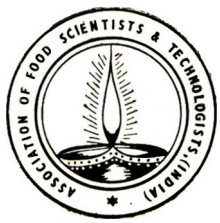
JOURNAL OF FOOD SCIENCE AND TECHNOLOGY



ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS, INDIA

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(INDIA)

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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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Research Papers

Research Notes

An Improved Method for the Determination of Nitrate and Nitrite in Fresh and Canned Fruit and Vegetable Products

W. E. EIPESON, M. MAHADEVIAH, R. V. GOWRAMMA AND L. V. L. SASTRY
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Manuscript Received: 18 April 1974

The interference by natural constituents of fruits and vegetables as well as tin in canned products in the l-naphthyl amine method for the determination of nitrate/nitrite are studied. An improved method to eliminate the interferences is described. This enables use of larger quantities of samples thereby increasing the sensitivity of the method. Further, it makes it possible to estimate nitrate and nitrite separately in canned products also.

Nitrate accumulation in foods has attracted attention of both medical men as well as food technologists since the mid 1950's. Nitrate accumulation in food is due to the excessive use of nitrogen fertilizers[1-3]. Nitrate has been shown to cause methaemoglobinaemia in infants[4], and nitrate found in foods may combine in the body with secondary amines to form carcinogenic nitrosamines[5].

The interest in nitrate accumulation in foods arises out of the possible role of nitrate in rapid detinning of tinplate containers. Culpepper and Moon[6], as early as 1928, concluded from their studies that the detinning properties of pumpkin and beets might be attributed to nitrates. Stroditz and Henry[7] who studied the relation of nitrate in foods to tinplate container corrosion, concluded that pH is the determining factor in nitrate-induced corrosion. Lambeth *et al*[8] have reported the acceleration of corrosion by nitrate in canned spinach. Roquest *et al*[9], Thomas *et al*[10], and Massani[11] also have shown the role of nitrate in tinplate container corrosion. Britton[12] has studied the role of nitrate on hot-dipped and electrolytic tinplate can corrosion and concluded that the difference in the extent of corrosion is due to the tin crystal size. Horio *et al*[13] have reported nitrate corrosion in the case of canned orange juice soft drinks in Japan. Due to the importance of this corrosion problem an extensive research programme was initiated in the United States[14]. Sherlock *et al*[15] have shown the corrosive effect of nitrate and nitrite using polarisation technique.

A number of methods for the determination of nitrate and nitrite in foods have appeared in the published literature. The method originally developed by Grau and Mirha[16] and subsequently modified by Kamm *et al*[17] involving reduction of nitrate on a cadmium column and subsequent coupling of nitrite with 1-naphthyl amine, and colorimetric determination is a convenient one for the determination of nitrate in foods. However, many products give low recoveries of added nitrate and nitrite when more than 2 g per 200 ml sample is taken[17]. This makes it difficult to determine low levels of nitrate and nitrite. Further, there is the draw-back that in many products, nitrate or nitrite, which may be present already can be masked by the interfering substances. Moreover, we have found that tin interferes in nitrate determination in canned foods.

In the present study, interference by some of the constituents in nitrate and nitrite determination has been investigated and suitable methods suggested to eliminate them. These studies were undertaken to enable the use of larger quantity of samples thereby increasing the sensitivity of the method, formation of stable emulsions during chloroform extraction and to make the method suitable for canned products.

Materials and Methods

The following reagents were used :

1. *Buffer (pH 9.6-9.7) :* The buffer consisted of 20 ml concentrated hydrochloric acid and 50 ml concentrated aqueous ammonia per litre of water[17].

2. *1-naphthyl amine :* This reagent consisted of 0.5 per cent 1-naphthyl amine in 1+3 aqueous ammonia[17].

3. *Methanol-hydrochloric acid solution :* This was prepared by mixing 17 ml concentrated hydrochloric acid with 2 l of methanol[17].

4. *Alumina cream :* This was prepared by precipitating aluminium hydroxide from aluminium potassium sulphate with aqueous ammonia, washing the

precipitate with water to free it from sulphate and suspending it in an equal volume of water[17].

5. *Cadmium reduction column :* This was prepared by filling 7 cm × 1.2 cm glass columns with 20-40 mesh cadmium[17].

6. *Cation exchange resin column :* Dowex 50-X8 was filled into a 10 cm × 1.2 cm glass chromatographic column to about 6 cm height. The resin was converted to the hydrogen form by passing about 50 ml of 5 per cent hydrochloric acid and washing the column with distilled water till the pH of the eluate came up to about 6.5.

7. *Tin solution :* Stock solution of tin was prepared by dissolving 0.7969 g of analytical grade $SnCl_2$ (anhydrous) in water with addition of HCl till a clear solution was obtained and the solution made up to one litre. This gave a solution containing 500 μg tin per ml.

Interference by tin in canned products and its removal: The interference by tin was studied over a range of 10 to 1000 μg tin by adding it to 100 ml buffered solutions containing 20 μg nitrite N and developing colour with 1-naphthyl a mine (Fig 1).The in terference was also studied using clarified extracts of some canned fruit and vegetable products (2 g per 250 ml) with known tin content. To remove dissolved tin, the solutions containing tin were passed through a cation exchange resin column in the hydrogen form. The eluate from the column along with water washings was taken for nitrate estimation (Table 2). Tin was estimated by the iodate method of McKenzie[18].

Interference by ascorbic acid and its removal : The interference by ascorbic acid was studied over a range of 0.1 to 1 mg by adding it to 100 ml buffered solutions containing 20 μg nitrite N and developing colour with 1-naphthyl amine (Fig 2). Ascorbic acid was destroyed by heating the solutions with copper sulphate. Ascorbic acid was estimated by the indophenol xylene extraction method of Robinson and Stotz[19].

Interference by constituents other than ascorbic acid present in fresh fruits and vegetables and their removal : Tomato paste was taken for this study as it is a typical product which interferes in the nitrate/nitrite estimation. The interference was studied over a range of 2 to 25 g per 250 ml with respect to 20 μg nitrite N (Fig 3). The interfering substances were removed by activated charcoal.

Recommended procedure for nitrate and nitrite estimation in canned products : Blend the product in a Waring blender and take a sample (1 to 25 g depending on nitrate/nitrite content), transfer into a 250 ml volumetric flask, using 100 ml distilled water. Add 5 ml buffer and 50 ml alumina cream, shake for 5 min and make up to volume. Filter through Whatman No. 41 filter paper. Take an aliquot of about 100 ml from the filtrate add 5 mg of activated charcoal, stir and filter through Whatman No. 42 filter paper. Take 40 ml of the filtrate in a 100 ml volumetric flask, make up to volume with acetone, allow to stand for 5 min and filter through Whatman No. 41 filter paper. Take 50 ml of the filtrate in a 100 ml separating funnel and extract thrice with 20 ml portions of ether and combine the ether layers. Extract the ether extract with 20 ml distilled water and combine the aqueous layer with the earlier aqueous layer and quantitatively transfer to a 100 ml conical flask. Add 2 mg $CuSO_4$ 5 H_2O, heat to 80°C, keep at that temperature for 5 min and cool to room temperature. Pass this solution through a cation exchange resin column which has been converted to hydrogen form as explained earlier. Wash down the column with two 15 ml portions of distilled water. Combine the eluate and washings. Pass the combined eluate and washings from cation exchange column through a cadmium reduction column after adding 5 ml buffer. Collect the eluate in a 250 ml conical flask and wash down the column with two 25 ml portions of distilled water into the same flask. Add 2 ml of the 1-naphthyl amine reagent and mix. Place the flask in the dark for 2 hr for colour development. Transfer the solution to a 250 ml separating funnel. Rinse the flask with about 5 ml chloroform and add to the separating funnel. Shake until most of the colour is transferred to the chloroform layer. Transfer the chloroform layer into a 50 ml volumetric flask. Repeat the extraction with 10 ml portion of chloroform until the chloroform layer is colourless which indicates complete extraction of the developed colour (total volume not to exceed 40 ml). Transfer the chloroform layers to the 50 ml volumetric flask. Add 10 ml methanol/HCl mixture make up to volume and shake. Measure the absorbance of the chloroform extract at 555 nm against a suitable blank. From a standard curve for nitrate, the nitrate value for the aliquot is calculated. This gives the value for nitrate N (A). For nitrite estimation the sample is taken through all the steps as above except passing through the cation exchange column. From a standard curve for nitrate (the standard curve for nitrite and nitrate is the same for all practical purposes) the nitrite value for the aliquot is calculated. This gives the total value for nitrite N and nitrate N (B). Subtracting (A) from

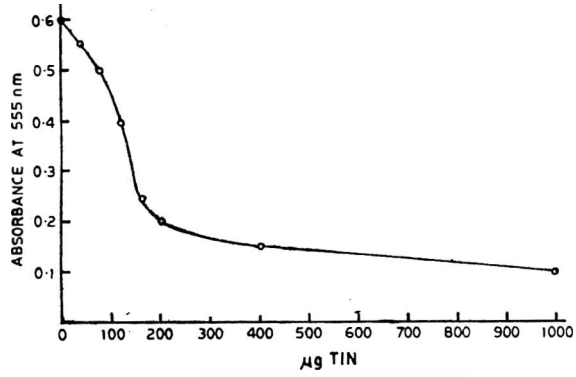

FIG 1. Interference by tin ions in l-naphthyl amine-nitrite reaction.

(B) the value for nitrite is obtained. The calculations are done as follows :

$$\text{Ppm nitrate N in the product} = \frac{25\ N}{2\ W}$$

When N = µg nitrate N in the sample aliquot taken for colour development.

W = Weight of the sample.

Results and Discussion

Fig 1 shows the interference by tin in the 1-naphthyl amine nitrite reaction. Tin in canned foods exists partly in dissolved and partly in undissolved form[20]. Only the dissolved tin interferes in the estimation as the undissolved tin is removed during the clarification of the extract. Table 1 gives the quantities of dissolved and undissolved tin in some typical canned fruit and vegetable products. The results show that in most of the cases more than 50 per cent of the tin is present in the dissolved form. This means that even in the initial stages of tin dissolution, sufficient tin will be present in the canned product to interfere in the nitrate/nitrite estimation.

TABLE 1. TOTAL AND DISSOLVED TIN IN VARIOUS CANNED PRODUCTS

Canned product	Tin (ppm) Total	Dissolved*
Tomato juice ..	317	188
Tomato paste ..	434	275
Mango nectar ..	572	445
Orange juice ..	487	362
Pineapple slices in sugar syrup ..	148	85
Peaches in sugar syrup ..	78	40
Potato in brine ..	105	48
Ivy gourd in brine ..	273	112
Bitter gourd in brine ..	89	40
Beans in brine ..	114	49
Asparagus in brine ..	168	92

* Dissolved tin refers to the tin present in the solution obtained after filtration through Whatman No. 41 filter paper.

TABLE 2. RECOVERY OF ADDED NITRATE IN THE PRESENCE OF TIN

Product	Added tin (µg)	Recovery of added nitrate % Before passing through cation exchanger	After passing through cation exchanger
Buffer ..	1000	10	100
Tomato juice ..	,,	12	97
Orange juice ..	,,	10	99
Ivy gourd extract ..	,,	9	100
Beans extract ..	,,	15	96

Table 2 gives the recovery of added nitrate from different fruit and vegetable extracts containing 1000 µg tin, before and after passing through cation exchange resin column. The results show that in all the cases the recoveries are about 96 per cent as compared to 9–15 per cent for samples before passing through cation exchange resin column. Iron does not interfere in the estimation of nitrate and nitrite even up to 1 mg in the solution taken for colour development which is far higher than the quantity found in canned foods.

Fig 2 shows the extent of interference by ascorbic acid in the nitrate/nitrite estimation. Organic acids like citric, malic, tartaric, oxalic, succinic, etc., which are commonly found in fruits and vegetables do not interfere in the estimation of nitrate and nitrite even up to 0.1 g in the solution taken for colour development.

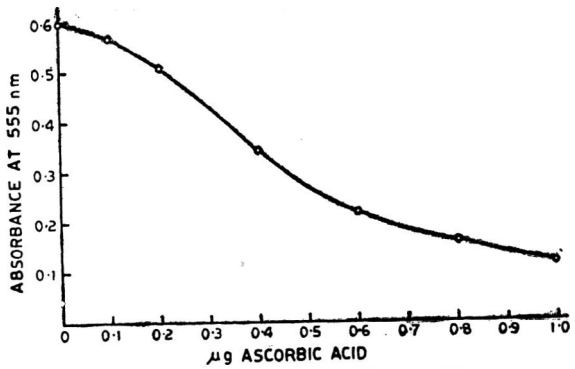

FIG 2. Interference by ascorbic acid in l-naphthyl amine-nitrite reaction.

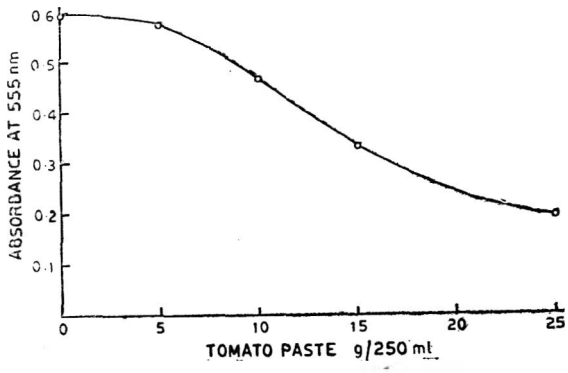

FIG 3. Interference by tomato paste in l-naphthyl amine-nitrite reaction.

Treatment of extracts containing ascorbic acid with copper sulphate (2 mg per 100 ml) and warming to 80°C almost quantitatively removes the inhibitory effect of ascorbic acid. The recovery of added nitrate/nitrite was 99 per cent as compared to 21 per cent for samples without this treatment.

Fig 3 shows the extent of interference by tomato paste in the nitrate/nitrite estimation. In the method of Kamm *et al*[17] the quantity of sample is restricted to2 g per 200 ml because of interference by some constituents present in the products. This limits the sensitivity of the method as well as results in the possibility that nitrate or nitrite already present may be masked by the interfering substances. Activated charcoal (5 mg per 100 ml) removes the interfering substances. The recovery of added nitrate/nitrite in tomato paste after charcoal treatment was 95 per cent as compared to 33 per cent in the case of samples without the treatment. When samples larger than 2 g per 250 ml are taken, during the chloroform extraction of the coloured complex of 1-naphthyl amine and nitrite, very stable emulsion is produced, which makes it almost impossible to separate the aqueous layer from the chloroform layer. This can be avoided by precipitating the foam producing constituents at 60 per cent acetone concentration and subsequent removal of acetone before colour development, using ether.

When solutions containing nitrite are passed through cation exchange resin column, the nitrite is destroyed completely, as nitrous acid is unstable. When solutions containing tin are passed through cadmium column, tin is deposited on cadmium even though the life of the cadmium column is reduced and the flow is affected. These two factors were made use of in the estimation of nitrate and nitrite separately in samples containing tin.

Acknowledgement

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Colorimetric Estimation of Tryptophan Content of Pulses

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The colorimetric procedure of Spies and Chambers (1948, 1949) for determination of tryptophan content of proteins has been examined in its applicability to proteins of pulses. It is observed that the amount of sodium nitrite to be added for the reaction needs to be increased to 0.1 ml of 0.02 per cent solution as against the recommended 0.1 ml of 0.05 per cent solution. This increase brings out higher amount of colour. With this modification, the tryptophan content of the pulses investigated was in the range 0.7–1.7 g/16 g N as against the literature values of 0.5–0.8 g/16 g N.

Conditions for chemical determination of tryptophan by using its colour forming reactions with p-dimethyl-amino benzaldehyde (DMAB) and sodium nitrite in sulphuric acid were exhaustively investigated by Spies and Chambers[1]. In a subsequent paper[2], the same authors described the chemical determination of the amino acid in various intact proteins and in the presence of several added substances such as other amino acids, and carbohydrates and recommended a general procedure for tryptophan determination in proteins. It was also recommended that for the greatest accuracy, each type of protein must be analysed for its tryptophan content by using pre-determined optimum conditions.

Tryptophan is often reported as a limiting amino acid in the proteins of the pulses that form an important protein source of the Indian dietary[3,4]. One pulse examined by us recently[5] indicated higher tryptophan content than hitherto reported when the conditions for tryptophan determination by the Spies and Chamber method were suitably modified. It needs no emphasis that an accurate determination of the tryptophan content in foodstuffs is necessary for assessing the protein score by FAO/WHO procedure[6]. In this paper we report tryptophan content of a few *dhals* by the colorimetric procedure of Spies and Chambers, with the slight modification described by them for special cases.

Materials and Methods

Samples of the commonly used *dhals*, viz., *toor dhal* (*Cajanus cajan*), Bengal gram (*Cicer arietinum*), green gram (*Phaseolus aureus*) and black gram (*Phaseolus mungo*) procured from the local market, were utilised in the study. Three qualities graded as A, B and C depending on the prices quoted for each were used. For purpose of comparison, freshly harvested whole grain samples (with husk) in triplicate of green gram and Bengal gram were also obtained from the University of Agricultural Sciences, Bangalore. The samples were powdered to pass through a 100 mesh sieve.

Protein content: The nitrogen content of the samples was determined by the micro-Kjeldahl method. The percentage protein content on moisture free basis was calculated using 0.25 as the conversion factor.

Tryptophan content: The general procedure of Spies and Chambers[2] for determination of tryptophan in proteins was followed. Defatted material containing approximately 50-100 μg of tryptophan was dissolved in 10 ml of 19 N sulphuric acid in 25 ml glass stoppered Erlenmeyer flasks and to the solution 30 mg DMAB was added. The solution was left overnight for 18 hr at room temperature. To the solution was added 0.1 ml of freshly prepared solution of sodium nitrite of varying concentrations (0.05, 0.1, 0.2 or 0.3 per cent) and the mixture allowed to react in the dark for 2 hr as per the modification of Miller[7]. The colour developed was measured using Filter No. 56 on a Klett-Summerson photoelectric colorimeter, there being no need to filter the solution, all the material being soluble. A blank was run simultaneously for each determination, without addition of DMAB to account for any interfering colours developed by components other than tryptophan present in the raw materials. Standard curve was constructed using DL-tryptophan. Sulphuric acid and sodium nitrite used were of A grade and others chemically pure. Absorption curves of the colour complex were determined on a Hilger UV spectrophotometer.

Results and Discussion

The final blue colour developed for measuring the amounts of tryptophan quantitatively is a two step reaction, the initial one being the reaction between the tryptophan present with added DMAB and the second one is the oxidation of the leuco-complex formed by the addition of soduim nitrate. Spies and Chambers[1,2] investigated the various molar ratios of nitrite to tryptophan necessary for complete and maximum colour development from the oxidation of DMAB-tryptophan complex, using pure tryptophan solution as well as proteins. The authors recommended that 0.1 ml of 0.05 per cent freshly prepared solution of sodium nitrite was adequate for maximum colour development for samples containing 50-100 μg of tryptophan. Excepting zein, which is devoid of tryptophan they had not investigated any vegetable protein. However, they demonstrated in model systems of mixtures of proteins with added carbohydrates or fructose as reducing substance, an interference in the amount of nitrite added needs to be correspondingly increased for reacting with extraneous reducing materials also ; optium conditions for the maximum colour development have therefore to be predetermined for any new material.

The tryptophan content of *toor dhal* has been investigated by various methods including that of Spies and Chambers and the values reported fall in the range of 0.2 to 0.9 g/16 g N. In Table 1 is given the value obtained by us following the general procedure wherein 0.1 ml of 0.05 per cent nitrite is added for the colour development. It will be seen that the value of 0.5 g/16 g N for the sample size of 50 mg analysed is in the expected range. When confirmation of this value is sought by working with half sample size (25 mg), the value obtained failed to agree, being 0.78 g/16 g N. Similarly, when tryptophan was added, the recovery was 100 per cent with 25 mg sample but with 50 mg sample the recovery was 85 per cent.

TABLE 1. RECOVERY OF TRYPTOPHAN (PER CENT) IN VARYING SAMPLE SIZES OF DHALS WITH THE ADDITION OF SODIUM NITRITE IN USUAL AMOUNTS *

Sample size (mg)	Tryptophan added (μg)	Tryptophan found (μg)	% Recovery of tryptophan
25	..	40	..
25	10	50	100
25	20	59	95
50	..	62	..
50	10	71	85
50	20	79	85

* Other conditions of the estimation were as described in methods.

TABLE 2. TRYPTOPHAN CONTENT ESTIMATED WITH THE ADDITION OF SODIUM NITRITE IN VARYING CONCENTRATIONS

Sample size (mg)	Klett readings* [% of $NaNO_2$ (0.1 ml) added]			
	0.05	0.1	0.2	0.3
25 ..	96	101	106	96
50 ..	150	190	188	187

* Average of at least three determinations.

In Table 2 are given the data on the determinations of tryptophan content using increasing amounts of sodium nitrite, *viz.*, 0.1 ml each of 0.05, 0.1, 0.2 and 0.3 per cent solutions added for the reaction. For purpose of easy comparison, Klett readings are given and it is seen that for maximum colour development, 0.1 ml of 0.05 per cent nitrite solution is not adequate. Further, there is no appreciable difference in the readings obtained using the three higher concentrations of nitrite solutions, although there is trend towards slight decrease with 0.3 per cent nitrite solution. This leads on to the problem of finding out how much increase in the addition of nitrite is desirable and whether the increase beyond a limit will affect the colour development.

In Table 3 are given the results obtained with the addition of the various amounts of nitrite using standard solutions of tryptophan. It will be observed the amount of colour developed decreases with the increasing amounts of nitrite. However, at each molar concentration of nitrite added, the tryptophan solutions follow the Beer-Lambert law. It would, therefore, mean that nitrite addition should be increased to the minimum amount necessary and also that standard curve needs to be made out using the same amount of sodium nitrite.

The other reaction conditions such as, ratio of DMAB to tryptophan, concentration of sulphuric acid, time of reaction for formation of tryptophan-DMAB complex were investigated by Spies and Chambers and the optimum conditions recommended by them have been followed excepting that the reaction was allowed

TABLE 3. EFFECT OF ADDITION OF SODIUM NITRITE ON OPTICAL DENSITY WITH STANDARD TRYPTOPHAN SOLUTIONS

$NaNO_2$ solution, conc %	Klett readings ml of 0.6 m M solution of tryptophan			
	.2 ml	.4 ml	.6 ml	.8 ml
.05 ..	62	126	181	252
.1 ..	50	115	175	230
.2 ..	50	112	168	225
.3 ..	50	112	165	225

TABLE 4. EFFECT OF REACTION TIME FOR FORMATION OF DMAB–TRYPTOPHAN COMPLEX ON THE FINAL OPTICAL DENSITY

Reaction time* hr	Klett readings ml of 0.6 m M solution of tryptophan			
	.2 ml	.4 ml	.6 ml	.8 ml
2 ..	62	126	181	252
18 ..	60	118	178	193

* Reaction time after adding 0.1 ml of 0.05% of sodium nitrate was 2 hr.

for 18 hr instead of 12 hr to facilitate working schedules.

Effect of various reaction times on colour development: It is reported that during the reaction period of 12 to 24 hr for the formation of DMAB complex starting with pure tryptophan solutions, a slight destruction was observed; this is confirmed by the data reported in Table 4. It is therefore necessary to restrict the reaction time to 2 hr for pure tryptophan solutions in constructing the standard curve.

Next, the time of reaction after the addition of the predetermined amounts of sodium nitrite was investigated. Half an hour was recommended as sufficient for complete oxidation but Miller[7] re-investigating the method in its application to determination of free tryptophan after hydrolysis has shown that 20 min reaction time is adequate. In Table 5 are given the values obtained using 30 min and 2 hr reaction time. Though there is no change in the readings obtained after either period, the two hour reaction time was adopted to ensure complete development.

