Technology—Challenges and Opportunity'.

Seminar

Under the joint auspices of the Association of Food Scientists and Technologists (India) and the Technical Seminar, CFTRI, Mysore, the following seminar was arranged on Monday 15th October, 1973: Speaker: Dr C. W. Hesseltine, Chief, Fermentation Lab, N.R.R.L., Peoria, Illionis, U.S.A. Subject: 'Mycotoxins in Cereals'.

Annual General Body Meeting of the Western Region

The Annual General Body Meeting of the AFST, Western Region was held on 3rd October 1973 at the University Department of Chemical Technology, Bombay.

Vice-President, Dr B. P. Baliga welcomed the gathering and Hon. Secretary, Dr G. A. Sulebele gave the report of the activities. As a part of the plan to encourge the student community, special cash awards

and certificates were given to Sri Iqbal Manekia, Sri Dilip Mundkur and Sri Susheel Kumar of the B.Sc., (Tech.) Food Technology Course of Bombay University.

The region plans to hold a Seminar on Food Machinery in January, 1974. Dr G. B. Ramasarma, who was the chief guest, delivered his address, in which he traced the origin of the present protein controversy. Dr D. V. Tamhane, Hon. Treasurer, presented the audited statement of accounts.

The following have been unanimously elected to the committee for the coming year: President—Dr B. P. Baliga, Associate President—Dr D. V. Rege, Vice-President Dr D. V. Tamhane, Hon. Secretary, Dr G. A. Sulebele, Hon. Jt. Secretary, Dr K. S. Holla, Hon. Treasurer, Sri R. D. Shenoy. Committee members: Dr K. T. Achaya, Dr A. G. Mulgaonkar, Dr (Miss) Thangam E. Philip, Dr D. V. S. K. Rao, Dr M. R. Vora, Sri L. K. Shah, Sri G. R. Shah, Sri S. R. Padwal-Desai.

Dr D. V. Tamhane, the New Vice-President proposed the vote of thanks.

Annual General Body Meeting of the Eastern Regional Branch

The Annual General Meeting of the AFST, Eastern Regional Branch was held on 1st July 1973 at the Birla Industrial and Technological Museum, Calcutta.

Mr B. S. Narayana took the chair. Secretary's report and audited statement of accounts were passed by the meeting. The new committee of the Regional Branch is as follows:

President—Mr N. C. Roy, Vice-President—Mr K. C. Dé, Hon. General Secretary—Mr A. K. Sen, Hon. Joint Secretary—Mr P. K. Bose, Hon. Treasurer —Mr P. Chattopadhayay. Committee Members: Prof. R. N. Ghosh, Mr S. K. Das Gupta, Dr G. C. Bhattacharya, Mr B. N. Srimani, Mr B. S. Narayana, Dr R. Dutta, Dr N. D. Banerjee, Dr A. N. Bose and Prof. Sumit Mukherjee.

