JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS, INDIA

VOL. 12 NO. 5 SEPTEMBER–OCTOBER 1975

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(INDIA)

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The Editor assumes no responsibility for the statements and opinion expressed by the contributors.

Manuscripts for publication and books for reviewing in the Journal should be addressed to the Editor, Journal of Food Science and Technology, AFST, Central Food Technological Research Institute, Mysore-570013. The Editor reserves the privilege of editing the manuscript to make it suitable for publication in the Journal.

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REVIEW

Adjuncts in Brewing

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Brewing is undergoing rapid changes. Before 1880 brewing was confined to the use of barley malt, hops, yeast and finings. Malt adjuncts were later allowed in brewing. Barley, maize, rice, etc. are used in brewing today with advantage. Attempts are being made to produce beer using microbial enzymes and suitable starchy material, without sacrificing the quality of beer. This is going to revolutionise the brewing industry. Several adjuncts used in brewing have been reviewed.

Until the middle of the nineteenth century, ingredients used in the production of beer were: barley malt, hops, yeast and finings. From 1880 onwards the brewer was free to use any unmalted cereals in his mashtun, provided they were not deleterious to health[1]. Today most of the beer is made with adjunct cereals. An 'adjunct' in brewing can be defined as any material which can be used in place of fully kilned malt during the production of beer with or without the addition of enzymes. Green malt, barley, maize, wheat, sorghum, rice, etc. are some of the adjuncts used.

The use of unmalted cereals has become indispensable and contribute 10-25 per cent of the total extract in the conventional brewing, the rest being barley malt. The addition and proportion of different adjuncts depend on the character of the beer to be produced. For example, sugars leave residual sweet flavour even when completely fermented. Corn flakes reduce the tendency to early haze formation particularly if the malt is under-modified. Addition of roasted barley imparts a drier flavour preferred by some brewers. When malt contains high nitrogenous constituents, it is diluted by adding raw grain.

I. Adjuncts without added external enzymes

a) *Barley* (Hordeum vulgare L.): In order to utilise the maximum activity of malt rich enzymes, unmalted barley has been used as an adjunct up to 25 per cent[2,3]. To get satisfactory yields unmalted barley has to be ground very finely. This has the disadvantage of grinding the husks also too fine, which causes difficulty during run off. The experimental worts are slightly lower in degree of fermentation than the controls and are 15-20 per cent lower in nitrogen. Beer possesses better shelf life and head retention than all malt controls. The flavour properties compare favourably with those of commercial scales. Mostek and Dyr[4] used finely ground barley up to 40 per cent in the mash with success, while Enari[5] claimed to have replaced malt by barley upto 60 per cent in the infusion mashing. The yield extract was reported to be good and saccharification satisfactory. Scully and Lloyd[6] successfully brewed about 20 per cent unmalted barley together with 18 per cent wheat flour. A loss of extract, however, has been reported by Tudeusz *et al.*[7] when unmalted barley was used as an adjunct. The loss increases not only with the increase in the amount of barley used, but also with the decrease in the degree of grinding. An increase in viscosity of wort and prolonged time of saccharification were also noticed. Addition of coarse ground barley decreased the protein content, improved the attenuation and increased the extract in the wort. A process for producing a light coloured American lager beer using unmalted barley and malt, with high amylolytic activity has been described by Lewis[8].

b) *Green malt:* Green malt is the germinated barley which is not kilned or dried. It is economical to use green malt instead of kilned malt in brewing. Drizinia and Kalashnikova[9] have used with success green malt, mashing it at 40°C to utilise the activities of cytolytic enzyme effectively. Fifty per cent green malt has been used by Mostek *et al.*[10] to manufacture 10 per cent pale and dark beer which compared in qualities favourably with beer obtained from kilned malt. Beer brewed from incompletely crushed green malt had a lower fermentability, a higher content of dextrose and bitter compounds, a lower content of nitrogen, higher alcohols and glycerol besides having a lower acidity. A successful brew was obtained when 48 per cent green malt, 48 per cent barley and 4 per cent special malt was employed. Such studies indicated the effect of rate of crushing on the biochemical process and organoleptic properties of light beer. Substitution of barley at a higher level of 68 per cent, resulted in a higher content of nitrogen in the brew with good colloidal stability and stable foam.[11] A process and apparatus for producing beer using green malt has been described by Ziemann[12]. Pre-treatment of green malt to remove undesirable aromatic and flavouring substances by applying vacuum was necessary prior to mashing. Green malt has been subjected to high temperature and pressure to produce wort similar in properties to wort produced from kilned malt. A continuous production