Absorption curves of the coloured complex: The absorption curves of the coloured complex obtained using pure tryptophan (A, B) as well as *dhal* (C, D) are shown in Fig 1. It will be seen, that with increase in the nitrite concentration from 0.1 ml of 0.05 per cent (Curve C) to 0.1 ml of 0.2per cent (Curve D) the OD has increased from .165 to .32 without altering the absorption maximum of the complex at 590 mμ or the general characteristics of the absorption curve. Curves A and B for pure tryptophan solutions indicate

TABLE 5. EFFECT OF REACTION TIME FOR OXIDATION OF DMAB-TRYPTOPHAN COMPLEX ON THE FINAL COLOUR DEVELOPED

Reaction time	Klett readings ml of 0.6 m M solution of tryptophan			
	.2 ml	.4 ml	.6 ml	.8 ml
30 min ..	62	126	181	252
2 hr ..	62	126	181	252

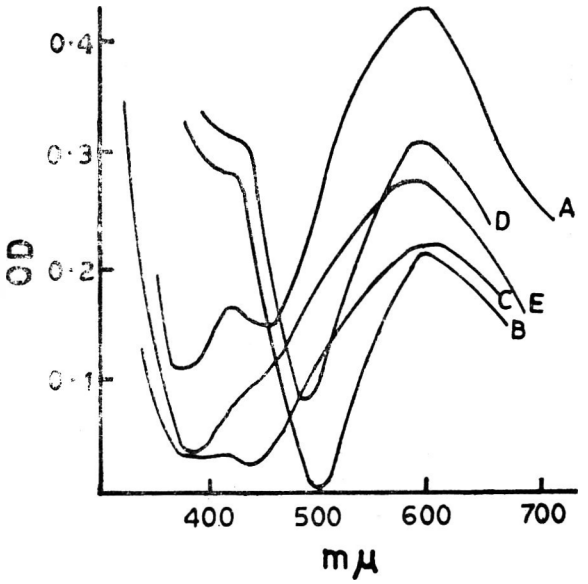

FIG 1. Absorption curves of Tryptophan-DMAB complex obtained with (1) using pure tryptophan 100 μg (A) and 50 μg (B) and adding minimum amount of Nitrite; (2) using 50 mg dhall and adding same amount of nitrite (C) and dhal with higher amount of nitrite (*viz.*, 0.1 ml of 0.2% solution) (D); and (3) using Casein and adding minimum amount of nitrite (E). Other details as in text.

similar trends. In the same figure are plotted the absorption curves for the complex obtained by using casein (E) as an example of isolated protein. It may be seen that in this case also the absorption maximum at 590 mμ is the characteristic of the complex.

Tryptophan content of various dhals: Table 6 (column 2) gives the values of the tryptophan contents of the pulses determined after incorporating the modifications discussed so far. *Toor dhal* gave a lower range of values (0.65–0.73) as compared to the others (1.08–1.55); black gram dhal gave the highest value of 1.40–1.55. For purpose of comparison the range of values compiled by Orr and Watt[4] as well as those accepted by the FAO are also indicated. It may be noted that the values for the tryptophan content as obtained by the present modified procedure are uniformly higher in all cases than the average values recommended by FAO. They are however within the range of values reported in the literature. It would appear that the inherent defects in the different methods adopted for tryptophan estimation may have contributed to low values.

No marked difference was seen in the tryptophan contents of the different quality of each of the pulses investigated. However, the tryptophan contents of the

TABLE 6. TRYPTOPHAN CONTENT OF THE PULSES FOLLOWING MODIFIED PROCEDURE ALONG WITH VALUES REPORTED IN LITERATURE

Pulse		Protein %	Tryptophan (g/16 g N) Found	Reported values
Toor dal	A ..	24.8	0.73	0.2–0.9
,,	B ..	24.9	0.65	0.5 (FAO [6])
,,	C ..	23.9	0.71	
Bengal gram	A ..	24.6	1.26	0.2–1.2
,,	B ..	22.6	1.17	0.8 (FAO [6])
,,	C ..	22.8	1.13	
Green gram	A ..	25.3	1.40	0.5–1.4
,,	B ..	23.0	1.39	0.8 (FAO [6])
,,	C ..	26.2	1.23	
(Fresh)	1 ..	27.0	1.11	
,,	2 ..	26.3	1.72	
,,	3 ..	25.3	1.08	
Black gram	A ..	26.1	1.44	0.3–1.3
,,	B ..	25.3	1.40	0.8 (FAO [6])
,,	C ..	23.4	1.55	
(Fresh)	1 ..	24.9	1.30	
,,	2 ..	23.9	4.31	
,,	3 ..	26.2	1.36	

Details of procedure given in text.

samples of the green gram and black gram(whole grains) were nearly in the same range as that of market samples.

Acknowledgement

The authors are grateful to Dr H. Nath, Director; for his guidance and to Dr J. V. Goud, Agricultural University, Bangalore, for the supply of freshly harvested samples.

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Studies on the Protein Content and Electrophoretic Analysis of Various Protein Fractions of High Yielding Varieties of Bajra *(Pennisetum typhoides)**

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Seven high yielding varieties of bajra, *viz.*, HB_1, HB_4, T-55, HB_3, S-530, A 1/3 and R-559, grown in northern part of India were analysed for total proteins and their fractions like albumin, globulin, prolamine and glutelin. Prolamins and glutelins were the major endosperm proteins accounting for about 50 to 60 per cent of the total proteins, whereas, albumin and globulin fractions, contributed about 13 and 10 per cent, respectively. Remarkable variations were observed in the relative proportions of various protein fractions in different varieties. In general, the amount of total proteins as well as of albumin, prolamine and glutelin was comparatively much higher and globulin content lower in hybrid varieties. Polyacrylamide gel electrophoretic patterns as revealed by various protein fractions of different varieties were also different at least in certain respects.

Bajra (*Pennisetum typhoides*) is an important millet crop in dry and semi-dry regions of northern and peninsular India and occupies about 12 per cent of the area under cereals. In recent years, considerable interest has been shown in breeding cereal grains with high protein content and good nutritional quality[1]. As shown earlier[2-6], the nutritional quality of cereal grains in terms of amino acid make up of their proteins is largely dependent upon the relative distribution of the various protein fractions like albumin, globulin prolamine and glutelin. The present investigation was, therefore, undertaken to study the distribution of protein fractions in some high yielding varieties of bajra. Electrophoretic analysis of various protein fractions of these varieties was also carried out. This knowledge could be of value in evolving new varieties having higher protein content with superior quality.

* The work forms a part of the thesis submitted by Sarla Popli to Haryana Agricultural University, Hissar, in partial fulfilment of the requirements for the M.Sc. degree.

Materials and Methods

Grain samples of high yielding varieties of bajra, *viz.*, HB_1, HB_4, T-55, HB_3, S-530, A 1/3 and R-559 obtained from the Bajra Breeder, Haryana Agricultural University, Hissar, were thoroughly cleaned, ground to pass through a 100 mesh sieve and preserved in air tight polythene bottles.

Total protein (N×6.25) was determined by AOAC methods[7]. The grain flour was defatted by shaking 20 g of the flour with 60 ml of n-butanol for 2 hr. After keeping overnight at room temperature, the solvent was removed by centrifugation and the powder was air dried. Buffer soluble proteins from this air dried powder were extracted by 0.05 M tris-buffer (pH 7.6) containing 5 mM of β-mercapto ethanol. Solubility fractionation of the proteins was done using a modification of the classical procedure of Osborne and Mendel[8], as employed by Sastry and Virupakasha[9] for fractionation of sorghum proteins. The solvents used for extraction of albumin, globulin, prolamine and glutelin protein fractions from defatted flour were, distilled water, 1 per cent sodium chloride solution, 60 per cent ethanol and 0.4 per cent sodium hydroxide solution, respectively. The protein content in these fractions was again determined by AOAC methods[7].

The polyacrylamide gel electrophoretic runs were carried out on freshly prepared protein fractions by following the procedure of Davis[10]. A suitable aliquot was layered on 7.5 per cent polyacrylamide gel and electrophoresis was done by using cationic buffer system (β-alanine acetic acid buffer, pH 4.5) in cold (4 °C) for 90 min at current of 5 mA per gel column. At the end of the run, which was indicated by the movement of the tracking dye (methyl green), the gels were removed and stained for 30 min in 1 per cent amido black in 7 per cent acetic acid (by vol.). The excess of stain was removed by repeated washings of the gels in 7 per cent acetic acid.

The mobility of protein bands are referred to that of methyl green taken as 1.

Results and Discussion

The data on the quantitative distribution of total proteins and various protein fractions, *viz.*, albumin, globulin, prolamine and glutelin in different varieties of bajra are included in Table 1. The protein content which ranged from 8.42 to 14.49 per cent was comparatively much higher in hybrid varieties. Similarly remarkable variations were noticed in the relative proportions of various protein fractions. The values for albumin, globulin, prolamine and glutelin varied from 1.10 (R-559) to 2.16 (HB_1), 1.02 (R-559) to 1.24 (S-530), 2.64 (S-530) to 5.10 (HB_4) and 1.75 (R-559) to 3.68 (HB_4) per cent, respectively. Albumin, prolamine and glutelin contents were comparatively higher and globulin content lower in hybrid varieties when compared to the other varieties. The data further demonstrate that prolamine and glutelin were the major proteins of the bajra endosperm accounting for about 50 to 60 per cent of the total proteins. Albumin and globulin fractions on an average contributed about 13 and 10 per cent, respectively.

TABLE 1. DISTRIBUTION OF PROTEINS IN DIFFERENT PROTEIN FRACTIONS

Variety	Total protein %	Albumin %	Globulin %	Prolamine %	Glutelin %
HB_1 ..	14.25	2.16 (15.2)	1.09 (7.7)	4.99 (35.0)	3.49 (24.5)
HB_4 ..	14.49	2.05 (14.2)	1.15 (7.9)	5.10 (35.2)	3.68 (25.4)
T-55 ..	11.30	1.45 (12.8)	1.21 (10.7)	3.24 (28.7)	2.92 (25.8)
HB_3 ..	13.78	2.10 (15.2)	1.12 (8.1)	4.60 (33.4)	3.42 (24.8)
S-530 ..	8.42	1.12 (13.3)	1.24 (14.7)	2.64 (31.4)	1.78 (21.1)
A 1/3 ..	10.34	1.20 (11.6)	1.14 (11.0)	2.80 (27.1)	2.95 (28.5)
R-559 ..	9.42	1.10 (11.7)	1.02 (10.8)	2.95 (31.3)	1.75 (18.6)

Figures in the parenthesis indicate protein in each fraction as per cent of total proteins.

Since it is a well established fact that albumin and globulin proteins of bajra contain higher concentrations of basic amino acids such as lysine, arginine and histidine, as well as of sulphur amino acids, cysteine and methionine, as compared to the other two fractions[11], the relative deficiency of lysine and sulphur amino acids in this millet as reported earlier[12-13] may, therefore, solely be due to the relative abundance of prolamine and glutelin fractions. Earlier studies on the quantitative distribution of various protein fractions in corn[14-15] and sorghum[6] have also shown a similar trend indicating thereby a similarity between the proteins of pearl millet and these cereals. However, striking differences in certain other respects may not be ruled out.

The electrophoretic patterns of the buffer soluble proteins and various protein fractions of different varieties represented in Figs 1–5 show remarkable variations in the number of bands observed in each case. The buffer soluble proteins separated into three groups of bands on the gels arbitrarily classified as slow moving bands having relative mobilities less thna

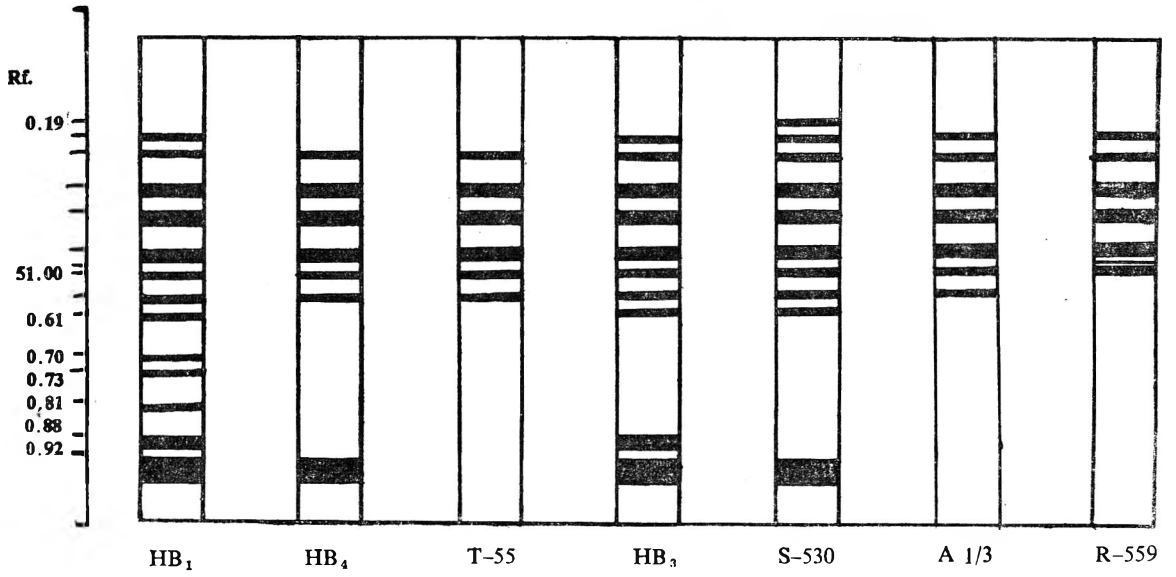

FIG 1. Electrophoretic patterns of buffer extracted proteins of different varieties.

0.50, bands with intermediate mobility (between 0.50 and 0.75) and fast moving bands with mobilities more than 0.75 (Fig 1). In slow moving region, four bands were common in all the varieties, whereas, a band with relative mobility 0.19 was detected only in S-530 and another band with mobility 0.22, was observed in HB_1, HB_3, S-530, A 1/3 and R-559 and was absent from HB_4 and T-55. Similarly in medium region one band was common in all the varieties. The band with mobility 0.51 was detected only in R-559. Another band with mobility corresponding to 0.61 could be detected only in HB_1, HB_3 and S-530. Similarly two more bands with mobilities 0.70 and 0.73 were observed only in HB_1. In fast moving region, no band could be observed in T-55, A 1/3 and R-559. A thick band with mobility 0.92 was present only in HB_1, HB_4, HB_3 and S-530. Protein band with relative mobility 0.88 was detected only in HB_1 and HB_3 and another band with mobility 0.81 could be observed only in HB_1.

Varietal differences were observed in electrophoretic patterns of albumin, globulin, prolamine and glutelin fractions also. The water soluble proteins (albumins) were separated into three distinct slow moving bands and two bands with intermediate mobility in all the varieties (Fig 2). However, one slow moving band with relative mobility 0.35 was observed only in T-55 and HB_3. Two fast moving bands with mobilities corresponding to 0.85 and 0.91 were detected only in hybrid varieties (HB_1, HB_3 and HB_4). The protein fraction extracted in sodium chloride solution (globulin) showed two bands with intermediate mobility and one slow

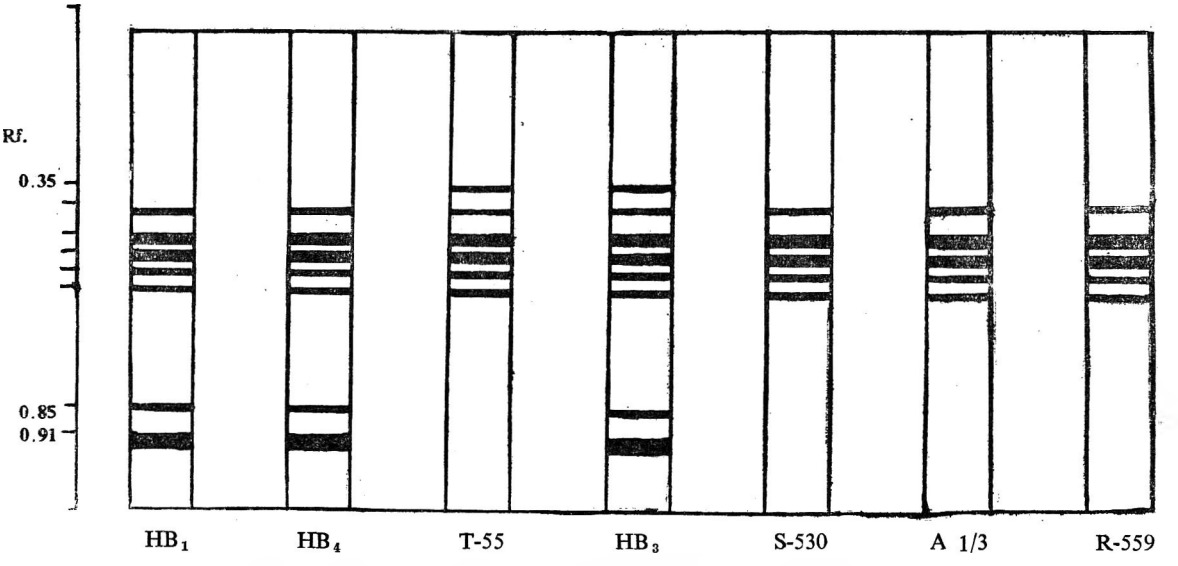

FIG 2. Electrophoretic patterns of albumins of different varieties.

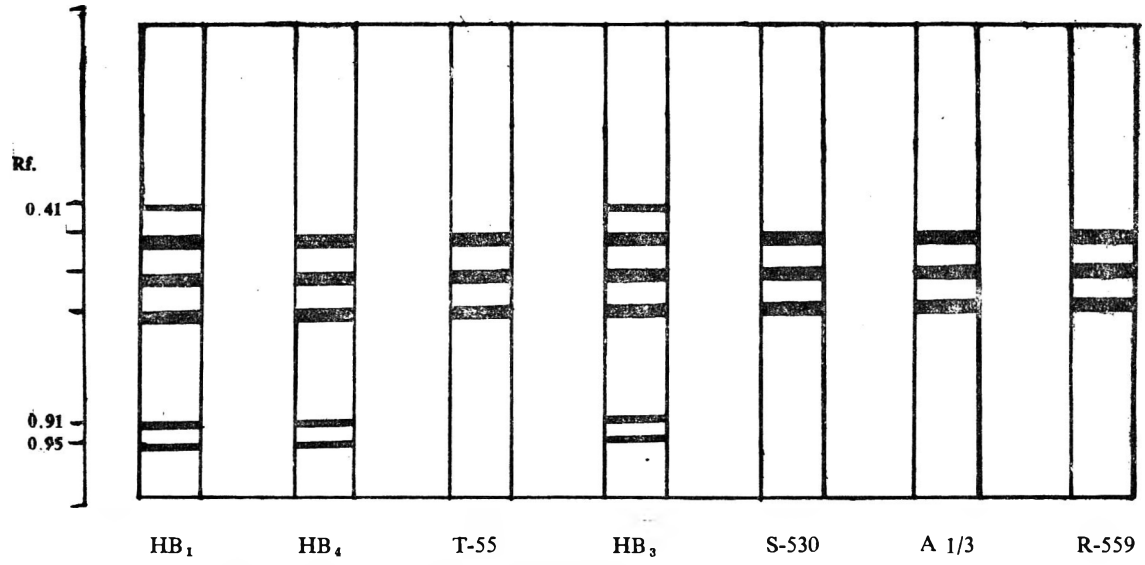

FIG 3. Electrophoretic patterns of globulins of different varieties.

moving band in all the varieties (Fig 3). However, a slow moving band with mobility 0.41 was detected only in HB_1 and HB_3 and two other fast moving bands with mobilities 0.91 and 0.95, respectively were observed in all the three hybrid varieties. The alcohol soluble proteins (prolamins) exhibited one slow moving band and two bands with intermediate mobility in all the seven varieties (Fig 4). Another slow moving band with mobility 0.33 was present in all the varieties except in A 1/3. Similarly, other two bands with mobilities 0.75 and 0.84, respectively were present only in hybrid varieties. Protein bands corresponding to mobilities 0.76 and 0.94 were detected only in HB_1. Glutelin fraction separated into three slow moving bands in HB_1 and HB_4 (Fig 5). In other varieties, the band with mobility 0.20 was missing.

The albumins and globulins seem to have certain components with similar mobilities in the cationic buffer system used in the present investigation. It was not clear whether the bands having same mobility represent the same components or different components with similar mobilities. Elton and Ewart[16] analysed wheat proteins electrophoretically in starch gels and suggested that fast moving components are due to globulins, the intermediate components to the albumins and slow moving components to gliadins. It is not very clear from the results reported here whether a similar relationship exists in the mobilities of various protein-fractions of bajra during the polyacrylamide gel electrophoresis.

Solubility fractionation of defatted bajra proteins and their quantitative estimation have indicated that

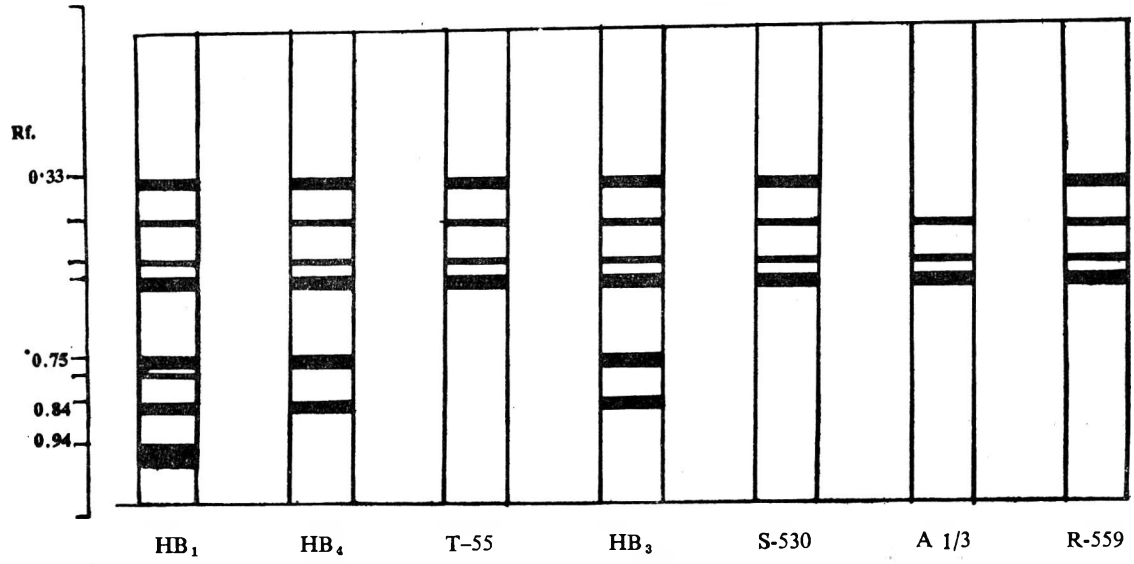

FIG 4. Electrophoretic patterns of prolamins of different varieties.