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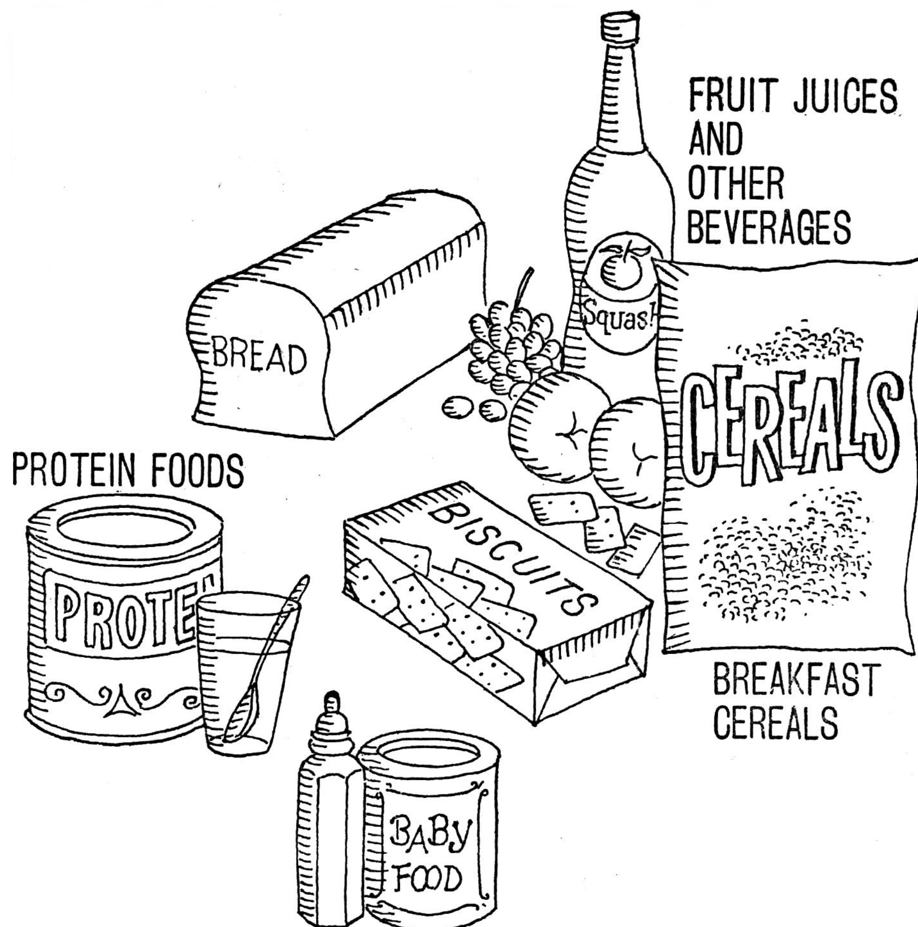

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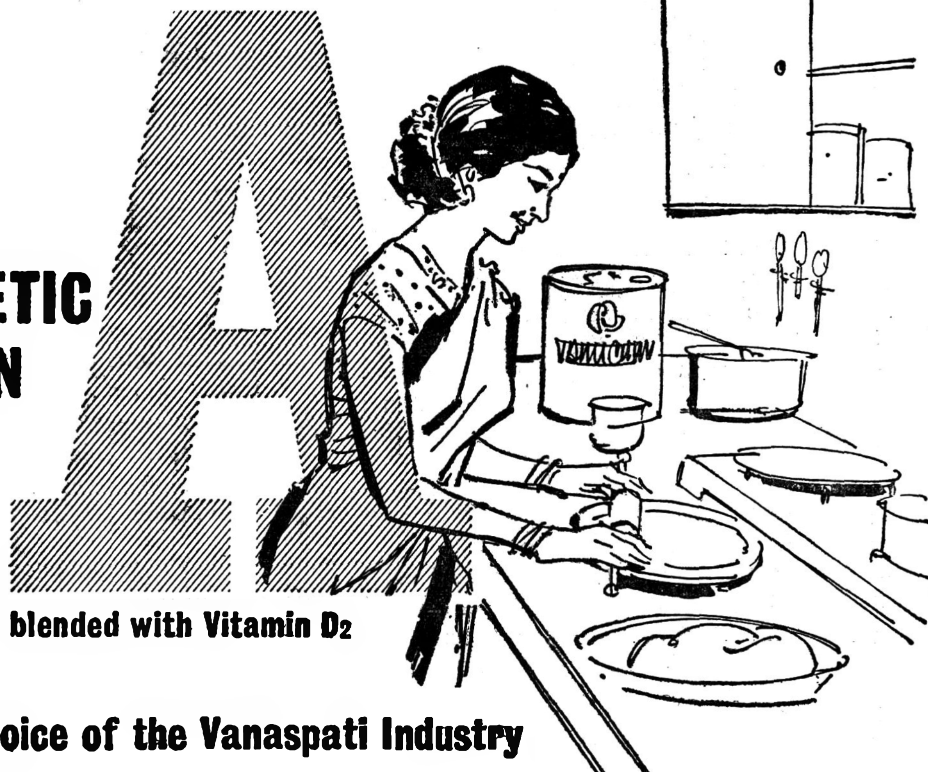

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(*e*) *Thesis:* Sathyanarayan, Y., *Phytosociological Studies on the Calcicolous Plants of Bombay, 1953*, Ph.D. thesis, Bombay University.

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