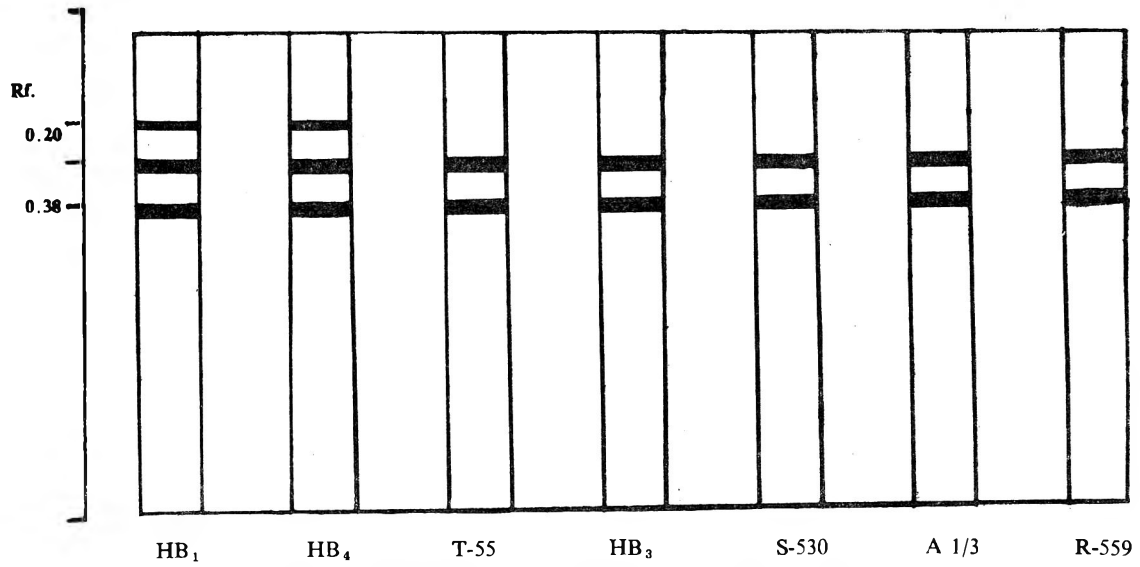

FIG 5. Electrophoretic pattern of glutelins of different varieties.

prolamines and glutelins are the major endosperm proteins. However, the results of electrophoretic analysis represented here indicate only a few bands in case of glutelin fraction. This indicates the poor resolution of these proteins in cationic buffer system which may be due to the poor mobility of these proteins. As quite a large number of buffers have been tried by different workers[16-18] in electrophoretic analysis of cereal proteins, there is every possibility of getting better resolution of these proteins in other buffer systems. Since this was the first attempt on electrophoretic analysis of bajra proteins, we could not go into further details. However, studies are in progress in this laboratory, where β-alanine acetic acid buffer (pH 4.5) will be replaced with buffers like tris-glycine (pH 8.3), glycine acetic acid (pH 4.6) and aluminium lactate-lactic acid (pH 3.1) and their effect on the resolutions of various protein fractions.

The results represented here show varietal difference in the protein make up of different bajra varieties. Earlier studies have also demonstrated the protein composition to be influenced by varieties of the same species[16-23]. The importance of varietal differences in electrophoretic patterns of various protein fractions of cereals is still controversial; some authors report it to be of little significance[18-19], others as very significant[17].

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Studies on the Stabilisation of Calcium Sensitive α_s-Casein by *k*-Casein Isolated from Fermented Milk in Making Curd

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Sialic acid content of whole caseins and *k*-casein decreased as the fermentation proceeded in making curd. The stability of calcium sensitive α_s-casein in *k*-casein (one of the major sialic acid containing milk protein) gradually decreased with the progress of fermentation. The *k*-casein can stabilise the α_s-casein better when both are isolated from same fermented milk than when *k*-casein from fermented milk and α_s-casein from fresh milk. Also *k*-casein from fermented milk was less susceptable to rennet action and there is less release of sialic acid compared to fresh *k*-casein.

Considerable amount of literature are available on the mechanism of rennet clotting of milk. Marrier *et al*[1] reported that proteose-peptone and *k*-casein are the only two major fractions containing sialic acid. They postulated that the stabilising power of *k*-casein preparations seem to be related to their sialic acid contents. Zittle[2] made an investigation on the stabilisation of calcium sensitive α_s-casein by *k*-casein. Interaction of α_s-casein with *k*-casein in a model system before and after freezing was also observed by El-Negoumy[3]. He showed that the stability of α_s-casein increased directly with the increase in *k*-casein to α_s-casein ratio in the presence of 0.02 M calcium. Addition of 0.2 per cent citrate along with calcium caused a 14 per cent increase in solubility of α_s-casein, whereas addition of citrate plus 16 per cent lactose increased it by 45 per cent. Again addition of 0.035 per cent phosphorus along with calcium resulted in 11 per cent loss in α_s-casein solubility. More drastic losses (about 18 per cent) resulted from the addition of 0.14 per cent chloride with calcium. Freezing of the above system caused a substantial loss in α_s-casein solubility except in case of those containing Ca+ citrate+lactose.

In the absence of calcium ions *k*-casein stabilised α_s-casein to an extent dependent upon the other ionic species present. Again Joshi *et al*[4] showed that cow milk *k*-casein has greater ability to stabilise cow and buffalo milk α_s-casein compared to buffalo milk *k*-casein. They have also showed that other sialic acid containing fraction proteose-peptone does not appear to stabilise α_s-casein.

The present knowledge on the mechanism of rennet clotting of milk revealed that rennet reduces the stabilising capacity of *k*-casein by releasing sialic acid in bound form, from the casein component as glycopeptide[5-8] and thereby converting *k*-casein to insoluble para *k*-casein. Proteolytic enzymes like pepsin, chymotrypsin are also capable of destroying the stabilizing property of *k*-casein[6,9]. Gupta *et al*[10] showed that trypsin can also release sialic acid both in bound and free form from casein. They differentiated a proteolytic enzyme from rennet by the fact that proteolytic enzyme will release more of sialic acid in free form than in bound form whereas a rennet type of enzyme will release sialic acid in bound form only.

But no information is available as regards the mechanism of the formation of curd by fermentation. Hence, it is worthwhile to study whether any change of *k*-casein is taking place with regard to its sialic acid content during fermentation as the inoculated culture (lactic acid bacteria) have also certain amount of proteolytic activity[10].

Materials and Methods

Fermentation of milk : Buffalo milk was fermented and curd was prepared by inoculating 5 per cent (v/v) inoculum having total acid ranging from 0.80 to 0.85 per cent (as lactic acid) using pure and active culture of *S. thermophilus* grown in sterile milk (5 psi for 30 min) and incubated at 40°C for various lengths of time as described before[11].

Whole casein : The method adopted for the preparation of sample was essentially that of Dunn[12]

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using isoelectric precipitation of casein from raw milk and fermented milk as described by Ganguli[13].

α_s-Casein : α_s-Casein was prepared following the method of Zittle *et al*[14].

k-Casein : *k*-Casein was prepared from whole casein according to the method of Zittle and Custer[15].

Determination of sialic acid: Warren's thiobarbituric acid method[16] was employed for the estimation of sialic acid.

Determination of sialic acid in casein : The procedure adopted was same as described by Marier, Tessier and Rose[1].

Assay system for stabilisation of α_s-casein by k-casein: For the stabilisation study assay system of Zittle[2] was followed with certain modifications as suggested by Joshi *et al*[4].

Estimation of protein: The protein estimation in assay tube was carried out by the method of Lowry *et al*[17] using Folin-Ciocalteu reageut.

Assay system for rennet action on k-*casein isolated from fermented milk and subsequent analysis of the released sialic acid :* Cow rennet (E. Merck) was used. The method followed was same as described by Gupta and Ganguli[8].

Results and Discussion

Sialic acid content of buffalo milk whole casein and *k*-casein isolated at different stages of fermentation are given in Tables 1 and 2.

TABLE 1. SIALIC ACID CONTENT OF FERMENTED BUFFALO MILK CASEIN

Fermentation time hr.	Sialic acid content mg/g casein	Total sialic acid released %
0 ..	2.98	..
3 ..	2.60	12.8
5 ..	1.59	46.6
6 ..	1.23	58.7
7 ..	0.98	67.1
8 ..	0.91	69.5

TABLE 2. SIALIC ACID CONTENT OF BUFFALO *k*-CASEIN ISOLATED FROM FERMENTED MILK

Fermentation time hr	Sialic acid content mg/g of *k*-casein	Total sialic acid released %
0 ..	11.89	..
2 ..	10.23	14.0
4 ..	7.28	38.8
6 ..	5.68	52.2
8 ..	4.32	63.7

The stabilisation studies were conducted with α_s-casein isolated from fresh milk and *k*-casein from fermented milk and also with α_s-casein and *k*-casein, both isolated from fermented milk and the results were expressed as per cent α_s-casein in solution and shown in Figs 1 and 2.

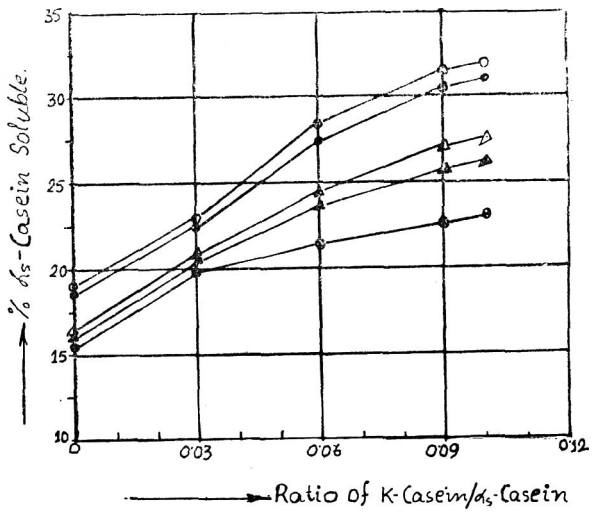

FIG 1. Stabilisation of α_s-casein by *k*-casein. α_s-Casein from fresh milk and *k*-casein from fermented milk.

-○- of fresh milk.
-●- of 2 hrs fermented milk
-△- of 4 hrs fermented milk.
-▲- of 6 hrs fermented milk
-⊗- of 8 hrs fermented milk.

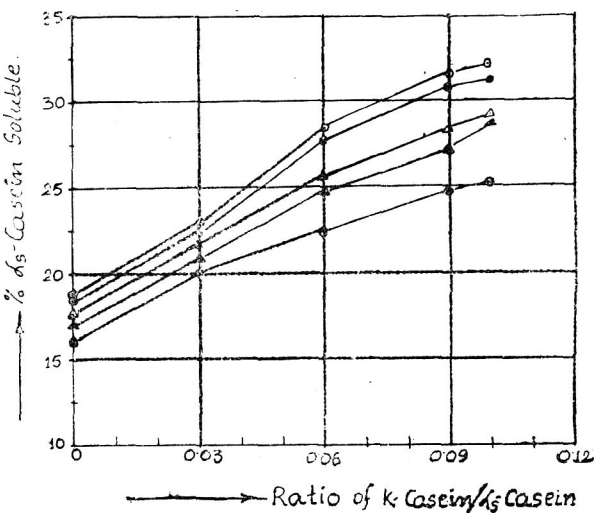

FIG 2. Stabilistaion of α^s-casein by *k*-casein when both α_s and *k*-caseins were isolated from same fermented milk.

-○- of fresh milk.
-●- of 2 hrs fermented milk
-△- of 4 hrs fermented milk
-▲- of 6 hrs fermented milk
-⊗- of 8 hrs fermented milk

The rate of release of sailic acid in the bound form as the glycopeptide from the buffalo milk *k*-casein isolated at different stage of fermentation when treated with cow rennet was also investigated. The results were expressed as per cent release of total sialic acid present in the respective casein samples and was shown in Fig 3.

From the results shown in Tables 1 and 2 it appears that there is release of sialic acid from casein with the progress of fermentation due to proteolytic activity of the inoculated culture. About 69.5 per cent of total sialic acid of whole casein and about 63.7 per cent of the total sialic acid of *k*-casein were released by the time (8 hr) curd gel is formed.

McKenzie[18,19] suggested that the casein micelles are spherical and that the *k*-casein exists mainly near the surface of the micelle and α- and β-caseins are protected from the precipitation by calcium that occurs in absence of *k*-casein. He also rejected the view of Waugh[20] that "the constituents of the micelles are in equilibrium with their counterparts in solution". It was also reported[21,22] that the *k*-casein interacts with the β-lactoglobulin during heating. Also Kannan *et al*[23] showed that in heated milk β-lactoglobulin can react with casein micelles sufficiently to delay clotting of the milk by rennin and the maximum stability of milk to heat was usually in the pH range of 6.6–6.75.

Keeping the above observations in mind it can thus be concluded that due to removal of sialic acid from *k*-casein during fermentation it gradually loses its stabilising power and at certain acid concentration it cannot protect other casein fractions to precipitate out in presence of metal ions such as calcium. Also curd is usually prepared from boiled milk and the temperature is sufficient to cause interactions between *k*-casein and β-lactoglobulin making other casein fractions very susceptable to micelle formation.

From the results (Fig 2) it appears also that the *k*-casein can stabilise the α_s-casein better when both were isolated from the same fermented milk than when *k*-casein from fermented milk and α_s-casein from fresh milk. These findings obviously indicate that not only the *k*-casein but also α_s-casein have undergone some change during fermentation. Thus unlike rennet destabilisation here in cultured acid milk both α- and *k*-casein take important part in the formation of curd gel.

The changes of *k*-casein (Fig 3) during fermentation are further established by studying the action of cow-rennet on fresh *k*-casein and *k*-casein of fermented milk as regards the rate of release of sialic acid in bound form as microglycopeptide. It is observed that there is less release of glycopeptide from *k*-casein of fermented milk than when it is from fresh milk. This proves that the *k*-casein from fermented milk is less susceptable to rennet action. This might be due to the proteolytic action and denaturation during fermentation, the bound sialic acid still remained with *k*-casein is not open for the rennet action.

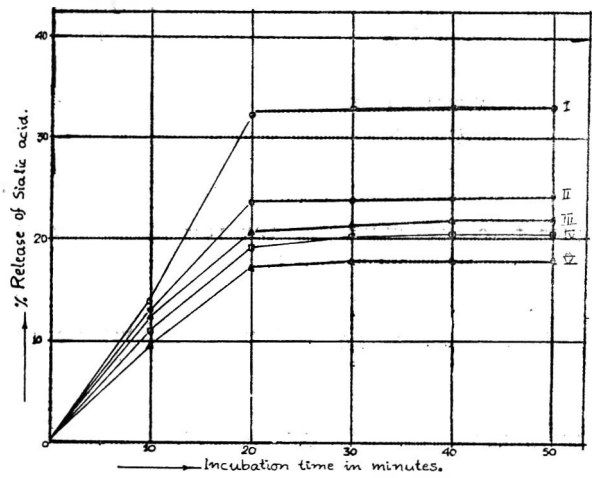

FIG 3. Rate of release of micro-glycopeptide by rennet from buffalo *k*-casein isolated from fermented milk in making curd.

I -○- fresh milk K-Casein
II -●- 2 hrs fermented milk K-Casein
III -▲- 4 hrs fermented milk K-Casein
IV -□- 6 hrs fermented milk K-Casein
V -▲- 8 hrs fermented milk K-Casein

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Effect of Thermal Oxidation on the Fatty Acid Composition of Ghee

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Analysis of component fatty acids of heated cow and buffalo ghee by gas-liquid chromatography showed that there was a decrease in 10 : 1, 14 : 1, 16 : 1, 18 : 1, 18 : 2 and 18 : 3 and an increase in 14 : 0, 16 : 0 and 18 : 0 fatty acids.

Spectrophotometric estimations by alkali isomerization technique showed that there were significant decreases in the poly-unsaturated fatty acids, *viz.*, dienoic, trienoic, tetraenoic and pentaenoic acids. The losses in the polyunsaturated fatty acids depended upon the degree of unsaturation, higher the unsaturation greater being the loss.

Ghee heated at high temperatures, as is done in cooking and frying operations, can undergo considerable changes in its fatty acid composition. Studies have been made on the changes in fatty acid composition of different vegetable oils and fats during heating at high temperatures[1-5]. Very little information is available about the changes taking place in the fatty acid composition of ghee under similar conditions[6]. Therefore, a systematic study was carried out to know the changes taking place in the fatty acid composition of ghee during its heat-treatment at high temperatures and the results obtained are presented here.

Materials and Methods

Cow and buffalo ghee samples were prepared by the creamery-butter method from cow and buffalo milk respectively. Two hundred grams of each ghee sample were taken in one litre capacity stainless steel beaker and separately heated and maintained at 150, 200 and 225°C for 2 hr. These heated ghee samples were analysed for fatty acid composition by gas-liquid chromatography (GLC) and poly-unsaturated fatty acids by the alkali-isomerization method of Herb and Riemenschneider[7] as adopted by AOCS[8] using Carl-Zeiss spectrophotometer. Fatty acid methyl esters of ghee were prepared by the method of deMan[9]. The GLC analysis was carried out using an F and M model 609, hydrogen flame ionization gas chromatograph. The 5 ft×3/16 in column was packed with 20 per cent diethylene glycol-succinate (Lac 728 F and M Corporation) on Diatoport-P (F and M Corporation). Three μl of the methyl ester mixture was injected at a column temperature of 80°C. After 3 min run, the column temperature was raised at the rate of 18°C/min to 195°C. The temperature of both injection port and detector were 250°C. The rate of flow of carrier gas, nitrogen was 70 ml/min. The peaks were measured by triangulation. It was assumed that the area under each peak was proportional to the weight of the corresponding fatty acid methyl ester.

Results and Discussion

Three samples each of cow and buffalo ghee in duplicate were studied and average values are presented here. The effect of thermal oxidation on the major fatty acids of cow and buffalo ghee at 150, 200 and 225°C for 2 hr is shown in Table 1. This Table indicates that in fresh ghee the initial fatty acid composition of buffalo ghee was found to differ from that of cow ghee. The buffalo ghee was higher in 4:0 and

TABLE 1. EFFECT OF THERMAL OXIDATION AT DIFFERENT TEMPERATURES ON THE FATTY ACID COMPOSITION OF COW AND BUFFALO GHEE

Fatty* acid	Fatty acids (%) at indicated temperatures of heating							
	Cow ghee				Buffalo ghee			
	Control	150°C	200°C	225°C	Control	150°C	200°C	225°C
4 : 0 ..	3.2	3.2	3.3	3.2	4.2	4.3	4.3	4.3
6 : 0 ..	2.2	2.2	2.2	2.2	1.7	1.7	1.7	1.7
8 : 0 ..	1.3	1.3	1.3	1.3	1.1	1.1	1.1	1.1
10 : 0 ..	2.7	2.7	2.8	2.8	1.9	1.9	1.9	1.9
10 : 1 ..	0.3	0.2	0.2	0.2	—	—	—	—
12 : 0 ..	2.9	2.9	2.9	2.9	1.8	1.7	1.8	1.7
14 : 0 ..	11.9	12.1	12.2	12.3	10.8	10.8	10.8	10.9
14 : 1 ..	1.8	1.7	1.6	1.5	1.1	1.1	1.0	1.0
15 : 0 ..	1.2	1.2	1.2	1.2	1.1	1.1	1.1	1.1
16 : 0 ..	28.9	29.0	29.3	29.4	32.5	32.8	32.9	33.8
16 : 1 ..	1.9	1.8	1.7	1.7	2.1	2.0	1.9	1.8
17 : 0 ..	0.2	0.2	0.2	0.2	0.6	0.6	0.6	0.6
18 : 0 ..	11.3	12.3	12.5	12.7	12.5	13.3	14.4	14.3
18 : 1 ..	27.3	26.5	26.1	26.0	25.8	25.0	24.4	23.8
18 : 2 ..	2.5	2.3	2.0	1.9	2.6	1.9	1.6	1.6
18 : 3 ..	0.7	0.5	0.4	0.3	0.5	0.4	0.3	0.3

* The first number refers to total carbons and second to the number of double bonds in the fatty acid.

lower in 6:0 to 12:0 fatty acids than those in cow ghee. Another difference between the two species was observed in the case of 10:1 fatty acid. It was not detectable in buffalo ghee, whereas it was found to the extent of about 0.3 per cent in cow ghee. Similar differences in the major fatty acid composition of cow and buffalo milk fats have also been reported by Rama Murthy and Narayanan[10].

On thermal oxidation of both cow and buffalo ghee (Table 1), there was no appreciable effect on the lower saturated fatty acids (4:0 to 12:0). However the unsaturated fatty acids such as 10:1, 14:1, 16:1, 18:1, 18:2 and 18:3 were found to decrease and an increase in the corresponding saturated fatty acids was observed. In general higher the temperature and unsaturation in the fatty acid greater was the reduction in the fatty acid. The average percentage decreases from the initial value in 18:1, 18:2 and 18:3 acids varied from 2.7 to 7.4, 8.3 to 38.6 and 23.1 to 49.7 respectively irrespective of the two species. The corresponding percentage increase in the 18:0 acid varied from 7.1 to 15.1. Similarly, slight decreases were also noted in 14:1 (4.3 to 17.9 per cent) and 16:1 (2.0 to 10.7 per cent) and increases in 14:0 (1.6 to 9.3 per cent) and 16:0 (0.4 to 4.0 per cent) acids. During thermal oxidation of ghee, its unsaturated fatty acids may get oxidised and result in the decrease of these fatty acids.

The effect of thermal oxidation on the poly-unsaturated fatty acids of cow and buffalo ghee showed that in ghee samples of both the species, conjugated and non-conjugated types of dienoic, trienoic, tetraenoic and pentaenoic acids were detected and estimated. All the ghee samples used for the studies were also analysed for phospholipid content. These ghee samples contained only traces of phospholipids (4–6 mg/100 g of ghee).

The initial values showed that among the poly-unsaturated fatty acids, in both cow and buffalo ghee the dienoic constitued the maximum (72-75 per cent), followed by trienoic (18–21 per cent), tetraenoic (3–4 per cent) and pentaenoic (2–3 per cent) acids. In all the polyunsaturated acids non-conjugated acids were higher than the conjugated ones of the corresponding acids. In ghee samples of both the species, the bulk of trienoic, tetraenoic and pentaenoic acids were in the non-conjugated form. Similar results in poly-unsaturated fatty acids of milk fats from Indian cows and buffaloes have also been reported by Rama Murthy and Narayanan[11]. However, they could not detect the presence of conjugated pentaenoic acid in any of the cow milk fats.

Heating of ghee for 2 hr at the various temperatures studied showed that there were considerable decreases in all the acids. Generally, higher the temperature

and unsaturation in the fatty acid, greater was the effect. However, within the limited number of samples studied no definite trend in the changes of fatty acids with regard to species and form of fatty acids could be established. The average percentage decreases in dienoic, trienoic, tetraenoic and pentaenoic fatty acids from the initial value, irrespective of the species, varied from 22.4 to 37.9, 22.6 to 44.0, 18.6 to 61.8 and 37.0 to 70.0 per cent respectively. The decrease noticed in dienoic and pentaenoic polyunsaturated fatty acids during thermal oxidation is also, in general, agreed with the results obtained in the fatty acid composition by gas-liquid chromatography (Table 1). It can be concluded, therefore, that considerable decreases in the polyunsaturated fatty acids are taking place during thermal oxidation and this may affect its nutritional properties.

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Interaction of Malonaldehyde in Foods. II. Its Relative Reactivity towards Amino Acids

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Relative reactivity of malonaldehyde with amino acids at pH 2.8, 4.2 and 7.0 has been studied by measuring the percentage decrease in primary amino groups and the absorbance at 390 mμ. Basic amino acids and cysteine have been found to react faster than acidic amino acids. Various amino acids have been found to have maximum reaction rates between pH 4.0 to 4.6. Fluorescent derivatives of various amino acids have been separated by paper chromatography and their Rf values in three solvent systems are reported.

Malonaldehyde (MA) originates from the oxidative degradation of polyunsaturated fatty acids and its concentration in foods like meat, fish, poultry and dairy products has been widely used as a measure of lipid peroxidation and off-flavour development during storage[1-3]. However, in a complex food system, the relationship between lipid peroxidation and accumulation of malon aldehyde is not simple as MA is known to interact with other food constituents like proteins[4,5], amino acids[6,7], phospholipids[8] and nucleic acids[9,10]. Although the exact mode of binding of MA in foods is not well established[11], in aqueous systems it has been shown to react with the free amino groups of proteins and amino acids forming an enamine, which on further condensation with another amino group forms imines having N, N'- disubstituted-l-amino-3-imino-propene linkage[7]. In a particular food system, extent of MA interaction with different food constituents will depend upon their relative reactivity, concentrations, pH and temperature. Studies have been initiated in this laboratory about the relative reactivity of malonaldehyde and other carbonyls with nitrogenous constituents of foods and their role in taste and flavour of foodstuffs. The present paper describes the relative reactivity of malonaldehyde with amino acids at different hydrogen ion concentrations.

Materials and Methods

Reaction conditions : Malonaldehyde was prepared by mixing of 1, 1, 3, 3-tetraethoxypropane (0.66 g) with

0.01N hydrochloric acid (100 ml) and heating the mixture in a stoppered flask at 50°C for two hours. Amino acid-malonaldehyde reaction was carried out by treating hydrolysed tetraethoxypropane solution (50 ml) with the respective amino acid (0.002 moles) in 0.2 M citric acid-disodium hydrogen phosphate buffer (50 ml) at pH 2.5, 4.2 and 7.0 respectively. The reaction mixtures were incubated at 40±1°C. Both malonaldehyde and amino acid blanks were run along with the reaction mixtures at each hydrogen ion concentration. For studying relationship between pH and reaction velocity, the reactions were also carried out in buffer solutions having pH ranging from 3.4 to 5.4 for 2 hr at 40°C. The relative rates of reaction of malonaldehyde with different amino acids were measured by determining the concentration of primary amino group and the absorbance at 390 mμ of the reaction mixtures against malonaldehyde blank. The fluorescence intensities of the reaction mixtures were measured in Klett fluorimeter using 5970 and 3389 filters for lamp and photocells respectively. Instrument was calibrated using quinine-sulphate as reference standard. Primary amino groups were determined by the trinitrobenzene-sulphonic acid method of Satake *et al*[12].

Chromatographic separation and absorption spectra of reaction products: The reaction products from the above reaction mixtures were separated by employing both ascending and circular paper chromatography using Whatmann No. 1 filter paper and n-butanol : acetic acid : water (12:3:5) ; isobutanol : acetic acid : water (27:3:75) and ethyl acetate : n-butanol : water (1:1:1) as irrigating solvents. After development, the chromatograms were dried at room temperature and seen under UV light (254 mμ) in a chromatoscan cabinet. The bands which gave intense fluorescence were accurately marked, cut into small pieces and extracted with distilled water. The absorbance of the water extracts were measured in a Perkin-Elmer model 124 UV spectro photometer against distilled water blank from 200–450 mμ.

Results and Discussion

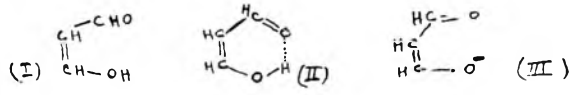
The reaction of malonaldehyde with amino acids has been reported to result in the formation of an enamine which further condenses with another molecule of amino acid producing a simple fluorochrome structure, the 1-amino-3-imino-propene system (R.N H-CH=CH–CH=NR) having an absorption maxima near 390 mμ

Since, in both these reactions primary amino groups are involved, the overall reaction velocity can be measured by determining the concentration of primary amino groups and the concentrations of fluorochrome linkage by measuring the absorbance at 390 mμ of the malonaldehyde-amino acid reaction mixtures, which has been followed in the present study. The relative reactivities of malonaldehyde with different amino acids expressed as percentage decrease in primary amino groups after 30 min., and 1, 2, 4 and 24 hr at pH 2.8, 4.2 and 7.0 are presented in Table 1. It is seen that overall reaction of primary amino groups of different amino acids with malonaldehyde is fastest in case of cysteine and slowest in aspartic, glutamic and

TABLE 1. PERCENTAGE DECREASE IN AMINO GROUPS IN AMINO ACID—MALONALDEHYDE REACTION MIXTURE

Amino acids	Reaction period														
	30 min			1 hr			2 hr			4 hr			24 hr		
pH	2.8	4.2	7.0	2.8	4.2	7.0	2.8	4.2	7.0	2.8	4.2	7.0	2.8	4.2	7.0
Glycine ..	7.0	13.7	7.5	14.3	19.1	16.7	23.4	31.7	20.9	25.8	47.6	25.0	39.1	64.2	43.3
Alanine ..	7.5	11.7	5.0	15.0	15.0	11.7	16.7	25.0	16.7	30.0	40.0	25.0	45.0	56.7	40.7
Phenyl alanine ..	5.0	21.7	6.7	10.0	33.3	13.3	16.7	43.3	20.0	35.0	63.3	36.7	50.0	76.7	63.3
Valine ..	5.0	10.0	5.0	8.3	16.7	8.3	12.5	33.3	14.2	23.3	49.2	24.2	40.0	60.2	40.0
Leucine ..	2.5	11.7	11.7	5.0	21.7	16.7	8.3	30.8	21.7	16.7	43.3	35.0	33.3	58.3	53.3
Serine ..	8.3	25.0	8.0	14.2	33.3	10.7	20.0	37.5	17.0	30.0	49.2	21.8	43.3	60.1	33.3
Threonine ..	5.6	21.7	5.6	8.9	25.6	8.9	16.7	33.3	17.8	25.6	50.0	24.4	44.4	64.5	40.0
Aspartic acid ..	2.4	6.0	1.2	6.0	23.8	2.4	9.5	26.2	6.0	16.7	32.2	15.5	28.6	44.1	31.0
Glutamic acid ..	4.0	9.6	2.2	11.1	23.4	4.4	13.9	33.0	8.9	18.9	38.3	15.5	31.1	50.0	32.2
Histidine ..	13.3	43.3	20.0	21.7	51.7	26.7	33.3	63.3	35.0	46.7	66.7	56.7	60.0	73.3	66.7
Arginine ..	9.2	13.3	10.0	20.8	36.7	13.3	23.3	57.5	23.3	33.4	66.7	30.0	49.8	73.3	56.7
Methionine ..	6.7	16.7	6.7	10.0	23.3	10.0	16.7	43.3	16.7	26.7	55.0	30.8	43.3	66.7	65.0
Cysteine ..	56.7	62.5	64.5	67.7	73.4	79.0	73.3	77.4	82.2	80.0	83.0	92.0	80.0	86.3	92.0
Cysteic acid ..	3.3	14.2	3.3	6.6	21.7	6.7	13.3	26.7	13.3	23.3	33.3	23.3	40.0	53.3	40.0
Glutathione ..	8.1	13.5	11.5	13.6	22.1	27.3	18.1	29.7	52.9	27.0	40.6	59.4	35.1	46.0	61.7

cysteic acids. Basic amino acids like histidine and arginine reacted much faster than neutral and acidic amino acids indicating that additional -NH or $-NH_2$ groups increase the reactivity of amino acids while additional -COOH or $-SO_3H$ groups decrease their reactivity towards malonaldehyde. Lower pK' (NH_3^+) values[13] of histidine (8.97) and arginine (9.09) compared to glutamic acid (9.67) and aspartic acid (9.60) may be responsible for their increased reactivity because the proportions of unionised-NH_2 groups responsible for nucleophilic attack will be higher in case of histidine and arginine than in case of aspartic acid and glutamic acid under the reaction conditions employed in the present study. Histidine has also been found previously to undergo maximum degradative changes in peroxidising system[14]. It may also be seen that except in case of cysteine, percentage decrease in amino group was more at pH 4.2 than at pH 2.8 and 7.0 indicating the pH dependancy of the rate determining step in amino acid-malonaldehyde reaction. The reaction of amino groups with malonaldehyde registers a maxima in the pH range of 4.0 to 4.6 and then commences to decrease as the hydrogen ion concentration is changed from pH 3.4 to 5.4 (Fig 1). Crawford *et al*[6] have also reported the maximum reaction rates at pH 4.2 in glycine-malonaldehyde reaction. These workers have explained that protonated enolic form of malonaldehyde (I) will be most susceptible to nucleophilic attack of amino group of glycine as malonaldehyde in cyclic chelated enol (II) or enolate ion form (III) would tend to stabilize the enolic hydroxyl group and make it difficult to displace by nucleophilic attack.



However, at pH below 4.5, malonaldehyde has been reported to exist mainly in the cyclic chelated enol form[15] and therefore reaction maxima at pH 4.2 may not be only due to the higher susceptibility of protonated enol towards nucleophilic addition of amino group. The reaction of carbonyls with amino compounds has been reported to proceed *via* two step mechanism, nucleophilic addition of amino group and the removal of water[16]. In neutral and mildly acidic solutions probably the nucleophilic addition of amino group to malonaldehyde is fast and the rate determining step is removal of water molecule. But as the acidity is increased dehydration becomes very fast and the nucleophilic addition step becomes the rate limiting. This along with the appreciable hydrolysis rates of imines and enamines in strongly acidic solutions may also be responsible for the reaction maxima in the pH-reaction rate profile.

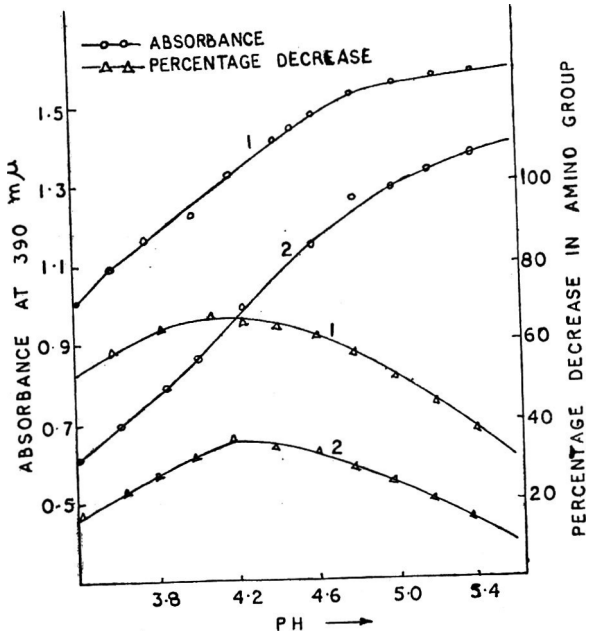


FIG 1. Effect of pH on the reaction of malonaldehyde with amino acids: 1, Histidine; 2, Methionine.

The changes in the absorbance of amino acid-malonaldehyde reaction mixtures at 390 mμ and fluorescence intensity which are the measure of N, N'-disubstituted-1-amino-3-imino-propene linkage produced by the condensation of two molecules of amino acids with one molecule of malonaldehyde are shown in Tables 2 and 3. Among the amino acids studied, histidine, arginine, lysine and phenyl alanine gave comparatively higher flourescence intensity and absorbance at 390 mμ. It may also be seen (Fig 1) that even though maximum decrease in the amino groups were near pH 4.2, the fluorescence intensity and absorbance at 390 mμ were higher at pH 7 after 2 hr of reaction at 40°C. However, during initial reaction periods (30 min and 1 hr) the absorbance at 390 mμ were comparatively lower at pH 7 than at pH 4.2. This indicates that even though the formation of fluorescent derivatives is faster at pH 4.2, their instability in strongly acidic solutions leads to the overall decreased reaction rates. In the cysteine-malonaldehyde reaction mixture, though the absorbance at 390 mμ was very high after 30 min at 40°C, the fluorescence intensity was very low and the reaction mixtures did not show absorbance maxima at 390 mμ indicating the negligible amounts of 1-amino-3-imino-propene linkages in the reaction products. Both cysteic acid and glutathione (reduced) gave appreciable

TABLE 2. ABSORBANCE AT 390 mμ OF AMINO ACID–MALONALDEHYDE REACTION MIXTURES

Amino acid	Reaction period: 30 min			1 hr			2 hr			4 hr			24 hr		
pH	2.8	4.2	7.0	2.8	4.2	7.0	2.8	4.2	7.0	2.8	4.2	7.0	2.8	4.2	7.0
Glycine	0.23	0.335	0.24	0.375	0.475	0.29	1.0	0.805	1.01	1.6	1.22	2.1	11.0	12.1	13.4
Alanine	0.03	0.09	0.16	0.09	0.215	0.205	0.3	0.635	0.36	0.815	1.26	1.59	6.6	10.6	13.5
Phenyl alanine	..	0.355	0.57	..	0.59	0.57	..	1.1	0.91	..	1.34	2.1	..	9.5	14.4
Valine	0.09	0.19	0.53	0.165	0.36	0.535	0.475	0.65	0.69	1.06	1.09	1.68	6.65	9.35	14.5
Leucine	0.085	0.165	0.435	0.155	0.36	0.465	0.475	0.755	1.05	0.99	1.16	2.1	8.3	9.7	15.2
Serine	0.17	0.183	0.27	0.28	0.365	0.31	0.59	0.68	0.84	1.17	1.06	2.02	8.5	10.7	13.8
Threonine	0.195	0.23	0.385	0.31	0.41	0.385	0.615	0.81	0.735	0.995	1.08	2.05	6.7	6.75	13.4
Aspartic acid	0.20	0.13	0.225	0.345	0.265	0.245	0.75	0.66	0.64	1.4	1.07	2.0	7.5	11.8	14.2
Glutamic acid	0.22	0.27	0.37	0.35	0.555	0.405	0.65	1.13	0.635	1.23	1.47	1.82	6.35	11.7	12.6
Histidine	0.68	1.05	0.395	0.925	1.2	0.52	1.29	1.36	1.77	1.74	1.45	4.11	10.1	0.75	11.4
Arginine	0.34	0.4	0.265	0.55	0.68	0.34	1.10	1.12	0.6	1.82	1.44	1.88	8.7	9.1	12.0
Methionine	0.29	0.635	0.42	0.435	0.92	0.535	0.83	1.28	1.19	1.54	1.34	1.67	7.5	10.4	14.1
Cysteine	1.64	1.19	0.355	1.94	1.39	0.575	1.94	1.41	0.84	1.76	1.19	0.8	1.33	0.9	0.69
Cysteic acid	0.12	0.135	0.37	0.235	0.275	0.41	0.51	0.565	0.94	1.02	1.06	2.08	4.8	9.0	13.6
Glutathione	0.395	0.34	0.605	0.52	0.55	0.88	0.82	0.96	1.16	1.32	1.14	1.34	5.55	7.45	2.4

TABLE 3. FLUORESCENCE INTENSITY OF AMINO ACID–MALONALDEHYDE REACTION MIXTURES

Amino acids	30 min			1 hr			3 hr			4 hr			24 hr		
pH	2.8	4.2	7.0	2.8	4.2	7.0	2.8	4.2	7.0	2.8	4.2	7.0	2.8	4.2	7.0
Glycine	63	62	6	65	68	34	96	235	132	208	135	173	164×10^2	326×10^2	408×10^2
Alanine	35	20	2	35	39	13	67	95	85	110	95	84	113×10^2	210×10^2	312×10^2
Phenyl alanine	..	38	2	..	60	19	..	94	105	..	98	105	..	176×10^2	348×10^2
Valine	38	16	2	38	39	7	82	80	67	113	81	89	101×10^2	230×10^2	404×10^2
Leucine	20	24	3	38	55	14	78	101	147	122	104	151	130×10^2	230×10^2	424×10^2
Serine	31	30	4	46	52	20	83	95	143	131	95	145	118×10^2	220×10^2	572×10^2
Threonine	34	28	4	45	51	19	69	92	122	107	92	127	105×10^2	200×10^2	440×10^2
Aspartic acid	35	28	3	44	40	22	97	88	122	139	90	124	125×10^2	250×10^2	512×10^2
Glutamic acid	38	37	3	50	68	19	85	117	122	108	118	124	141×10^2	242×10^2	448×10^2
Histidine	71	79	6	83	85	37	109	124	92	155	124	135	141×10^2	272×10^2	336×10^2
Arginine	48	36	6	50	57	14	103	104	136	137	106	136	143×10^2	222×10^2	452×10^2
Methionine	28	46	0	34	47	5	80	83	93	86	94	108	68×10^2	98×10^2	170×10^2
Cysteine	0	0	0	2	0	0	8	0	0	3	0	0	300	0	0
Cysteic acid	38	..	..	42	..	..	78	..	..	119	..	..	93×10^2	..	..
Glutathione	21	38	0	30	41	0	81	95	46	91	95	66	42×10^2	88×10^2	20×10^2

fluorescence intensity on treatment with malonaldehyde and the reaction mixtures had absorption maxima near 390 mμ. In cysteine the reaction of both primary amino and sulphydryl groups was very fast but in glutathione there was slow decrease in their concentration. Since the amino groups of cysteic acid also reacted very slowly compared to that cysteine, the amino and sulphydryl groups in cysteine somehow influence their reactivity towards malonaldehyde. The same effect has been observed previously by Shin *et al*[17] who observed that reaction of -SH group in N-acetyl cysteine was very slow compared to that in cysteine.

The R_f values of the fluorescent derivatives of various amino acids in the solvent systems are given in Table 4. The fluorescent derivatives had higher R_f values than the parent amino acid and none of them gave colour with ninhydrin. These fluorescent compounds had absorption maxima in the vicinity of 240, 260 and 390 mμ indicating N=C-C=C-N chromophoric linkage. However, fluorescent derivative of cysteine had absorption maxima at 325 mμ and also migrated slower than cysteine on paper chromatogram in the solvent systems employed.

It is therefore evident from the data presented above that relative reactivity of malonaldehyde with various amino acids depends upon the nature of the amino acid and the hydrogen ion concentration of the reaction system. Basic amino acids and cysteine react faster than acidic amino acids. All the amino acids react with malonaldehyde forming fluorescent deriva-

TABLE 4. RF VALUES OF THE FLUORESCENT DERIVATIVES OF AMINO ACIDS

Amino acid	R_f value		
	1	2	3
Glycine ..	0.77	0.78	0.34
Alanine ..	0.82	0.88	0.40
Phenyl alanine ..	0.88	..	0.32
Valicne	0.88	0.95	0.73
Leuine ..	0.95	0.96	0.85
Serine ..	0.72	0.69	0.29
Threonine ..	0.79	0.81	0.38
Aspartic acid ..	0.71	0.70	0.21
Gutamic acid ..	0.77	0.81	0.31
Hlstidine ..	0.50	0.37	0.16
Ariginine ..	0.60	0.51	0.17
Methionine ..	0.86	0.93	0.60
Csteine ..	0.37	0.31	0.24
Lyysine ..	0.52	0.40	0.26

1. n-Butanol : acetic acid : water in 12 : 3 : 5.
2. Isobutanol : acetic acid : water in 27 : 3 : 7.
3. Ethyl acetate : n-butanol : water in 1 : 1 : 1.

tives having N, N'-1-amino-3-imino-propene chromophoric linkage.

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Examination of Oil-soluble Colours from Foods by Solvent Partitioning and Chromatography

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Fats and oils interfere in the detection of oil-soluble colours in foods by chromatography. The methods of separating fats and oils from colours usually practised are either by alcoholic potash digestion or by silica gel adsorption: both sometimes pose problems in extracting the colours fully. In this communication fats and oils and other interfering substances are removed from the colours by solvent partition technique using dimethyl formamide and hexane (3:1), followed by alumina adsorption. Finally reversed phase paper chromatography detects the colours. This method is rapid and simple and can detect minute quantities of colours in foods.

While some of the oil-soluble natural colours are permitted for use in certain foodstuffs, use of oil soluble synthetic dyes are prohibited under the provisions of Indian Food Rules[1]. There being a tendency to use such prohibited dyes in different foodstuffs, particularly in prepared foods, some quicker methods for their detection will therefore be helpful inasmuch as these will ease the work of regulatory analysts in detecting permitted as well as non-permitted oil soluble colours in foods in the course of their routine analysis.

There are a few preliminary sorting tests for detecting oil soluble colours[2], spot tests on dyed silk[3] also give a,

preliminary idea about the oil soluble coaltar dyes present in foods. But for confirmatory identification the method has to be comprehensive. Less elaborate methods have been used for fat soluble dyes in some specific foods[6, 7]. The usual methods of removing fats and oils from colours are either by alcoholic potash digestion[4] or by silica gel adsorption[5], both of which sometimes pose problems in extracting the colours fully. This paper deals with a rapid clean-up using solvent partition technique followed by chromatography for detecting oil soluble colours from foods.

Materials and Methods

(i) *Extraction of colours from foods :* About 2–3 g of food materials are taken in a conical flask and shaken with hexane (approximately 50 ml) for about 10 min in a mechanical shaker. It is then filtered and filtrate concentrated to about 10 ml. In the case of samples of fats and oils, 1 to 2 ml are dissolved in hexane (10 ml).

(ii) *Separation of colours from interfering materials :* Three volumes of dimethyl formamide (DMF) with one volume of concentrated hexane extract are shaken ; two operations of partitioning are generally sufficient. DMF layer is separated and shaken with hexane and water. Saturated sodium chloride solution is also added to help the separation of layers in case any emulsion is formed. Then the hexane layer is separated and concentrated to about 20 ml.

Though this technique removes fats and oils, it is not intended to separate the oil soluble natural colours from the oil soluble synthetic dyes. To separate the natural colours, the extracted hexane layer is passed through activated alumina column. Alumina (Brockmann, activity I) is activated at 100°C for 4 hr and then cooled in a desiccator. A chromatographic tube is clamped and the fritted glass disk covered with anhydrous sodium sulphate (about 1 in. layer). Activated alumina is then filled to a depth of 4 in. with tapping. Hexane (30–40 ml) is added to pre-wet the adsorbent. When this had just sunk in, a beaker is placed under the column and concentrated hexane extract is transferred to the column. The oil soluble coaltar dyes pass down through the adsorbent and collected in the beaker while the natural colours are adsorbed on the column. The solution collected in the beaker is concentrated, and the concentrated solution and some known oil-soluble colours are spotted on the reversed phase chromatogram prepared by soaking Whatman No. 1 filter in 5 per cent paraffin in petroleum ether (b.p. 40–60°C) and subsequent drying[8]. Ascending chromatography is carried out using one or more of the following solvents : (*a*) Diethyl ether : ethanol : water (5:3:2) ; (*b*) 80 per cent ethanol in water ; (*c*) isoamyl alcohol : ethanol : water : ammonia (4:4:2:1).

(iii) *Detection of natural colours :* The natural colours adsorbed on the column are eluted from the column by the solvent methanol : ammonia (9:1) and then examined for natural colours. Turmeric and annatto are tested as described by Jacobs[9] while others depending on surmise, compared with known natural colours chromatographically and by chemical reactions.

Results and Discussion

The method involving solvent-partitioning, alumina adsorption and paper chromatography or TLC has been found to be simple and rapid. Oil soluble colours (both artificial and natural) tried are Sudan IV, Butter Yellow, Cres Orange GN, Cress Yellow 3 G type, Cres Yellow GRN type; and turmeric and annatto. These are isolated from the food materials and subsequently seprarated and identified by this method.

Advantage of this solvent partition method over the classical alcoholic potash digestion[4] is that while there is a chance of missing some of the colours which are amenable to saponification, such possibilities are eliminated in the present method. In case of silica gel adsorption[5] natural and artificial colours cannot be adsorbed simultaneously; further it has been observed that sometimes natural colours are co-extracted with artificial colours and confuse the subsequent analysis. These difficulties are removed by solvent partition and subsequent alumina adsorption and hence this is more advantageous.

Use of alumina of the exactly desired activity and treatment as prescribed here is essential. Though it separates natural and artificial colours, broad generalisation, it may be appreciated, is not possible because chemical nature of both categories is so varied. It is expected that this method will also work for similar or related colours in addition to the ones mentioned.

It must have been noticed that the clean-up procedure of this method has been influenced by ideas of general methodology of clean-up by solvent partitioning so widely used in pesticide analysis[10]. Banerjee *et al*[11] used a similar method to examine carotenoids.

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Variability for Cooking Characteristics in a Collection of Green Gram (*Phaseolus aureus* Roxb)

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Out of the 60 green gram cultures screened *ML*-6, *PS*-16, *P*-30, 10045, 10106 and 10509 were found to cook within 36 min and also increase in volume by more than 200 per cent after cooking. The increase in volume after cooking was found to be positively associated with the specific gravity of seeds and increase in weight after cooking. But specific gravity (particle density) of seed was negatively associated with the weight and volume of 100 seeds (before cooking). Specific gravity of seeds and increase in volume after cooking was proposed as important criteria in selection programmes.

Many of the high yielding varieties recently evolved in various crops have drawn criticism from the consumers regarding their quality. This is largely because the objective to breed varieties according to the consumers preference is not given due importance. In green gram, consumers prefer the varieties which cook early and expand greatly in volume after cooking. This calls for breeders to consider these characteristics in addition to high yielding abilities while breeding for new varieties. Therefore with the object of providing information regarding the cooking characteristics in green gram, the present investigation was undertaken.

Materials and Methods

The material comprised of 36 cultures from the germplasm and 24 elite varieties from all over India. The weight and the volume of seeds were recorded and their ratio and specific gravity (particle density) was computed. The specific gravity was determined from the weight and volume of 100 seeds. The volume was determined in a graduated flask containing 10 ml of water and the difference between final and initial readings gave the volume. The specific gravity was then computed as

$$\frac{\text{Wt. of 100 seeds in g}}{\text{Volume of 100 seeds in ml}}$$

Each variety was triplicated in the experiment. Cooking tests were performed in test tubes with 10 ml of water and 2.5 g of seeds, in a hot water bath at 96°C, and the following observations were recorded.

Time taken for cooking: Intermittently, with the help of a long needle, seeds were taken out and squeezed in between fingers. When the seeds were completely soft and became a homogenous mass, the time taken for cooking was recorded.

Increase in weight after cooking: After cooling the tubes, the water was completely drained out on a sieve and the weight of the cooked seeds was recorded. The increase in weight after cooking over the initial weight of seeds was expressed in per cent.

Increase in volume after cooking: The volume of cooked seeds was recorded as the volume of water displaced by them and the increase over the initial volume was expressed in per cent.

Simple correlation co-efficients were worked out for all the possible character pairs.

TABLE 1. CORRELATION CO-EFFICIENTS AMONG VARIOUS CHARACTERS ALONG WITH MEAN VALUES AND RANGE FOR 60 CULTURES IN GREEN GRAM

Characters	100 seed vol	Cooking time	% Increase in cooking wt	% Increase in cooking vol	Sp. gr. of seeds (particle density)	Mean	Range
1. 100 seed wt ..	0.9644**	0.0184	0.1242	—0.1335	—0.6168**	3.37 g	2.06 — 5.09
2. 100 seed vol ..	..	0.0547	0.0978	—0.2155	—0.4404**	2.51 ml	1.50 — 3.88
3. Cooking time ..	..	..	— 0.0457	—0.0416	—0.1747	39.01 min	29.7 — 55.0
4. %Increase in wt after cooking ..	..	..	..	0.5979**	0.1222	141.48%	73.2 —236.0
5. % Increase in cooking volume ..	..	..	..	..	0.3978**	176.77%	100.0 —311.8
6. Sp. gr. of seeds (particle density) .	..	..	..	..	..	1.35	1.192— 1.470

** Significant at 1 per cent level.

Results and Discussion

The mean values of various characters studied for 60 cultures of green gram are presented in Table 1. The cultures ranged from 2.06 to 5.09 g and from 1.50 to 3.88 ml respectively for weight and volume of 100 seeds. Variety *Kopergoan* had the maximum seed weight and seed volume, and *P*-244 had the lowest values. The specific gravity (particle density) of seeds ranged from 1.192 to 1.470 with *P*-23 having the maximum specific gravity and *Madira Mung* having the minimum. The cultures took 29 to 55 min to cook. While the cultures 10,870 took 29 min to cook followed by 10,381 (29.7 min) and *P*-37 (30 min) the varieties *Khedda*-10 and *PS*-7 needed 55 min to cook. The percentage increase in weight of seeds after cooking was maximum for *ML*-6 (236 per cent). In comparison to these, the increase was only 73.2 per cent for *P*-152. There was a lot of variability for percentage increase in the volume of seeds after cooking among the varieties. While in the culture *P*-30 the increase in volume was 311.8 per cent, it was only 100 per cent in *B*-105 and *P*-28. Such varietal differences in time taken for cooking and increase in volume after cooking were also reported by Kurien *et al*[1] in rice, Sankaran and Srinivasan[2] and Rathnaswamy *et al*[3] in red gram.

From the consumer's view point, it is very important that the maximum volume of cooked product must be achieved with unit weight or volume of seeds. It is also important that the time taken for cooking should be minimum. Therefore the varieties *ML*-6, *PS*-16, *P*-30, 10045, 10106 and 10509 which are some of the best among the cultures studied, could be utilized in breeding programmes to meet the needs of the consumers.

The correlation co-efficients among various characters are presented in Table 1. Very close positive correlation between the weight and volume of 100 seeds was observed. These two characters were negatively correlated with the specific gravity (particle density) of seeds. Significant positive correlations were observed beween percentage increases in weight and volume of seeds after cooking and also between the specific gravity of seeds and increase in volume after cooking.

Sankaran and Srinivasan[2] observed that medium and small sized dhals (red gram) cooked easily and expanded in volume more than big sized ones, and the latter increased in weight more than the former. Similar trend was also shown by the correlations in the present work, but the co-efficients were not significant.

The correlation co-efficients revealed that the increase in volume after cooking was positively associated with the specific gravity (particle density) of seeds and increase in weight after cooking. This indicates that specific gravity and increase in weight after cooking could also form important criteria in selection programmes. The correlations of weight and volume of seeds with specific gravity of seeds indicated that smaller seeds would have higher specific gravity and thereby would help in getting maximum increase in volume of seeds after cooking.

The reason for the undue increase in volume of seeds after cooking in certain varieties needs explanation and work in this direction is in progress to explain this phenomenon.

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Minor Seed Oils. VI. Component Acids of Six Seed Oils*

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The six seed oils have been examined by reversed phase partition chromatography with the following results: *Cassia renigera* seeds yielded 3.8 per cent oil and the mixed fatty acids of the oil had an iodine value of 123.2 and were estimated to contain mostly linoleic (67.7 per cent) and palmitic (18.7 per cent) acids. *Dolichos biflorus* seeds yielded 8.0 per cent oil and the mixed fatty acids of the oil had an iodine value of 73.8 and were found to consist mainly of palmitic, oleic and linoleic (28.6, 31.1 and 27.2 per cent respectively) acids. *Pavonia spinifex* and *Thespesia populnea* seeds yielded 14.0 and 20.0 per cent oil respectively and the mixed acids had iodine values 103.8 and 92.8 respectively and were found to contain many palmitic (29.2 and 31.6 per cent); oleic (16.9 and 15.0 per cent); linoleic (50.8 and 45.6 per cent) acids respectively. *Thevetia neriifolia* seeds yielded 57.0 per cent oil and the mixed fatty acids of the oil had an iodine value of 75.4 and were found to be rich in palmitic (24.5 per cent) and oleic (39.6 per cent) acids, while *Zizyphus oenoplia* seeds contained 3.2 per cent oil and the mixed acids of the oil were found to contain mainly of palmitic (23.9 per cent); oleic (32.2 per cent) and linoleic (34.3 per cent) acids.

In our search for new industrial oils and oils containing acids of novel structure, we have examined six seed oils for their component acids by the application of reversed phase partition column chromatography.

Cassia renigera, Linn (N.O. Leguminosae) is a very handsome, quick growing cassia. It has the most ornamental habit of growth among cassia. There are no reports on the component acids of this seed oil.

Dolichos biflorus, Linn (N.O. Leguminosae) (Horse gram)is a native of India and is also distributed throughout the tropical regions. Horse gram is extensively used in South India as feed for cattle and horses in the same way as gram. The seeds are astringent, diuretic and tonic and are a rich source of urease. The plant is used as a valuable protein supplement to bulky straw fodders[1]. Recently this seed oil has been analysed by Dube *et al*[2].

Pavonia spinifex, Cav and *Thespesia populnea*, Soland, both belong to the family Malvaceae. *Pavonia spinifex* is a shrub, not indigenous to India[3]. There are no reports on the component acids of this seed oil. *Thespesia populnea* is known for its medicinal value. The root of this plant is good for heart disease and throat trouble. It is also used as a tonic. The flowers are employed in the cure of itch. The fruit yields a yellow, viscid juice, which forms a valuable local application in scabies and other cutaneous diseases[4]. The general characteristics and the fatty acid composition of the oil have been studied by a few workers[5-7]. The latest available report is that by Raikar *et al*[8], who used gas-liquid chromatography as a method of analysis. The results obtained by the above authors are compared with the results reported in the present investigation (Table 3).

Thevetia neriifolia, Juss (N.O. Apocynaceae) is a large evergreen, glabrous shrub or small tree, native of south America. The plant is bitter, pungent, acrid, hot, astringent to the bowels, useful in urethral discharges, worms, skin diseases, lucoderma, wounds, piles, eye troubles, itching, fevers, etc. The oil from the seed is emetic and purgative[9]. The seeds are chewed as a purgative. The seed also contains two glucosides, thevatin and thevetidin. There are two reports[10,11] on the component acids of this seed oil.

Zizyphus oenoplia, Mill (N.O. Rhamnaceae): This is a straggling shrub distributed throughout the hotter parts of India, Ceylon, tropical Asia and Australia. A decoction of the bark is used to promote the healing of fresh wounds. The fruit is used as an ingredient in the preparation of stomach-ache pills[12]. There are no reports on the component acids of this seed oil.

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Materials and Methods

The air dried seeds (about 100 g each) were powdered and extracted thoroughly with light petroleum, at room temperature. The solvent was removed from the clear filtered extract in a rotary film evaporator under reduced pressure to yield the oil. A portion of the oil in each case was hydrolysed by allowing it overnight at room temperature with 5 per cent ethanolic potassium hydroxide solution and the non-saponifiable matter and mixed acids free from non-saponifiable matter were recovered.

The mixed acids free from non-saponifiable matter (i) without any treatment ; (ii) after hydrogenation ; (iii) after oxidation[13] were each examined by reversed phase partition column chromatography as previously described[14].

Oleic and linoleic acids isolated from the appropriate chromatographic fractions, when the mixed acids were being chromatographed, were characterised as 9:10 dihydroxy stearic (m.p. 129–130°C) ; 9:10:12:13 tetrabromostearic (m.p. 113–114°C) acids respectively[15].

When thin layer chromatography of the mixed acids and the esters of the samples of oils examined in this investigation was carried out on Silica-gel G plates using petroleum-ether-ether-formic acid (70:30:1) as the developing solvent system, it did not reveal the presence of any oxygenated acids. In reversed phase partition column chromatography the oxygenated acids usually elute in aqueous acetone fractions (35, 43 and 53 per cent respectively) when mixed and hydrogenated acids are being chromatographed. Since no such oxygenated acids were detected by TLC, the eluates of the above fractions were characterised as lower saturated fatty acids.

Results and Discussions

Of the six seed oils reported in this work, two belong to the family Leguminosae (*Cassia renigera* and *Dolichos biflorus*), two to the family Malvaceae (*Pavonia spinifex* and *Thespesia populnea*) and one each to the families Apocynaceae (*Thevetia neriifolia*) and Rhamnaceae (*Zizyphus oenoplia*).

Palmitic, oleic and linoleic acids are the major constituents of all the seed oils except the seed oil of *Cassia renigera*, which is very rich in linoleic acid. (67.7 per cent) and poor in oleic acid (5.2 per cent). The values reported by us for the seed oils of *Thespesia, populnea* and *Thevetia neriifolia* agree closely with those reported earlier by Raikar *et al*[8] and Qazi *et al*[11], except that we have been able to detect also the presence of myristic, arachidic and behenic acids in these seed oils. The presence of epoxy acid in the seed oil of *Thespesia populnea*, as reported by Hopkins and Chisholm[7] was not found in the sample of oil analysed in this investigation. As expected[16], the seed fats belonging to the family Malvaceae are found to be rich in palmitic, oleic and linoleic acids.

TABLE 1. EXTRACTION DATA AND CHARACTERISTICS OF MIXED ACIDS FREE FROM NON-SAPONIFIABLE MATTER

Source of oil	Oil content %	Unsaponifiable matter %	Iodine value (Obs)	Iodine value (Calc)	Saponification equiv* (Obs)	Saponification equiv* (Calc)
Cassia renigera ..	3.8	1.2	123.2	125.1	278.5	275.9
Dolichos biflorus ..	8.0	0.8	73.8	74.5	271.6	269.5
Pavonia spinifex ..	14.0	1.2	103.8	104.3	275.1	273.3
Thespesia populnea ..	20.0	1.0	92.8	93.5	275.1	273·2
Thevetia neriifolia ..	57.0	1.8	75.4	76.8	276.0	275.ε
Zizyphus oenoplia ..	3.2	0.6	88.2	89.5	277.3	275.8

* These values are measured on mixed acids free from non-saponifiable matter and calculated from the composition determined in this investigation.

TABLE 2. CHROMATOGRAPHY RESULTS ON PARAFFIN COLUMNS (MOLE PER CENT)

Treatment	Load mg	Recovery %	Eluting solvent (per cent aq acetone) 35	43	53	62	67	73	78	83
Cassia renigera:										
Hydrogenation	12.2	100.0	..	..	..	2.4	17.6	74.5	2.2	2.3
No. treatment	12.7	97.3	..	..	..	69.0	25.2	2.8	1.1	1.9
Oxidation	75.7	..	..	..	..	1.4	20.1	4.6	1.2	1.5
Dolichos biflorus:										
Hydrogenation	11.5	100.0	1.7	1.2	0.9	2.4	30.1	60.8	1.0	1.9
No. treatment	12.2	99.9	1.6	0.5	0.9	28.6	59.8	5.7	1.4	1.5
Oxidation	82.5	..	..	..	0.8	1.2	33.1	5.4	0.7	1.0
Pavonia spinifex:										
Hydrogenation	17.4	99.3	..	..	..	0.4	31.2	67.0	0.9	0.5
No. treatment	15.1	98.8	..	..	..	49.9	47.6	1.9	0.2	0.4
Oxidation	60.2	..	..	..	..	1.0	29.1	3.2	0.8	0.9
Thespesia populnea:										
Hydrogenation	12.4	97.4	..	..	..	1.5	33.7	61.7	1.4	1.7
No. treatment	12.7	100.0	..	..	..	45.9	48.3	3.0	1.2	1.6
Oxidation	86.2	..	..	..	..	0.5	32.9	4.2	1.0	2.7
Thevetia neriifolia:										
Hydrogenation	11.2	100.0	..	..	..	1.8	24.2	68.8	3.3	1.9
No. treatment	12.2	99.0	..	..	..	25.0	65.0	6.8	2.0	1.2
Oxidation	86.5	..	..	..	..	1.6	26.3	8.0	0.8	0.3
Zizyphus oenoplia:										
Hydrogenation	13.9	100.0	..	..	..	1.1	25.7	69.8	1.5	1.9
No. treatment	13.1	97.9	..	..	..	34.8	57.2	5.0	1.3	1.7
Oxidation	75.6	..	..	..	..	0.8	28.3	3.9	1.0	1.2

TABLE 3. COMPONENT ACIDS (MOLE PER CENT AND WEIGHT PER CENT)

Acids	8:0*	10:0	12:0	14:0	16:0	18:0	20:0	22:0	18:1	18:2
Cassia renigera:										
Mole %	..	..	..	2.4	20.1	2.8	1.1	1.9	5.1	66.6
Wt %	..	..	..	2.0	18.7	2.8	1.2	2.4	5.2	67.7
Dolichos biflorus:										
Mole %	1.6	1.2	0.9	2.4	30.1	5.7	0.7	1.5	29.7	26.2
Wt %	0.9	0.8	0.7	2.0	28.6	6.0	0.6	1.9	31.1	27.2
Pavonia spinifex:										
Mole %	..	..	..	0.4	31.2	1.9	0.2	0.4	16.4	49.5
Wt %	..	..	..	0.4	29.2	1.9	0.3	0.5	16.9	50.8
Thespesia populnea:										
Mole %	..	..	..	1.5	33.7	3.0	1.2	1.6	14.6	44.4
Wt %	..	..	..	1.2	31.6	3.2	1.4	2.0	15.0	45.6
Wt %**	..	..	..	..	33.8	3.2	..	..	13.6	49.4
Thevetia neriifolia:										
Mole %	..	..	..	1.8	26.3	6.8	2.0	1.2	38.7	23.2
Wt %	..	..	..	1.5	24.5	7.0	2.3	1.5	39.6	23.6
Wt %***	..	..	..	..	25.9	7.1	1.1	..	41.6	24.3
Zizyphus oenoplia:										
Mole %	..	..	..	1.1	25.7	5.0	1.3	1.7	33.7	31.5
Wt %	..	..	..	0.9	23.9	5.1	1.5	2.1	32.2	34.3

* These figures indicate the number of carbon atoms and the number of double bonds in the acids.
** Values reported by Raikar *et al*[8].
*** Values reported by Qazi *et al*[11].

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A COLOUR REACTION FOR ASSESSING RANCIDITY IN COCONUTS*

It was found that coconut gives a pink colour with hydrochloric acid. The intensity of this colour decreased with the development of rancidity as indicated by FFA and carbonyls as also by organoleptic evaluation.

During the course of experiments aimed at determining the total lipids in coconut by different methods, it was observed that a characteristic pink colour was produced when coconut reacted with concentrated hydrochloric acid. While the formation of such a colour may not be a speciality of coconuts, preliminary experiments indicated that the intensity of the pink colour was less with rancid samples of coconut and fherefore it might serve as an index of rancidity in coconuts. This communication summarizes the results of experiments demonstrating a correlation between rancidity in coconut and the intensity of the colour ormed with hydrochloric acid.

Mature coconuts were purchased from the local market. After removing the shells, the nuts were pared with stainless steel knives, cut into small bits and put through a Fryima mill. The fine shreds so obtained were divided nto two equal portions. One Portion was freze-dried in a Stokes freeze drier. Another portion was autoclaved at 5 psi for 15 min and then dehydrated in a through-flow drier at 60 °C. Each batch onsisted of 10 nuts so that the kernels from 5 were teken for freeze drying and an equal quantity was use for dehydration. Such a batch constituted one sample. The moisture content of these samples was 0.35-0.60 per cent in the freeze dried and 0.8-0.9 per cent in the dehydrated sample.

For storage studies, three samples of freeze dried ane three samples of dehydrated coconuts were equilibrated to 2 per cent moisture in a Gallenkamp humidifying oven. They were immediately transferred toscrew cap bottles and stored in an incubator at 37°C. Atdesired time intervals, samples were withdrawn for the determination of free fatty acids, peroxide value, forKreis test[1] and for carbonyls by Hennick's procedure as described by Berry *et al*[2]. For measuring the intensity of pink colour with hydrochloric acid,100 mg sample was mixed with 10 ml of 8.8 N hydrochloric acid in stoppered test tubes. After occasional mixing, the solution was filtered after 27-28 min. The absorbance was determined at exactly 30 min (after addition of acid) in a Bausch and Lomb spectrophotometer at 520 nm.

As is evident from Fig 1, the FFA increased from an initial value of about 0.14 to 0.75 per cent at the end of 9 weeks or to 1.1 per cent at 12 weeks storage. At 5 weeks, the samples showed clear signs of rancidity as indicated organoleptically. The intensity of colour with hydrochloric acid decreased rather sharply during the initial stages of storage and then plateaued off. The point of inflexion of this curve was very close to the storage period at which the samples were found to be organoleptically rancid.

With a view to having parameters other than FFA for the indication of rancidity, the peroxide value, Kreis value and the TBA value as also the carbonyls were determined. With the exception of the carbonyls, the other indices of fat deterioration showed no change as expected for coconut fat which has such a low degree of unsaturation. The changes in carbonyls during storage are shown in Fig 2. The concentration of saturated carbonyls dropped markedly in the beginning, becoming negligible at 12 weeks of storage. On the other hand, unsaturated carbonyls which were absent to begin with started increasing at a point close to the

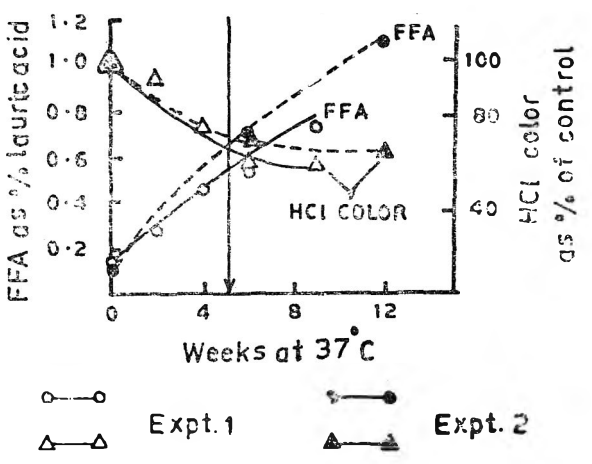

FIG 1. Changes in FFA and colour with HCl on storage of freeze-dried coconut. Each point is the average determination on 3 samples. Arrow mark indicates rancidity organoleptically perceptible.

* Preliminary results were presented at the Annual Scientific Meetings of the Society of Biological Chemists (India), November 1-2, 1973.

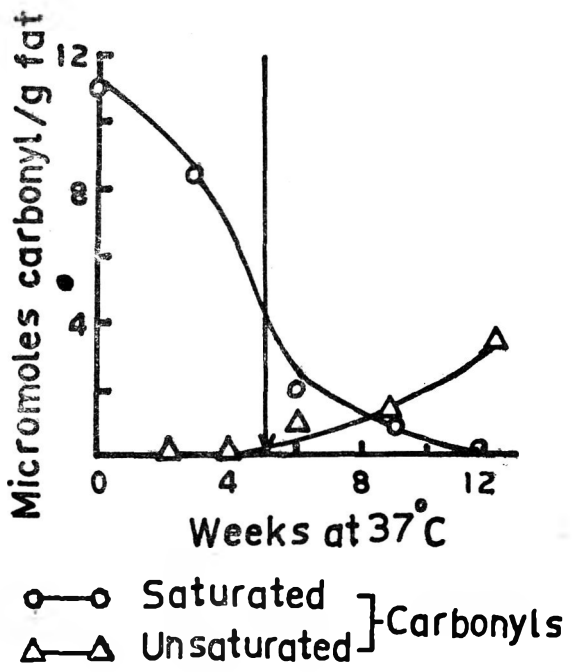

FIG 2. Changes in carbonyls on storage of freeze-dried coconut. Arrow mark indicates rancidity organoleptically perceptible.

time of storage when the samples could be pronounced rancid. Though Rasper[3] found that very low degrees of rancidity in coconut butter could be detected by the determination of methyl ketones, there is no report regarding the changes in the saturated and unsaturated carbonyls during the storage of coconut. This point is of relevance because of the coincidence of appearance of unsaturated carbonyls with the onset of rancidity.

At 10 weeks of storage, samples were plated for microbial counts[4]. There was no significant change in the microbial population thereby ruling out the possibility of rancidity due to microbial action. This is in agreement with the observation of Rasper and Rasperova[5] who concluded that microbial rancidity occurred when desiccated coconut was stored at 20 °C at a moisture level higher than 2 per cent. While the data presented here are only for freeze dried coconut, similar results were obtained for dehydrated coconut also.

The chemical methods that are in vogue for evaluation of rancidity are involved and time consuming. On the other hand, because of its relation to rancidity in coconuts, the colour reaction with hydrochloric acid offers an easier and more convenient alternative to the methods now available. The mechanism of this colour reaction, why the intensity of the pink colour with hydrochloric acid decreases on the development of rancidity and how far this colour test would be applicable to other materials susceptible to become rancid are being studied.

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EFFECT OF FRESH EGG YOLK AND LECITHIN FROM EGG ON THE WHIPPING ABILITY OF ICE CREAM MIX

A study on the effect of fresh egg yolk and lecithin from egg on the whipping ability of ice cream mix showed that the mix having the fresh egg yolk whipped much more as compared to one containing the lecithin from egg.

The function of an emulcifying agent in the manufacture of ice cream lies mainly in improving the whipping quality of the mix, the production of a smooth texture, giving a dry stiff product at the time it is drawn from the freezer and a more exact control during the various manufacturing processes. The beneficial effect of egg solids on whipping ability has been demonstrated by DePew and Dyer[1], Sommer[2], Martin and Caulfield[3], Mueller and Button[4] and Pettee[5].

Bassett[6] showed that when eggs were used in sufficiently large amounts they did exert some emulsifying effect on the ice cream and the only effective part of the egg yolk was lecithin while Sommer and Horrall[7] concluded that egg lecithin alone did not improve the whipping property of the ice cream mix. These controversial observations induced the authors to conduct the present study to observe the effect of fresh egg yolk and lecithin from egg on the whipping ability of the ice cream mix.

The following base formulae were prepared and processed in the series of mixes prepared:

Constituents	Mix-1	Mix-2	Mix-3
Fat % ..	7.0	10.0	14.0
Serum solids % ..	12.0	11.5	10.0
Sugar % ..	15.0	15.0	15.0

In addition to these ingredients each sample of mix also contained either 0.6 per cent fresh egg yolk or 0.04 per cent egg lecithin. The quantity of lecithin in 0.6 per cent fresh egg yolk corresponded to 0.04 per cent egg lecithin, hence 0.04 per cent egg lecithin was taken. The egg lecithin used was from Germany. Control mixes (having neither fresh egg yolk nor egg lecithin) were also prepared. Ice cream mixes were prepared from fresh cow milk obtained from our Institute. The cream was obtained by separating cow milk by De-Laval tri-process machine. The spray dried skim milk was obtained from the Institute. To avoid difficulties in mixing pre-calculated quantities of dry ingredients, *i.e.*, milk powder and sugar were mixed in a lot. Similarly the liquid ingredients, namely, milk, cream and fresh egg yolk or egg lecithin were mixed separately in a container. The dry mixture was incorporated within the liquid ingredients mixture gradually with constant stirring and heating to the homogenization temperature of 65.5 °C. After proper mixing it was filtered through muslin cloth and homogenized in two stages, first at a pressure of 2500 psig and then at 500 psig. The mixes were then pasteurized at 71 °C for 30 min, and subsequently cooled to 20 °C by first dipping the cans containing mix in tap water and then immersing the cans in chilled water. All the mixes were prepared in the same manner. Three trials were conducted with each sample.

Schlagsahne-Prufgerat (Dr Mohr's) whipper was used to determine the whipping ability of the ice cream mixes. The mix samples were kept in the refrigerator for about 12–14 hr. The whipping cup was also chilled. Measured quantities of mixes were transferred into the cup and the blades were fixed; whipper was allowed to run for 10 min. The volume of the whipped mix in the cup was measured and the increase in volume of mix after whipping due to incorporation of air was observed.

It is seen from the Table 1 that the average per cent values for whipping ability obtained in case of Mix-1 for fresh egg yolk (FEY) and egg lecithin were 4.0 and 2.6 respectively. Similarly the average whipping values in case of Mix-2 for FEY and egg lecithin were 5.6 and 3.2 per cent respectively and the average whipping values for FEY and egg lecithin in Mix-3 were 6.5 and 4.0. It is clear from the above results that in all the three mixes the one containing fresh egg yolk whipped much more as compared to one having egg lecithin. The average whipping values for the control mix samples and the mix samples containing egg lecithin are almost same, confirming that lecithin alone did not improve the whipping ability of the ice cream mix.

TABLE 1. WHIPPING ABILITY OF MIXES

Trial	Whipping ability %		
	Mix-1	Mix-2	Mix-3
	Fresh egg yolk (0.6%)		
1 ..	3.5	5.7	6.3
2 ..	4.5	5.9	6.8
3 ..	4.0	5.2	6.4
Mean ..	4.0	5.6	6.5
	Egg lecithin (0.04%)		
1 ..	3.0	3.6	3.8
2 ..	2.6	2.8	4.2
3 ..	2.2	3.2	4.0
Mean ..	2.6	3.2	4.0
	Control		
1 ..	2.5	3.3	3.9
2 ..	2.8	3.3	4.4
3 ..	2.6	3.3	3.8
Mean ..	2.6	3.3	4.0

The results obtained are in agreement with the earlier findings of Sommer and Horall[7], who observed that the egg lecithin alone did not improve the whipping ability of the ice cream mix. This is due to the disruption of lecithin protein combination by ether alcohol which is essential for increasing the whipping ability of the mix. However, the results do not agree with the Bassett's[6] observation that lecithin is the only effective part of egg yolk to increase the whipping property of mix.

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EFFECT OF SUPPLEMENTATION OF LEAF PROTEIN EXTRACTED FROM BERSEEM (*TRIFOLIUM ALEXANDRINUM*) TO WHEAT FLOUR DIET

Supplementation of wheat flour with 3 per cent leaf protein extracted from berseem improved the protein quality compared to whole wheat flour indicating the possibility of utilisation of leaf protein to fortify the nutritionally poor diets.

Leaf proteins are considered to be nutritionally superior to grain proteins on account of their high lysine and tryptophan contents[1,2]. Therefore, these can provide an excellent source to fortify the poor quality diets. Various workers[3,4] have established that leaf proteins could be beneficially used as a supplement to cereal diets. In the present investigation the effect of supplementation of wheat flour with berseem leaf protein concentrate, rich in essential amino acids[2], was studied by feeding to rats.

Protein from fresh green leaves of berseem was isolated as described earlier[5]. The leaves were crushed in a domestic meat mincer and the juice was squeezed out through a thick cotton cloth. After coagulation and filtration, the wet cake was dried in an air oven at 60°C and ground to a fine powdery form. Wheat flour and berseem leaf protein concentrate (LPC) were found to contain 12.12 and 56.88 per cent of crude protein, and 3.40 and 8.40 per cent of ether extract respectively. Albino rats, about four weeks old, were individually weighed and randomly divided into three groups of six each and maintained on diets at 10 per cent protein level with adequate amounts of vitamins and minerals. Diet D_1, a control diet, contained casein whereas diet D_2 contained 3 per cent berseem leaf protein and 7 per cent protein from wheat flour, and diet D_3 had wheat flour only. The diets were fed *ad libitum* for a period of four weeks. Gain in body weight and food intake of individual rats were recorded weekly. At the end of the experiment, the animals were anesthetized with solvent ether. Blood and different organs were removed and analysed for total nitrogen, fat[6] and glycogen[7] contents. Haemoglobin content was estimated by acid hematin method[8].

The mean gain in body weight of experimental rats in various groups, quantity of food consumed and protein efficiency ratio (PER) are given in Table 1. The growth rate in the LPC supplemented group (D_2) was better as compared with the wheat flour diet (D_3). PER value of D_3 group was lower than that of control D_1 but was significantly improved from 0.82 to 1.26 by the addition of 3 per cent berseem leaf protein. The improvement in the PER value of the fortified wheat flour diet might be partially due to improved protein quality with a balanced amino acids make up. However, the improvement in the nutritive value of LPC supplemented wheat flour was comparatively less than the reported values[9].

The weights of total liver, spleen and right gastrocnemius muscle of the animals fed on D_2 and D_3 diets were appreciably smaller as compared with control D_1 group. However, when the weights of these organs were expressed in terms of 100 gram body weight, the difference was not significant. The data on total nitrogen, glycogen and lipids of liver and haemoglobin content of blood are given in Table 2. The nitrogen content of liver was observed to be comparatively less in D_2 and D_3 diets than the control D_1 diet. However, the nitrogen content of liver in D_2 group was higher than that of D_3 group. The perusal of the data indicates that the supplementation of wheat flour diet with berseem leaf protein has considerably increased the nitrogen whereas liver lipids are reduced when expressed on per gram basis. The increase in the

TABLE 1. BODY WEIGHT GAIN, FOOD INTAKE, PER AND WEIGHTS OF LIVER, SPLEEN AND RIGHT GASTROCNEMIUS MUSCLE* OF RATS FED ON VARIOUS DIETS

Diet**	Wt gain (g)	Food intake (g)	PER	Liver wt (g) Total	Liver wt (g) Per 100 g BW	Spleen wt(g) Total	Spleen wt(g) Per 100 g BW	Right gastrocnemius muscle wt (g) Total	Right gastrocnemius muscle wt (g) Per 100 g BW
Control (D_1) ..	46.50 ±5.65	194.00 ±0.67	2.37 ±0.27	3.74 ±0.18	3.93 ±0.16	0.25 ±0.02	0.27 ±0.03	0.46 ±0.03	0.49 ±0.02
LPC+wheat flour (D_2) ..	16.10 ±0.65	128.00 ±1.80	1.26 ±0.05	2.11 ±0.12	4.13 ±0.97	0.14 ±0.01	0.27 ±0.03	0.27 ±0.01	0.48 ±0.01
Wheat flour (D_3) ..	9.30 ±0.47	112.80 ±4.80	0.82 ±0.06	1.90 ±0.05	4.35 ±0.12	0.27 ±0.01	0.63 ±0.04	0.17 ±0.02	0.39 ±0.04

* Mean ± S.E. of mean. ** As given in the text. BW = Body weight.

TABLE 2. LIVER TOTAL NITROGEN, GLYCOGEN, LIPID CONTENTS AND NITROGEN OF SPLEEN, RIGHT GASTROCNEMIUS MUSCLE AND HAEMOGLOBIN CONTENT* OF RATS FED ON VARIOUS DIETS

Diet	Total liver N		Liver glycogen		Liver lipids		Spleen N	Rt gastrocnemius muscle N	Haemoglobin
	mg/g	mg/total wt	mg/g	mg/total wt	mg/g	mg/total wt	mg/total wt	mg/total	g/100 ml
Control (D_1) ..	24.44	90.85	77.41	296.48	30.40	104.30	7.56	15.90	14.20
	±1.65	±0.91	±8.28	±12.48	±3.11	±15.51	±0.77	±1.45	±0.41
LPC+wheat flour (D_2) ..	19.99	42.89	108.00	215.37	42.60	89.76	5.04	9.92	11.70
	±2.77	±7.45	±11.70	±22.60	±3.40	±7.75	±0.04	±0.22	±0.94
Wheat flour (D_3) ..	16.51	31.36	111.80	213.48	50.77	98.42	6.22	5.60	9.70
	±0.22	±0.80	±9.97	±23.98	±8.46	±18.02	±0.70	±0.63	±0.89

* Mean ± S.E. of mean.

nitrogen content of muscle and liver, and haemoglobin content of blood indicates an improvement in the nutritive value of wheat flour with leaf protein supplementation. It may, therefore, be concluded that leaf protein concentrates could be used as a supplement to the nutritionally poor cereal diets with beneficial effects.

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EFFECTS OF COMPLEX NUTRIENTS, VITAMINS AND AMINO ACIDS ON THREONINE PRODUCTION BY *MICROCOCCUS GLUTAMICUS*

Different nutrients like corn steep liquor, yeast extract, malt extract, rice bran extract, wheat bran extract lowered the production of threonine by *M. glutamicus* C, where as tomato extract, paddy soak liquor, beef extract and soybean meal were without effect. Of the different amino acids tested only L-lysine increased the production of threonine.

Complex nutrients, vitamins and amino acids are required for the production of many amino acids. One or more complex nutrients are required for the production of glutamic acid[1], valine[2] and lysine[3]. Vitamins like biotin and thiamine are required for the production of glutamic acid[4,5] and valine[6]. Udaka and Kinoshita[7] reported that the addition of certain organic nutrients in appropriate concentrations favours valine production and helps in the stabilization of the fermentation. But data available on the effect of complex nutrients, vitamins and amino acids on the production of threonine are limited. Hung[8] reported that the production of L-threonine by auxotrophic mutants of *E. coli* was increased with the addition of corn steep liquor. The effects of single vitamins and amino acids and combinations were studied on growth and threonine accumulation by a variant of *B. subtilis*[9].

In our previous paper we reported the effect of carbon and nitrogen sources on the production of threonine by *Micrococcus glutamicus*[10]. In the present work, a study has been made to assess the individual role played by complex nutrients, vitamins and amino acids on the growth of *M. glutamicus* and threonine production.

In the course of mutation studies with ethyl methane sulphonate and gamma rays (^{60}Co) of a glutamic acid producing strain of *Micrococcus glutamicus* ATCC 13032, the amino acid threonine was found to be produced by a biotin-dependent mutant *M. glutamicus* C_1. This mutant strain *M. glutamicus* C_1 was used throughout the study. The stock culture was maintained on a solid medium consisting of glucose, 1; urea, 0.2; K_2HPO_4, 0.1; $MgSO_4.7H_2O$, 0.25; $FeSO_4.7H_2O$, 0.002 per cent; biotin, 1 μg/ml; and agar

3 per cent at pH 7.2 and was subcultured at 27°C at monthly intervals.

The effect of complex nutrients on threonine production was studied in the synthetic medium consisting of glucose, 5; urea, 0.8; K_2HPO_4, 0.1; $MgSO_4.7H_2O$, 0.025; $FeSO_4.7H_2O$, 0.0005; $ZnSO_4.7H_2O$, 0.0001 per cent; biotin, 1.0 μg per ml at pH 7.0. To the synthetic medium was added a complex nutrient (yeast extract, beef extract, soybean meal, malt extract) in 0.1 per cent concentration. The nutrients like corn steep liquor, paddy soak liquor, rice bran extract, wheat bran extract, tomato extract were each added to the medium in such amount as to make 0.1 per cent solid (W/V). Vitamins like riboflavin, inositol, thiamine, nicotinic acid, pyridoxal hydrochloride, folic acid, choline, p-amino benzoic acid and vitamin B_{12} were each tested in concentrations of 0.2, 0.5, 1.0 and 3.0 μg per ml for their effect on amino acid production. For studying the effect of amino acids on threonine production, individual amino acid was added to the synthetic medium at a level of 0.5 mg per ml. The medium was dispensed in 30 ml in 100 ml Erlenmeyer flasks and was inoculated with 1 ml of cell suspension containing 10^9 cells. For the preparation of inoculum, the culture was grown in 30 ml of synthetic medium consisting of glucose, 1; urea, 0.2; K_2HPO_4, 0.1; $MgSO_4.7H_2O$, 0.025 per cent; and biotin, 0.5 μg per ml, at pH 7.0 in 100 ml flasks for 24 hr at 27°C on a rotary shaker (240 rpm). After harvest, the cells were washed 3-4 times with sterile water and then suspended in 30 ml of water. The fermentation flasks were incubated at 27°C on a rotary shaker at 240 rpm for 3 days.

After fermentation, the concentration of threonine in the broth was determined by the paper chromatographic method of assay, using a solvent system n-butanol-acetic acid-water (2:1:1)[11] and Klett Summerson photoelectric colorimeter with a green filter (540 mμ)[12] for the measurement of colour. The identification and concentration of threonine was also checked by periodate assay[13,14]. Cellular growth was determined by measuring dry weight of the cells. After centrifugation, the cells were washed 2–3 times with distilled water and dried at 70°±5°C for 24 hr.

It was observed that complex nutrients like corn steep liquor, yeast extract, malt extract, rice bran extract and wheat bran extract lowered the production of threonine and stimulated the cellular growth of *M. glutamicus* C_1. Tomato extract had no effect on the production of threonine but increased the cellular growth. Paddy soak liquor, beef extract and soybean meal were without any effect on growth but lowered amino acid synthesis. The complex nutrients used were either sources of amino acids or of vitamins and amino acids.

TABLE 1. EFFECT OF DIFFERENT AMINO ACIDS ON THE GROWTH OF *M. glutamicus* C_1 AND THREONINE PRODUCTION

Amino acid (0.5 mg/ml)	Cellular growth at 72 hr (mg/ml)	Threonine at 72 hr (mg/ml)
Control ..	5.5	5.6
L-Lysine monochloride ..	5.8	6.0
L-Methionine ..	5.3	3.0
L-Serine ..	5.4	5.5
L-Glutamic acid ..	5.5	5.6
DL-Phenylalanine ..	4.5	5.0
L-Aspartic acid ..	5.1	2.8
DL-Valine ..	4.3	3.7
L-Arginine ..	4.8	4.8
L-Cystine ..	5.4	5.6
L-Histidine ..	5.4	5.6
L-Cysteine ..	5.1	5.0
L-Proline ..	5.7	5.6
L-Leucine ..	4.0	4.5
DL-Alanine ..	5.3	5.5
L-Tyrosine ..	5.2	3.0
L-Tryptophan ..	5.1	5.4
DL-Threonine ..	5.3	3.6

Vitamins tested, *viz.*, riboflavin, inositol, nicotinic acid, folic acid, p-amino benzoic acid, vitamin B_{12} had no significant effect on the synthesis of threonine by *M. glutamicus* C_1 although folic acid, p-amino benzoic acid, vitamin B_{12} increased the cellular growth. Biotin was required both for the production of threonine and the cellular growth.

The effect of biotin on threonine biosynthesis may be due to the important role played by the vitamin in the fixation of CO_2, resulting in the formation of aspartic acid, an intermediate in threonine biosynthetic pathway.

Data given in Table 1 show that out of different amino acids tested only L-lysine increased the production of threonine, while amino acids like L-methionine, DL-phenylalanine, L-aspartic acid, DL-valine, L-arginine, L-histidine, L-proline, L-tyrosine and DL-threonine lowered the yield of the amino acid to different extents.

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Industrial Uses of Cereals, American Association of Cereal Chemists, St. Paul, Minnesota, 1973, pp. xxiii+483.

I

This massive volume is a record of a Symposium on the same subject held in St. Louis, Missouri on 4–8 November 1973 in conjunction with the 58th Annual Meeting of the American Association of Cereal Chemists. Interestingly, the material was neither edited nor proofread but directly reproduced from the typescripts as presented by the contributors. Y. Pomeranz acted as the Chairman of the Symposium.

The objectives of the Symposium were to provide the first comprehensive survey on industrial (non-food) uses of all cereals. Traditionally cereals have been considered only as food and feed grains—unlike cotton, flax, castor, jute, beet and many other cultivated crops which were developed primarily for industrial use. However, many recent developments have caused a rethinking in this matter. Already starches, cereal flours and proteins have found diverse industrial applications. Starch is ofcourse a major industrial commodity. Various physical and chemical modifications of these materials are adding to their potential industrial uses. Never challenges and economic considerations are also opening up newer possibilities of other uses.

However, it is probably other considerations of more far-reaching import, and of more recent origin, which are primarily at the root of such rethinking on industrial uses of agricultural commodities including cereals. As is already evident from the contributions in this volume, the newer awareness about the fast depletion of non-renewable energy sources and other raw materials and the hazards of pollution arising from industrial applications are causing serious rethinking in the industrialised countries for a different context of industrialisation. Agricultural commodities, including cereals, fit in well in this context. Cereals can be produced in very large quantities and in wide geographical areas. Cereal crops convert and store solar energy and can provide a source of renewable industrial energy as well as renewable raw material for other purposes. Further, the concern for environmental pollution has given impetus to more efficient disposal of agricultural wastes, *i.e.*, other than by open burning. The same concern necessitates, for example, newer paper additives which are completely retained in the sheet and thus do not pollute the effluent water or which give good wet strength to paper but at the same time break down easily on repulping thus enabling the recycling of waste paper. It is really such considerations which are directing attention to more widespread industrial use of cereal crops and their by-products.

While such discussions of industrial use of cereals may appear a little paradoxical at the moment for the underdeveloped countries, traditionally desperately short of food including cereals, the principles and ideas are no doubt of general applicability, particularly the newer ideas of a rational basis for industrialisation.

II

The papers presented in the Symposium provide an overview of the current status of the industrial utilization of cereal crops, the grains as well as the residue components, discussing the current as well as potential applications. The fundamental composition, structure and properties of cereals and their components as related to such uses are also discussed in much detail.

The proceedings are divided into 4 parts. In Part I are two papers : one reviewing the economic considerations in industrial utilization of cereals, and the other the physical properties of cereals and their products as related to their potential industrial use. Incidentally the first paper, on a subject of much potential interest, if probably the weakest in the volume containing some routine stereotypes but little intrinsic information. The second paper however deals its subject with thorough comprehensiveness.

Part II contains 4 papers dealing with the structure and composition of the major cereal components—*viz.*, proteins, starch, non-starch polysaccharides, and lipids—as related to their potential industrial use. These papers provide wealth of basic data concerning the subjects and a solid background on which the subsequent discussions as well as the potential uses can be built.

Part III also contains 4 papers which discuss some general industrial uses covering all cereals, *viz.*, production of furfural, certain specific uses of cereal proteins, and the use of cereal grains as a source of industrial energy. The last paper is of considerable interest where the potential production of energy from cereal

and cereal products either directly or via their fermentation to produce ethyl alcohol and/or fuel gases are discussed. The paper (by Dwight L. Miller) ends with the following prophetic lines:

> "The development of industrial energy sources has been as follows: First, after wood, wind and waterwheels, it was coal. Gas came next, then oil, then hydroelectric power, and a half century elapsed before another new source of energy was discovered —nuclear fission. If history continues to repeat itself, another new source of energy will begin to become important yet this century. Agriculture could possibly have an essential function in the future U.S. industrial energy supply."

Part IV contains as many as 11 papers dealing in much detail with specific commodities, their structures, compositions and properties, and current and potential uses. These include corn cobs, corn starch, corn proteins, dry-milled corn products, sorghum grain, wheat starch and gluten, wheat byproducts, total utlization of wheat, barley, oats and rice hull. From an Indian standpoint, rice stands out as the cereal least discussed, but this is probably a reflection of the paucity of literature on the industrial use of rice and rice by-products, no doubt because over 90 per cent of world rice is grown and consumed in the poverty-stricken south, east and south-east Asia.

The volume ends with an annotated bibliography of selected publications of the last 25 years (1947-72), on the industrial utilization of cereals, other than starches prepared by Chairman Y. Pomeranz. As can be well appreciated, this bibliography will be of tremendous help to research workers in the field.

All in all, this massive volume will be of great help to all concerned with cereal researches and cereal industries. It is a new-look symposium which not only collects together existing information on the fundamental properties as well as current industrial uses of cereals, but also projects itself into the future by analysing the basic properties and trends and visualising the possible future uses.

III

Another very interesting feature of the volume, one which struck the present reviewer greatly, is the great changes it portends about the philosophy and practices of industrialisation in the highly industrialised countries of today.

The theme not always articulate yet present, that runs through almost the entire volume is the concern for the exhaustion of non-renewable energy sources and other raw materials and pollution of the environment through current industrial practices and in that light to weigh future trends and search newer alternatives. An interesting point made was about the efficiency of modern agriculture. While we in the poor countries have been so long suitably overawed by the supposed great efficiency of modern agriculture, for the first time people are becoming aware, as mentioned in passing by D. S. Miller in his paper on cereal grains as a source of industrial energy, that modern agriculture, far from being a means for trapping energy, possibly uses up more energy in the form of fossil fuels than the energy equivalent of the cereal grains produced. A few years ago when prodigeous waste and gigantism were still in arrogant fashion, such discussion would have invited condescending laughter. But what is more significant, whereas discussion on exhaustion of energy sources, pollution, ecological change, etc., has been raised for a few years now, so long it has been confined only to the social scientists and what might be called the social scientists among natural scientists. But a measure of the speed with which these ideas are penetrating the societies in their entirety is the fact that such ideas are now affecting even the 'hard' scientists and in fact are explicitly discussed even in such formal Symposia as the present one and in which they actually form the background on which the discussions are projected. Clearly a revolutionary change in the social, economic and industrial organizations in the affluent countries are in the offing in the near future, of which the present volume is a clear straw in the wind.

K. R. BHATTACHARYA

Food Chemistry, by L. W. Aurand and A. E. Woods, The AVI Publishing Company, Inc., U.S.A., 1973, pp vi+363, Price $ 14

A book on 'Food Chemistry' has appeared after a considerably long time. "The intent of the authors of this book was to present various principles of Biochemistry together with such other facts that were deemed most important to an effective grasp of the subject of Food Chemistry". Even a quick reading of the book shows that at least in some chapters, the principles of Biochemistry have overshadowed the main theme of the book.

The first three chapters give a physical chemistry background and deal with water, solutions and colloids. While the special properties of water and the nature of the hydrogen bond and its significance have been pre-

sented adequately, no mention has been made of 'bound water'. Though different ways of expressing concentrations have been covered, one of the most commonly used units in Food Analysis—ppm is not included. The important point that pH values cannot be averaged, which is rarely mentioned in other texts. has been pointed out and explained well. The term micelle should have been explained. Good examples have been given for coagulation and syneresis.

The next three chapters deal with the bulk constituents carbohydrates, lipids and proteins. In general, coverage of material in these chapters is adequate. Tables 4.1 and 4.2 and figure 4.5 are of particular relevance to Food Chemistry/Technology. The section on Relationship between structure and sweetness is apt and well written. However the statement 'Molecules possessing an asymmetric carbon atom exist in two mirror images' is misleading. A serious lapse under carbohydrates is the absence of any reference to non-enzymatic browing particularly because of its relevance to colour and flavour of foods. The section on lipid metabolism is more extensive than is desirable in a book on Food Chemistry. An attempt has been made at a different way of defining proteins. Under the section on denaturation, a good example of its relevance to the dairy industry—cheese making—is mentioned. Whereas so much of the text is devoted to discussing DNA, RNA and protein biosynthesis, there is no mention of any food protein say like gluten, and its relation to the functional properties.

'Enzymes' is a very good section providing sufficient back-ground as well as considerable amount of information relevant to Food Chemistry. Table 7.3 and textual matter on pages 195-206 are particularly worth mentioning in this regard. It is a pity that in such an otherwise good chapter, there is an erroneous statement: 'glucose oxidases which catalyse the breakdown of β-D-glucose to gluconic acid' (p 200).

The chapter on vitamins is a good example for the general comment that proper balance is lacking between Biochemistry and Food Chemistry resulting in an overwhelming abundance of biochemical details. This is clearly seen in: (*a*) the detailed presentation on page 209 of the role of thiamine in carbohydrate metabolism, (*b*) the stereospecificity of the reduction of NAD on p 217, (*c*) the pathway of conversion of tryptophan to niacin, p 218 and (*d*) the biosynthesis of FH_4 on p 228 and its role described on p 229. Whereas so much emphasis is laid on biosynthetic pathways, even adequate structural representation of ascorbic acid, dehydroascorbic acid and diketogulonic acid is absent. There are factual errors in addition. The vitamin (B_{12}) contains a porphyrin nucleus (p 230); 3 keto-L-gulonolactone is not the precursor of L-ascorbic acid as shown on p 234, fig 8.6. The vitamin A potency of β-carotene is 1.6 and not 1 as stated: "but this symmetrical molecule is only one-half as active as vitamin A" (p 236).

While the presentation on minerals is unique because of the inclusion of an account of atomic absorption spectroscopy and flame photometry, it is grievously lacking in describing the role of minerals in the texture and colour of foods. Photosynthesis and Fermentation and Glycolysis are brief but could have been further abridged without any loss to the textual value.

The appropriateness of a chapter on Respiration in a Food Chemistry book would be to point out the manner in which most of the organic acids of importance in foods are produced. This is totally obscured by such a detailed presentation of the pyruvate dehydrogenase complex, α-ketoglutaric dehydrogenase complex, etc. This is to be contrasted with the very little information given regarding the role of organic acids in food quality. Yet information on pages 205-308 is a redeeming feature.

The last three chapters in the book 'Food and Energy', 'Food Glycosides' and 'Flavouring Compounds' are good examples of a judicious blend of Biochemistry and Food Chemistry.

A chapter on carotenoids is conspicuously absent in the book. Carotenes are so important as food colours and the technology of making water soluble 'beadlet' carotenoids is very well developed. Yet 'the biosynthesis and function of the carotenoids is still under investigation' is almost all that the authors have said about the carotenoids.

The text is not tersely written in several places. Some examples are: Glycogen is one of the most important *biochemical* substance (p 83); the *occurrence* of vitamin B_6 is so *wide-spread* (p 220); metal ion-ligand interactions and their role in *Biochemical* Nutrition (title on p 249); in the *metabolic* enzyme xanthine dehydrogenase (p 262); the reaction takes place in the soluble portion of the *extramitochondrial* cytoplasm of cells.

Bibliography is adequate and the get up as is usual with AVI publications, is good. In spite of the deficiencies mentioned above, the book will be a good and useful addition to any Food and/or Home Science Department Library or any Food Research Institute Library.

N. CHANDRASEKHARA

Establishing a Manufacturing Plant in Europe: Noyes Data Corporation, Mill Road at Grand Avenue, New Jersey 07656, U.S.A., 1974, pp 373, Price: $ 28.

The aim of the book is to provide factual information regarding the investment climate in the twenty-two countries of Europe. The countries covered are not only those with backward economies which are trying to catch up with the rest, but also the highly industrialised countries like Germany, France and U.K., which are trying to develop their backward regions and have comprehensive system of incentives linked to their regional development plans. These national authorities are trying to level off the regional discrepancies through fiscal incentives, investment grants, low-priced industrial sites, employment premiums, etc. The new nations like Malta, Yugoslavia and Cyprus are giving various incentives for industrial development to any one who wants to invest in these countries.

The book gives valuable information regarding the recent industrial development, forms of business organizations, taxation and tax concessions, cost factors, like land, labour, etc., infrastructure like power, communications, repatriation of capital and remittances of profits to foreign countries and agencies concerned with industrial growth, banks and financial institutions for each of the twenty-two countries.

The book may not be of any interest to the Indian entrepreneurs who may be considered as minors compared with their counterparts in America or Europe. The methods and systems of incentives for industrial growth, adopted by the European governments with capitalist economies where free play of private competition is the order of the day, may not be suitableto those developing nations of the third world who are trying to evolve a new strategy for solving their socio-economic problems. The book may be of much value to the American investors in European markets and it is mainly intended for them only.

A. NARASIMHA RAO

Food and the Consumer, by Amihud Kramer, The AVI Publishing Co., Inc., West Port, Connecticut, 1973, pp 256, Price domestic $ 6.50 and foreign $ 7.50.

According to the author the main purpose of his modest effort is to sort out food facts from food fancies in the field of food science and nutrition and provide a summary of educational material for the general consumer.

First chapter gives, the general outline of historical aspects of growth of population and foods and related problems in developed and developing countries emphasising the concepts of labour reduction, efficient transportation and newer packaging developments for the former and waste reduction and efficient management of water, sea foods, vegetable proteins and foods based on micro-organisms for the latter to obtain the over all objective of human survival and meeting the global food requirements for the coming century. Chapter two discusses the nutritional requirements of Americans and concludes that major malnutrition and health problems like anemia, obesity, defective teeth, atherosclerotic heart disease, diabetes mellitus and osteoporosis are amenable to dietary treatment combined with appropriate exercises. Chapter three discribes various aspects of quality control of foods from the stage of raw material production to the final consumption of processed item. In Chapter four, consumer protection aspects by suitable nutritional labelling, ensuring correct quantity, drained weight, identity, wholesomeness and freedom from toxicants enforced through Federal Food and Drug Law and standards laid down by Industry and Agricultural Marketing Service are discussed. Chapter five deals with food codes and habits and possible reasons for their origin and evolution through the ages. In Chapter six principles of food preservation based on control of microbial action, enzymatic degradation and chemical and physical changes are discussed along with development of techniques like canning, pasteurization use of ionizing radiations, refrigeration, freezing,, dehydration to low moisture and intermediate moisture foods, chemical inhibitors, fermentation, gas exchange by controlled atmosphere storage and control of non-enzymatic browning food poisoning and food infections. Chapter seven describes the convenience, foods like bread and wine, processed juices and drinks, cereal snacks and starches, instant coffee and tea, processed fruits and vegetables, egg, milk, meat and their products prepared specialities, imitation foods and nutritionally complete foods. Chapter eight deals with various aspects of packaging and marketing and describes tin, glass, paper, flexible films and pressurized containers and disposal problems. In Chapter nine, problems like minimising wastes, manufacture and preservation of by-products, conservation of food by minimising animals that compete with man and short-cutting nature's cycle and disposing of residual wastes are dealt with. In the last chapter, predictions of foods of the future. are made based on existing knowledge and past trends Science fiction predicted food pill is ruled out, though more and more of future foods will be concentrated, weigh less and occupy less space than today. Reduction

of form, transportation and packaging costs, automation of processing and ware housing, efficient marketing and distribution of food, utilization of fish and wild life, efficient control of insects, diseases and other pests, efficient water management, farming the seas and evolving overall global strategy to conserve foods are likely to solve the food problem to the satisfaction of future generations.

The book gives a general overall picture of present status of food problem affecting the consumers all over the world and speculates on future trends.

The general get up of the book is excellent. It will be a useful addition to the libraries of Agricultural Universities and Food Science and Technology institutions.

B. S. BHATIA

European Food Processing Industry: Noyes Data Corporation, Mill Road at Grand Avenue, New Jersey 07656, U.S.A., 1973, pp 263, Price: $36.

This book deals with the position of food industry in seventeen selected countries of Europe. So far, there is a lack of adequate information on Food Processing industries in Europe and this book tries to fill up this gap now. Complete information has been gathered in this book regarding the structure of the industry, statistics of production, consumption and trade and specific features of the industry for which certain countries are famous. It gives the export and import potential of the concerned countries in processed foods. There is an exhaustive list giving the names and complete addresses of about 2,000 leading food processors of Western Europe.

This book will be of much interest to the American investors, who may try to develop super markets, self-service stores and well-organized restaurants in Europe.

A. NARASIMHA RAO

Health Hazards of the Human Environment: World Health Organization, 1211, Geneva, 27, Switzerland, 1972, pp 387, Price $11.00 (SW. Fr. 44).

The publication includes a wide range survey of environmental hazards to human health for the benefit of health authorities and others concerned with environmental problems. The contributors and reviewers in the book are drawn from the list of 100 well-known exponents. Their review has made the content of the book not only suitable for a generalist but also specialist in the field. Some of the chapters although controvercial in content but due to the highly complex inter-relationships between the environmental factors and health, the results may pose appreciable uncertainty on many issues on which judgements and decisions are required by health authorities. It appears from the book that in general, prescription for action to protect human well beings will have to be adapated to the conditions existing in different countries. Nevertheless, the areas covered in the book offer sufficient information to contribute to the development of a rational and integrated approach both short-term and long-term to environmental health problems in individual countries and also at international level. The chapters in the publication are distributed in 4 parts. (i) The Community Environment; (ii) Chemical Contaminants and Physical Hazards; (iii) Surveillance and Monitoring; and (iv) Public Health Principles and Practice of Intervention.

The environmental hazards from the standpoint of the media such as air, water and food have been appropriately discussed comprehensively. The role of insects and rodent vectors, the home environment, the work environment, climate and altitude, transport hazards, environmental influences on mental health are important aspects of the problem. The sources and type of pollutants have been discussed in a general way. Even the air borne biological agents such as pollens and micro-organisms have been treated as the environmental factors that may have adverse health effects along with other pollutants dealing with the chemicals which are potentially hazardous.

In Part II, the chemical contaminants and physical hazards have been presented in six chapters such as laboratory toxicity tests; selected environmental pollutants; special problems mutagens, carcinogens and teratogens ; ionising radiation, non-ionising radiation and UV waves and lastly the noise.

The inter-relationships of the parasite and environment and inter-relationships between the host and environment are treated from the point of view of the effects on health, physical, chemical and social factors. Because of the special problems, the environmental contaminants which act as mutagens, carcinogens and teratogens are dealt at great length.

In the selected environmental pollutants—arsenic, cadmium, lead, mercury and other metallic contaminants, asbestos, carbon monoxide, ozone and photo-

chemical oxidase; oxides of nitrogen, fluorides, nitrates, nitrites and nitroso compounds DDT and related compounds, polychlorinated biphenyl have been cited as examples and relative literature have been reviewed.

Chemical teratogenesis like chemical mutagenesis is an emerging area of pharmacological investigation. The present understanding of mechanism of action in this field is inadequate. There is profound ignorance as to the mode of action of teratogens. Large number of teratogens listed in the chapter 13, could form the basis for further knowledge to be acquired on their biochemical events in normal embryogenesis and better understanding of teratogenesis.

In the Part III a prime need for the development of a system of integrated surveillance and monitoring on man's health and well being in relation to the environmental factors, so that advance action may be taken to avert major dangers that would otherwise take him by surprise. In the text it has been emphasised that the fundamental requirements of any such system is built in flexibility so that both orderly change and unexpected developments can be readily and profitably accommodated without comprising the entire structure.

The Part IV, standpoint of public health operations, the principle and practice of intervention and chemical control procedures, some approaches to evaluation and control environmental health hazards have been discussed. It has rightly recognised that the political, economic and cultural differences by themselves make it difficult to formulate criteria and standards that could be universally applied to problems such as air and water pollution and food contamination.

The environmental health standards acceptable or permissible limits of concentration have been established to protect a defined population from the undesirable effects of a specified exposure to one or several environmental hazards. This has been admirably treated in this publication. The primary protection standard, acceptable daily intake and maximum permissible intakes, are some of the important proposals which form components of regulatory measures. Many countries are now tending to consolidate their various items of legislation of pollution control and to introduce Laws with wide scope thereby covering the environment as a whole. The environmental Protection Law of Sweden; the Basic Law for Control of Environment Pollution of Japan and Singapore Environmental Public Act are the examples of such a procedure to achieve better standard of environment. The Clean Air Act of U.K. and Environmental Protection Agency of U.S.A., have already achieved significant improvement in the fast deteriorating environment. The subject like air quality, water quality, occupational exposures have been treated in this publication at depth of guidance particularly of the developing countries.

The last chapter in the book deals with the sanitation technology. It is indeed that filariasis, hookworm infestation, enteritis, amoebic infection are so common are needed to be controlled by systematic application of the sanitation technology. Sanitation technology is well established and provides protection against disease transmitted by contaminated water or food, or by insect vectors. In this chapter apart from dealing with the advanced technology in the field of 'Community water supplies', 'Excreta' and 'Waste water Disposal', also covers 'Solid Wastes', 'Vector Control', 'Food Sanitation' and 'Air Pollution'.

In general, this book is a comprehensive treatise on the subject of topical interest.

S. K. MAJUMDER

Toxicological Evaluation of Certain Food Additives with a Review of General Principles and of Specifications: Seventeenth Report of joint FAO/WHO Expert Committee on Food Additives, World Health Organization, 1211, Geneva 27, Switzerland, pp 40, SW. Fr. 5.

This is the Seventeenth Report of the Joint FAO/WHO Expert Committee meeting on 'Food Additives' held in Geneva from 25th June to 4th July 1973. This covers a review of the (1) principles of evaluating the safety of food additives; (2) toxicological re-evaluation of the food additives; (3) the specifications of some common additives; and (4) the approach to the re-evaluation of flavouring substances. The substances included in this re-evaluation are anticaking agents, antimicrobials, antioxidants and synergists, emulsifiers and stabilisers, thickening agents and other groups of substances unrelated functionally and structurally. The Committee examined under each of these categories acceptable daily intake in the light of new data ; the recommendations are compiled in a tabular form. In addition, procedures for testing additives particularly for short-term and long-term toxicities have also been outlined. For those eng-aged in toxicology of foods this is a very valuable book as it keeps them abreast with the current information on various related topics and provides valuable guidelines to assess the safety or otherwise of common food additives.

V. SREENIVASA MURTHY

Menu Planning, by Eleanor F. Eckstein, The AVI Publishing Co., Inc., West Port, Connecticut, U.S.A., 1973, pp. 318.

Menu planning is of great importance in providing a nutritious and acceptable diet to the average consumer. It helps to make the best use of available food supplies for feeding the different categories of the population both in food deficit and food surplus countries. The book under review gives an excellent account of the subject. The book is divided into four sections, namely, (1) Mechanics of menu planning, (2) Menu planning for sub-groups, (3) Menu planning and computerization, (4) Food ways of American sub-group cultures. In the first section, the author has discussed various aspects of the mechanics of planning menus such as nutritional requirements, food habits, number of persons to be served, funds available, avoidance of monotony, acceptability of the menus, availability of foods, menu planning, casting, etc. In the second section, the author has discussed the scientific aspects relating to the planning of menus for various groups such as hospitals, patients, home residents, children, college students, industrial workers and restaurant customers. For planning menus for patients in hospitals various factors such as psychological or emotional condition of the patient, nutritional requirements and meal pattern, adequate service facilities, etc., have to be taken into consideration and these are discussed in detail. The various problems involved in providing suitable menus for geriatric patients in nursing homes and retirement homes are discussed in the next two chapters. In the following two chapters, the nutritional and acceptability considerations in planning suitable menus for children in nurseries, elementary and secondary schools, adolescents in colleges and handicapped children are discussed. The next three chapters discuss the various aspects relating to the planning of suitable menus for industrial canteens, for adults in state institutions and restaurant customers. In the third section, the author has discussed the use of computer in menu planning and computerizing the menu planning system. The first two chapters discuss the basic concepts made by various workers in using the computer for menu planning. In subsequent chapters, collection of various types of data programme techniques and testing procedures are discussed. In Section four, the author has discussed the food habits and pattern of diets consumed by the various sub-group cultures settled in U.S.A., such as Native Americans, Puerto Ricons and Cubans, Jews, European-Americans, Chinese Americans, Japanese Americans, Russian Americans, Indians and other orientals settled in U.S.A. The data will serve as a guide in planning menu to meet the needs of various sub-group cultures.

The book will prove highly useful to Dietians and Administrators in planning suitable menus to meet the nutritional needs of various categories ofthe population and in the management of catering establishments.

M. SWAMINATHAN

Food Analysis Laboratory Experiments, by Clifton E. Meloan and Yeshajahu Pomeranz, The AVI Publishing Co., Inc., West Port, Connecticut, U.S.A., 1973, pp 143, Price: Domestic, $ 24, Foreign, $ 25.

This Manual plans practical experiments in food anlaysis and is a supplement to the authors' earlier book "Food Analysis: Theory and Practice". It is divided into thirty-three chapters, of which the first twenty-three exclusively cover various instrumental methods of analysis currently in vogue, *viz.*, visible spectroscopy, turbidimetry, fluorimetry, infra-red spectroscopy, flame photometry, atomic absorption spectroscopy, potentiometry, conductivity, polarography, disc electrophoresis, column, gel filtration, ion exchange, thin layer and gas chromatography, the Craig apparatus, dialysis, floatation, molecular distillation, polarimetry and refractometry. Chapters twenty-four to twenty-nine are devoted to proximate analysis, *viz.*, moisture, ash, carbohydrates, crude fibre, lipids and nitrogenous compounds. Chapters thirty to thirty-three include enzymatic methods, vitamin assays, determination of chemical oxygen demand and mycotoxins.

Methods given are well tried ones or those recommended by well-known organizations and need hardlyany comment. In a few chapters, alternative methods are given and the method to be chosen is generally dependent upon the food sample to be analyzed. The principle scope and applicability of the method are first outlined and is succeeded by equipment and chemicals needed for each experiment. This approach greatly simplifies the work of the student. The samples needed and the procedure then follow. Review questions asked at the end of each chapter ascertain the overall understanding of the experiment by the student. References given suggest the source(s) for further study.

The manual includes a number of figures, useful tables and abbreviations and symbols followed by American Chemical Society Journals and a subject index.

A number of general review questions are given at the end of the book which stimulate the interest of the

student in food analysis and justifies the need for the same.

Simplicity and direct approach to the experiments are the keynote of the methods followed. The Manual deserves to be read and followed by students taking courses in food analysis and food technology.

S. RANGANNA

Essentials of Food and Nutrition, Vol. 1, Fundamental Aspects, Vol. 2, Applied Aspects, by M. Swaminathan, Ganesh and Company, Madras-17, India, 1974, Price: Vol. 1 : Rs. 30 ($10.00), Vol. 2 : Rs. 20 ($8.00).

The above volumes entitled "Essentials of Food and Nutrition" have been just released for publication. Dr M. Swaminathan is an established Nutritionist in India and is well known for his contributions in the area of nutrition for more than two decades. Volume 1 deals with Fundamental Aspects of Food Science and Nutrition and Volume 2 deals with the Applied Aspects. These volumes are intended to be an introductory book for under-graduates and also for Post-graduates of Food Science, Nutrition, Food Technology and Home Science. A wide range of interest is covered in these two volumes.

The fundamental aspects of Food Science are briefly summarised in Volume 1. A brief history of Nutritional Sciences followed by a discussion of the nutritional significance of dietary constituents such as proteins, fats, carbohydrates, vitamins and minerals is covered in the first ten chapters. A brief account of the chemistry, metabolism requirement and nutritional significance of these constituents has been appropriately given.

A comparative view of recommended allowances for various nutrients is provided. Recommendations made by the Nutrition experts group of the Indian Council of Medical Research, the National Research Council, U.S.A. and the expert group of FAO/WHO are listed. The nutritional requirements of different age groups are adequately summarised. In particular the discussion of the nutrition of infants, pre-school children and expectant and nursing mothers who form the most vulnarable section of our population, is most useful.

The use of processed foods and other supplements for infants is well discussed. An appendix on the chemical composition of food materials in India is included in both volumes.

The summary on nutritional aspects of the vitamins, etc., are brief and lucid. The list of references listed at the end of each chapter, however, is mostly restricted to publications up to the years 1960-62. It is hoped that in subsequent editions of these volumes, recent published work will be added. Some examples of recent work of great interest in the area of nutritional research are (i) the transport of vitamin A in plasma by a specific protein (retinol binding protein), (ii) active metabilites of Vitamin D, and (iii) studies on significance of zinc deficiency in humans. The above aspects have not been fully elaborated.

Volume 2 covers a very wide range of interest. The first seven chapters are of interest to Food Technologists. Food preservation and storage aspects and fortification of foods tailored to meet specific nutritional needs and standards for food hygiene and quality are discussed.

The application aspects of Food Science and Nutrition particularly of interest to para medical personnel, workers in the applied nutritional programmes and students of public health are also adequately indicated. The chapters on diet surveys, methods of assessment of nutritional status and nutritional education should be of particular interest to field workers in the public health and nutritional welfare programmes.

It is my opinion that these two volumes fulfill the much needed introductory books or guides to students of nutrition and food sciences and to field workers in nutritional welfare programmes.

P. B. RAMA RAO

Proteins from Hydrocarbons : The Proceedings of the 1972 Symposium at Aix En Provence and Relevant Guidelines of the U.N. Protein Advisory Group, Edited by H. Gounelle De Pontanel, Academic Press, London and New York, pp 285, Price £ 5.

The Symposium was designed to review the work done on proteins from single cell organisms (yeasts) on hydrocarbon substrates. The occasion was used to take stock of results, and to carry out preliminary experiments on their use in human food under the supervision of qualified nutritionists and biologists.

The proceedings largely falls under two broad heads, namely,

(i) Papers presented on the topic of 'Proteins from Hydrocarbons' covering about 230 pages ; and

(ii) Protein Advisory Group Guidelines, covering the rest of 50 pages and a short subject index of 5 pages.

The main contributions of the Symposium centre round the papers presented on the following topics :

Single cell proteins, the nutritional value of the yeast, safety evaluation of the yeast, conventional and unconventional foods, yeasts grown on alkanes and food requirements, the psycho-sociological problem involved in relation to new foods alkane grown yeast, the future outlook for feeding the human race.

We have relevant discussions at the end of each paper and conclusions at the end. In addition to the above proceed ngs we have relevant guide ines of the UN Protein Advisory Group, which includes : Statement No. 4 on single cell protein, No. 7 for human testing of supplementary food mixtures, No. 8 on protein rich mixtures for use as weaning foods ; and No. 12 on the production of single cell protein for human consumption.

The present Symposium held in 1972, brings together the problems involved in the commercial production of proteins from hydrocarbons with a view to their use in animal feeds and human foods. Considered from the nutritional point of view, the paper presented by Shacklady and Gatumel on the nutritional value of the yeast and the companion paper on safety evaluation of the yeast by Engel are of great interest and importance. The two factors which limit the immediate use of protein from hydrocarbons relate to (*a*) margin of safety which can be related to the residual gas oil in the biosynthetic protein, and (*b*) the high level of nucleic substances in the yeast.

The present publication contains useful and well documented information on 'Proteins from Hydrocarbons'. The PAG Guidelines would form a ready reference material to all those who are interested in using new types of proteins in human nutrition. Sometime back, high hopes had been raised about the possibility of filling the protein gap by this source, but the current trend in respect of the cost and availability of hydrocarbons at economical rates would pose a problem for which no easy solution is in sight. The publication is welcome as a useful and up-to-date presentation of information on " Proteins from Hydrocarbons".

T. N. RAMACHANDRA RAO

The Microbiological Safety of Food : Edited by Betty C. Hobbs and J. H. B. Christian, Academic Press, 1973, pp 487, Price : £ 7.50.

This publication is a record of the Eighth International Symposium on Food Microbiology with special reference to the " Microbiological Safety of Food". This was organised by the Committee on " Food Microbiology and Hygiene of the International Association of Microbiological Societies" and held at Reading, England, in September 1972. The main objectives of the Symposium are as follows :—

(i) The microbial safety of the food, or more specifically the prevention of food poisoning.

(ii) To compare the prevalence and type of food poisoning in different countries, to analyse the differences, and to study the ways in which the disease may be prevented.

(iii) To consider what may be done—technically, educationally, or by legislation—to lower the incidence of food borne disease.

(iv) To pool the knowledge and experience of workers—medical, veterinary and industrial—in their various fields.

In all forty-one papers were presented in the following five Sessions. At the end of each paper the relevant references are given followed by a discussion. Finally we have summing up by Sir Graham Wilson :

1. Bacteriology of Various Commodities in Relation to Food Poisoning. Part 1 and Part 2. Chaired by : M. Ingram and E. Hess respec tively.
2. Epidemiology of Food-borne Infection in Man and Animals. Part 1 and Part 2. Chaired by : Graham Wilson and B. Weitz respectively.
3. Special Laboratory Techniques. Chaired by : Betty C. Hobbs.
4. Legislation and Non-legal Specifications. Chaired by : M. Ingram.
5. Education. Chaired by : F. A. Aylward.

The incidence of food poisoning is generally on the increase due to communal feeding, pre-cooked food, bulk distribution, importation of new foods and expanded laboratory services. We now have a fairly complete list of organisms responsible for food poisoning, though the mere presence of certain organisms in enormous numbers in the food may prove toxic. The symposium brought out the fact that Food

Epidemiology has lagged a long way behind food bacteriology and before we can learn the full extent of food poisoning, we shall have to institute a thorough system of surveillance. Measures for the prevention of food poisoning were discussed at length. The institution of training courses for all classes of food workers was considered desirable.

The papers presented, and the discussions that followed are well documented. The various facets of the problem 'Microbiological Safety of Food', have been covered in depth as well as in range. The scientists who presented the papers are the leading workers in the field and this lends a stamp of authority and urgency to the problems of food safety. We should congratulate the editors : Betty C. Hobbs and J. H. B. Christian for bringing the Proceedings of the Symposium in this form. One need hardly emphasise that the publication under review is a valuable document on this topic and deserves the serious attention of Scientists and Administrators interested in "Microbiological Safety of Food".

T. N. RAMACHANDRA RAO

Australian Scientists See Vegetable Protein as Substitute for Meat

Scientists in Australia are investigating large-scale use of vegetable protein as a substitute for meat.

They believe it may be more efficient to convert vegetable protein seeds into human food through a factory process than by feeding an animal for eventual slaughter and the dinner table.

One of the economic arguments supporting this theory is that meat production requires at least 10 times as much land as is needed to produce a similar quantity of soy protein product. This means more land would be available for growing other crops.

Textured vegetable protein (TVP) is made by extruding high-protein plant materials such as soy flour through a small nozzle at high pressure and temperature. The sudden release of pressure makes the protein fluff into an open-textured product.

Several Australian firms will begin production of TVP from soybeans within a few months. Previously such meat substitutes were imported from the United States, supplying mainly a small vegetarian market, and providing an ingredient for a variety of manufactured foods.

Among the advantages of TVP foods is their indefinite storage life, through either refrigeration or dehydration, and the fact that there is no waste in preparation for cooking. TVP foods are also virtually free of cholesterol. Nutritional properties such as amino acids, vitamins and minerals can be adjusted during manufacture.

Extruded TVP will look like ground meat, meat pieces, or meat chunks. According to the scientists, it will be ideal for making stews and casseroles. Eventually they expect to manufacture meat analogues—vegetable protein that looks like chicken or steak, complete with plastic bone and taste to match the real thing.

Seminar on Autoxidation and Stabilization of Fatty Foods delivered by Dr M. K. Kundu, D.Sc., Dept. of Applied Chemistry, Calcutta University, Calcutta-9, at CFTRI, Mysore, on 7 October 1974.

The mechanism of autoxidation and stabilization of fatty foods has been reviewed. The importance of the problem of efficient storage of fats and oils has been shown with special reference to the Indian context. It has been shown that a basic understanding of the mechanism of autoxidation is helpful in the practical tasks connected with the stabilization of fats and fatty foods during processing and storage. As regards mechanism of autoxidation the following aspects have been discussed:

(1) the key steps involved in autoxidation;

(2) reactions involving secondary degradation products of lipids which include cleavage of the hydroperoxy radicals—the first major group of oxidation products of the unsaturated fatty acids—to alkoxy and hydroxy free radicals and the subsequent reactions of the alkoxide radicals to form a host of compounds some of which are of the same, higher or lower molecular weights and some of which contribute to the off-flavour and off-odour associated with the autoxidized fats;

(3) the kinetics of autoxidation reaction and its dependence on the reaction conditions and the nature of the substrate moleculess;

(4) the various tests such as peroxide values, benzidine values, acid values, etc., available for determining the progress of autoxidation, *i.e.*, measuring the fat stability;

(5) reactions of oxidized lipids with proteins; detection and determination of the various fractions formed by gel permeation chromatography using a double detector system, namely, differential refractometer and UV detector;

(6) nutritional value of the lipid-protein interaction products;

(7) a possible reaction sequence involved in lipid-protein interaction; and finally,

(8) the factors determining the rate and course of autoxidation have been summarised.

As regards the problem of stabilization the following aspects have been discussed :

(1) Role of antioxidants in retarding or preventing autoxidation;

(2) inversion of autoxidative activity of antioxidants to prooxidative effects at high concentration;

(3) the mode of action of the antioxidants;

(4) the effect of synergists and their role;

(5) determination of protection factor for evaluating the antioxidative activity of an antioxidant;

(6) desirable qualities expected of a food antioxidant;

(7) classification of antioxidants based on their use in various industries such as food, petroleum, rubber industries;

(8) relationship between antioxidant activity and structure of the antioxidant compound;

(9) determination of the redox potential of an antioxidant as a criterion for predicting the antioxidant activity;

(10) comparative prooxidant effects of metal traces in oils; and finally

(11) the mechanism of prooxidant action of the metal traces and inactivation of the metals by binding them into inactive compounds.

Seminar on New Development in Analytical Methods : Direct Determination of Metal Traces in Oils by Nonflame Atomic Absorption Spectrophotometry delivered by Dr M. K. Kundu, D.Sc., Dept. of Applied Chemistry, Calcutta University, Calcutta-9, at CFTRI, Mysore, on 9 October 1974.

The influence of metal traces on the development of rancidity leading to spoilage of fats and oils has been well documented. Quantitative analysis of metal traces in fats and oils is therefore of importance for quality control. In this context, studies were made of several effects to enable direct determination of metal traces in oils at ppb levels with improved accuracy by atomic absorption spectrophotometry, using nonflame atomization. The results show that an improvement of the sensitivity level of the method is possible by proper adjustment of the nitrogen flow rate during atomization and that complete destruction of the organic matrix (lipid materials) can be achieved at lower temperature, within 500 °C in less than 2 minutes under an atmosphere consisting of 2 volumes of oxygen and 1 volume of nitrogen flowing at 0.9 l/min. Determination of iron presents a special complication in that its atomization is usually accompanied by a number of secondary peaks due perhaps both to chemical interaction and to optical interference. However, the problem of interference due to secondary peaks can be eliminated or minimized by proper adjustment of the inert gas flow rate. In case of copper, the absolute detection limit attained was 20×10^{-12} g, representing 20 μl of 1 ppb copper in 40 per cent solution of oil in i-amyl acelate. The relative standard deviation data determined for 100, 25 and 15 ppb of copper in 50 per cent solution of oil in i-amyl acelate were 3.5, 7.5 and 13.0 per cent respectively. The absolute detection limit attained for iron was 10×10^{-11} g, representing 20 μl of 10 ppb iron in 20 per cent solution of oil in i-amyl acelate.

Annual General Body Meeting of the Eastern Regional Branch

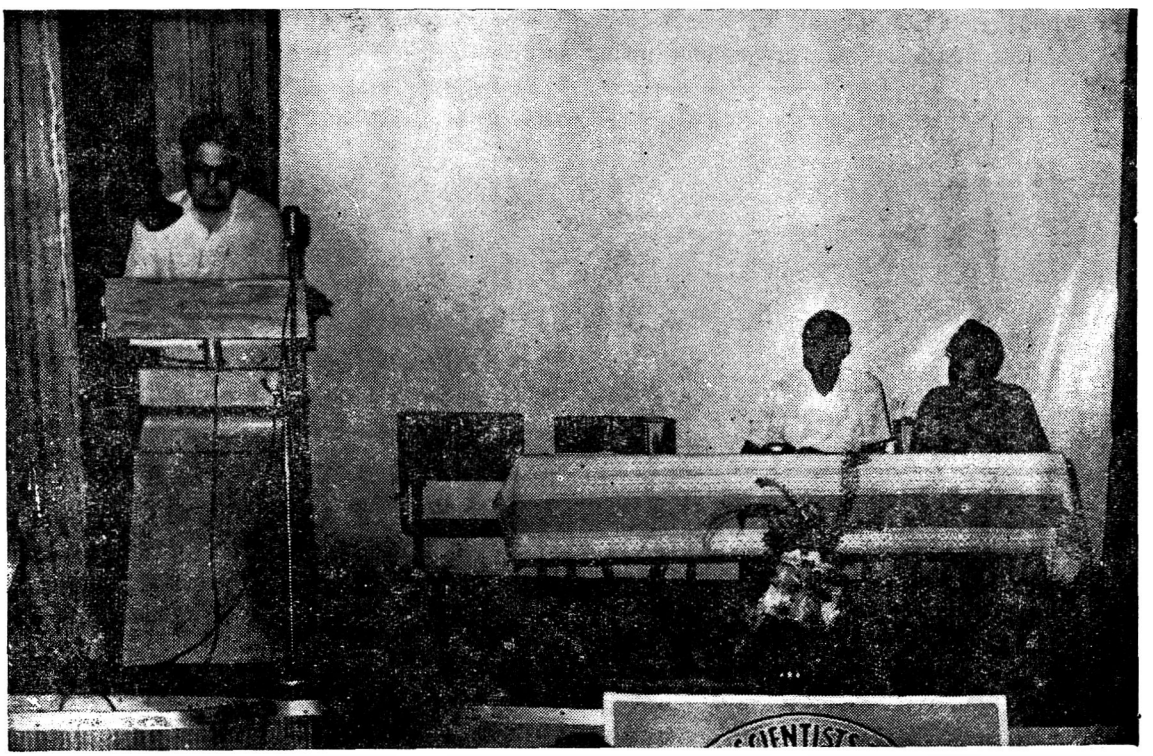
The Annual General Meeting of the AFST, Eastern Regional Branch, was held on 13th July 1974 at the Birla Industrial and Technological Museum, Calcutta.

Mr N. C. Ray, took the chair. Secretary's report and audited statement of accounts were passed in the meeting. The following members were elected as office-bearers for the year:

1.	Mr K. C. Dé ..	President.
2.	Prof Sunit Mukherjee	Vice-President.
3.	Mr S. K. Das Gupta ..	Hon. General Secretary.
4.	Mr A. K. Banik ..	Hon. Joint Secretary.
5.	Mr P. Chattopadhyay	Hon. Treasurer.
6.	Prof A. N. Bose ..	Committee Member.
7.	Mr N. C. Roy ..	Do
8.	Mr B. S. Narayana	Do
9.	Mr P. K. Bose ..	Committee Member
10.	Mr A. K. Sen ..	Do
11.	Mr R. N. Ghosh ..	Do
12.	Mr B. N. Srimani ..	Do
13.	Dr R. N. Dutta ..	Do
14.	Mr R. K. Rao ..	Do
15.	Dr G. C. Bhattacharyya	Do

Address by the Chief Guest, Shri Shankar Ghose, Minister-in-Charge, Finance, Development and Planning, at the Inaugural Session of the 14th General Body Meeting of the Eastern Regional Branch of the Association held in Calcutta, on 13th July 1974.

The problem of shortage of food in our country is not new. Shortly after independence it was realised that with limited agricultural land and increasing population there was no other alternative but to take recourse to more intensive cultivation of crops on scientific lines for increasing the yield from land. Since independence



Dr B. L. Amla, Director, Central Food Technological Research Institute, addressing the gathering as guest speaker at the 14th Annual General Meeting held at Calcutta on 13th July 1974

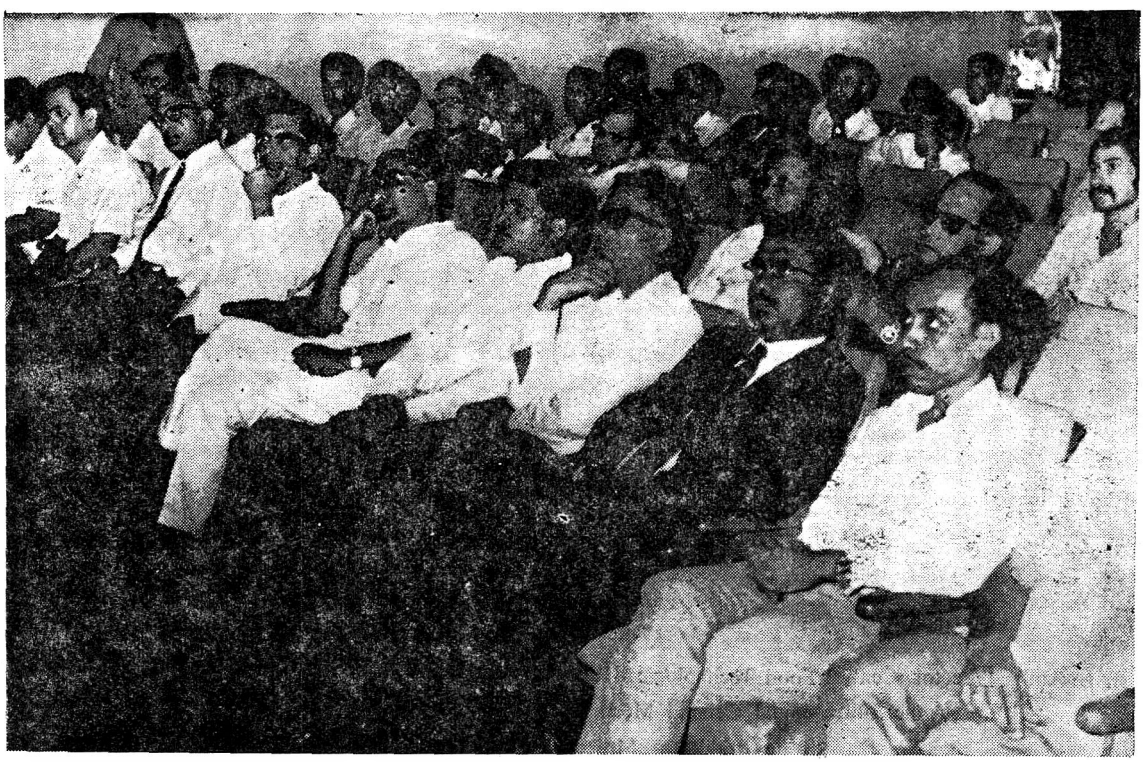
Sitting (Middle) : Mr N. C. Roy, Branch President.
Mr K. C. Dé, Vice-President.

dynamic changes have taken place in agriculture. The discovery of high yielding varieties of seeds and the adoption of modern technology of production have brought about these changes. The level of cereal production rose by nearly 110 per cent from about 45.8 million tonnes in 1950-51 to 96.6 million tonnes in 1970-71.

It is the pioneering research and hard work of food scientists, agricultural scientists and technologists that have given us high yielding variety seeds, pesticides and different types of fertilizers. In Punjab and other places of North India the introduction of new varieties of wheat and certain high yielding strains bread out of them were responsible for ushering in the revolution in regard to wheat.

In regard to rice also certain high yielding varieties developed by the International Rice Research Institute as well as our Indian Council of Agricultural Research are now being used extensively throughout the country. But in the case of rice the success has not been as spectacular as in the case of wheat. The all India average production of rice rose from 6.7 quintals per hectare in 1950-51 to 11.5 quintals per hectare in 1971-72. In the case of wheat the average yield during the same period rose from 6.6 quintals per hectare to 13.6 quintals per hectare. The likely achievement in 1973-74 in regard to areas under high yielding varieties of wheat and paddy in the country may be of the order of 10.8 million hectares and 9.5 million hectares respectively. In West Bengal the total area under high yielding variety of cereals in 1971-72 was 1.09 million hectares producing 28 lakh tonnes.

In order to bring about the full potential of these high yielding variety seeds it is also necessary to apply new techniques of cultivation. First of all large quantity of water supply accompanied by proper water control techniques like irrigation, drainage and proper management is essential. Heavy dose of fertilizers and pesticides are also indispensable. This necessarily implies that the new varieties demand not only modern technology but also much more capital to finance. The food scientists, agricultural scientists and technologists will now have to devise suitable means so that the high yielding variety seeds can be utilised with the minimum possible amount of chemical fertilizers which is increasingly becoming a scarce commodity. A co-ordinated effort of suitable crop rotation will also have to be introduced in order to increase our food production.



Gathering of the members and the invitees at the 14th Annual General Meeting held at Calcutta on 13th July 1974.

We have introduced various methods for increasing our food production with some success in certain areas, and we will have to continue to make our best endeavour in this direction. But production alone is not enough, we must also be careful about the extent of wastage between "the stalk and the stomach". Certain losses occur during reaping, threshing, winnowing and transport to the place of storage. Driage alone accounts for a loss of one per cent. Transportation from the farm site to the local market also causes some wastage. There has not been any firm estimate of wastage and whatever figure that is available seems to be based, more or less, on guess work. But it is believed by many that wastage from all causes may be as much as 5 to 6 per cent of the gross production. The all-India total gross production of cereals in 1972-73 was 85.7 million tonnes and 5 or 6 per cent of this is quite a substantial amount. We have to find out means for preventing this wastage.

Marketing, warehouses, cold-storage, etc. are yet to be developed on modern lines. A substantial amount of foodgrains can be saved from being wasted if proper warehousing facilities are made available. During the Fourth Plan period certain measures have been taken to improve storage facilities at the farm level. During the Fifth Plan period greater efforts will have to be made for giving storage facilities to the farmers. Post harvest technology would have to be developed for each area and should be tested and demonstrated in the pilot project areas so that the farmers can easily learn and adopt the techniques to their advantage.

I wish this Session all success.

Symposium on "Fish Processing Industry in India"

The above symposium being organized under the joint auspices of AFST and CFTRI is proposed to be held on 13th and 14th of February 1975 at Mysore. Papers pertaining to different areas of fish and fish processing will be presented in the following Technical Sessions:

1. Raw Material-Resources, availability, potentialities and handling.
2. Freezing of fish.
3. Processed fish products.
4. Traditional fish products, by-products.
5. Machinery and equipment needs of fish industry.
6. Quality control and marketing.

The organizers are overwhelmed by enthusiastic response received from the industry, R. & D. workers and other agencies. A Technical Committee with Dr M. N. Moorjani, Project Coordinator, Meat, Fish and Poultry Technology Division, CFTRI, is screening the papers received so far and the final technical programme is expected to take a firm shape by the end of December 1974.

A Souvenir containing invited articles from a few leading experts is being brought out on the occasion of the symposium. Assistance from the industry for conducting the above symposium is expected to be channelled through advertisements that can be inserted in the proposed souvenir. Members of the Association are requested to assist this venture by personal influence and persuation of their colleagues in the Industry in extending their support. Advertisement materials are to be received at this office by 31st December 1974.

Proceedings of the Seminar on Protein Fortification of Foods

The Seminar was organized by the Eastern Regional Branch of the Association in February 1969 at Jadavpur University. Few copies of the Proceedings are available now for sale. It contains articles on Ingredients of fortification, vehicles of fortification, sociological and economic aspects of fortifiction. The copies can be had from the Hon. General Secretary, AFST, Eastern Regional Branch, Dept. of Food Technology and Biochemical Engineering, Jadavpur University, Calcutta-700032, India. The price for members is Rs. 5 and for non-members Rs. 20 per copy. Foreign $ 3 Plus postage

New Members

Mr Sanjay Keshao Parmanand, C/o Dr L. R. Lakhkar, Zenda Chowk, Dharampeth, Nagpur-440010.

Dr K. Balakrishna Rao, Department of Chemistry and Soils, Agricultural College, Hebbal, Bangalore-560024.

Dr Ranjan Bhadra, Department of Food Technology and Biochemical Engineering, Jadavpur University, Calcutta-700032.

Mr Rajendra Singh Powar, Gandhi Gunj, Bidar, Karnataka.

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Mr Satya Ranjan Ray, 71, Sadar Buxi Lane, Howrah-1, West Bengal.

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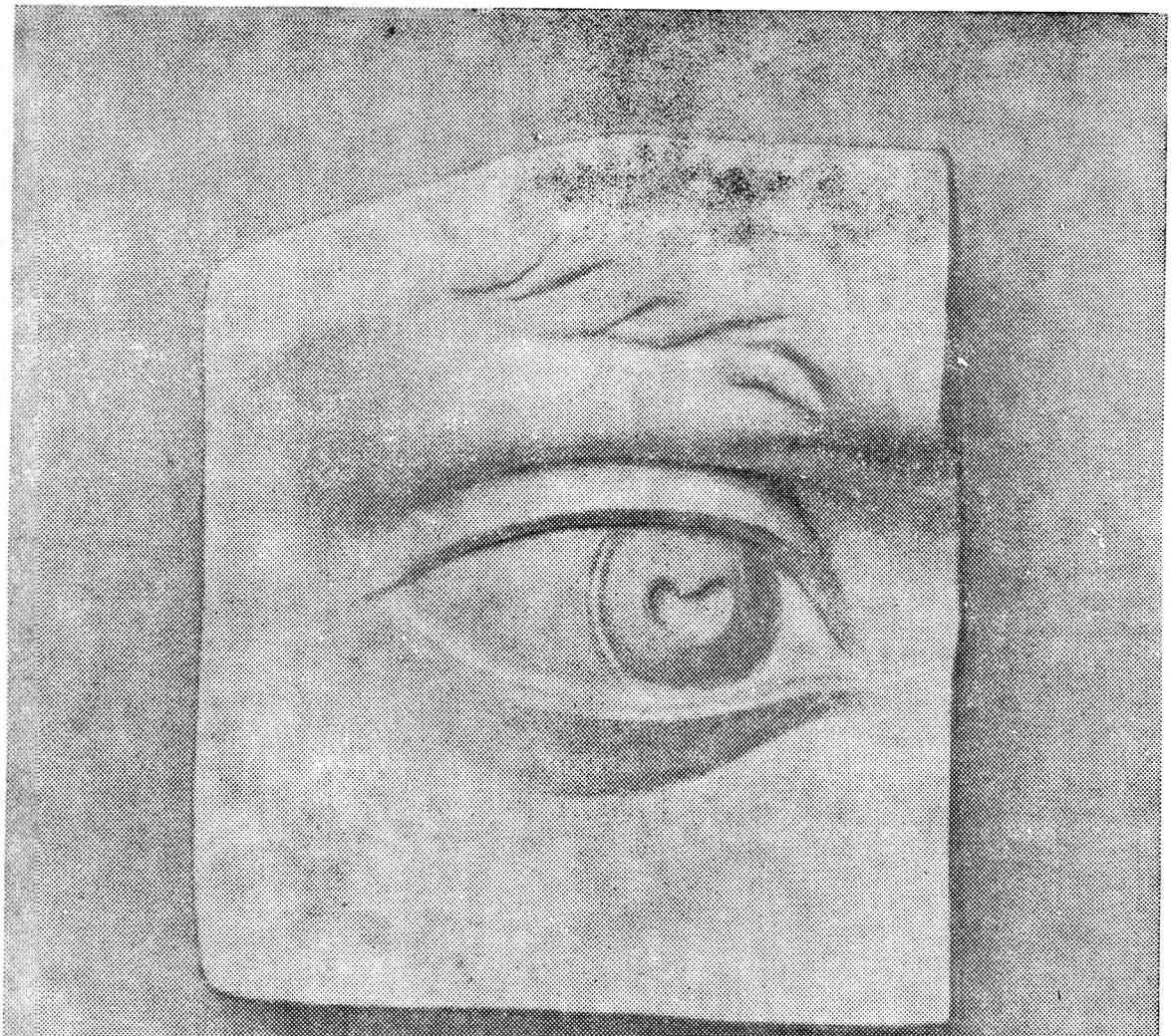

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