*M/s Hindusthan Milk Food Manufacturers Ltd., Bommuru, Rajmundhri, A.P.

of wort from green malt of seven days or even three days was attempted[13].

c) *Rice* (Oryza sativa *L.*): Perhaps the most common adjunct, apart from sugar, employed in most of the breweries is rice, either in the form of grits (ungelatinised product from degermed rice kernels), flakes or in broken condition. Gelatinised grits and thin white flakes are used as adjuncts. Thinner the flakes more rapid will be their conversion in the mashtun. Precautions, however, have to be taken to use flakes of uniform size and free from broken fragments or dust. Broken rice from milling and polishing industries can be used. An advantage of using rice or maize is that even though they have protein content equal to that of malt, proteolytic enzymes of malt are unable to degrade these proteins. As such no nitrogen is supplied to the wort. They can, therefore, be regarded as nitrogen diluents particularly when high nitrogen malts are used in brewing.

d) *Maize* (Zea mays *L.*): European countries adopting decoction process utilize 20–25 per cent maize, while in United States of America where high enzyme malts are used the figure may go upto 40 per cent.

Corn syrups: Among the liquid adjuncts used in the brewhouse, corn syrups have been recognised as an economical raw material which does not require processing and therefore, there is no demand on the mashing equipment of limited capacity. Corn syrup produced by acid hydrolysis suffered from serious defects such as low fermentable extracts, undesirable flavour and bitter after taste. Some of these defects were also encountered in beer. Mathes[14] has suggested an improved method of manufacture of corn syrup which is free from the above defects, with little dextrins and amylo dextrins and completely devoid of proteins. Beers brewed with modified syrup required less time for clearing during storage and showed equal stability and were preferred or equally rated in taste tests.

Corn grits: A portion of malt can be substituted by coarse and fine corn grits with advantage, along with a blend of 20 per cent alpha amylase and 80 per cent diastase[15]. Pre-gelatinised maize starch has been used for continuous fermentation under conditions of restraint yeast growth and limiting nitrogen content[16]. Corn flakes digested with malt without previous boiling has been successfully used by Mekis[17] for brewing. Harris[18] recommended that when lauter tun was in operation the conversion of maize grits could be carried out in a simple stirred converter with final attemperation secured by direct addition of cold water. Yakovenko *et al.*[19] achieved maximum yields and total saccharification when maize grits were mixed with malt enzymes and 25–50 per cent *Aspergillus oryzae*. Pure starch from refined corn grits was suggested by Latimer[20] as an adjunct but with considerable cost.

e) *Wheat* (Triticum aestivum *L.*): Use of wheat malts as adjuncts during brewing poses problems. Mould attack or damage to the acrospire during malting produces under-modified malts, while high protein content might contribute to haze and instability in beers. However, methods have been suggested to produce wheat malt[21]. Studies have been conducted by Harris and Williams[16] using soft wheat flour as an adjunct in brewing. Refined wheat starch, a relatively pure carbohydrate has been used, with advantage, as an adjunct. Geiger[22] has suggested a mash cycle to obtain an overall brew house yield increase of 1 per cent. Use of wheat starch slurries as a practical alternative adjunct to wheat has been proposed.

f) *Sorghum* (Sorghum vulgare *L.*): When brewing materials became scarce during second world war, sorghum was used as an adjunct by many breweries with variable results, chiefly attributed to the varieties of sorghum used, and also to the inadequate milling method then in use. Sorghum contains less oil and more proteins. After the war brewers switched back to the barley malt and adjuncts with which they were familiar. Of late there is a renewed interest among brewers to select an improved variety of sorghum and adopt improved milling operations[21]. Lager beer made from grits containing 60 per cent barley malt and 40 per cent sorghum compared well with the beer made from barley malt and corn.

g) *Oat* (Avena sativa *L.*): Some varieties of oats have been recommended[26,27] as suitable adjuncts but there is no complete agreement amongst brewers. Greater husk (30 per cent) content of oat limits its use. It has been reported that when oat is used the beer exhibited moderate gushing. Because of its flavour and nutritive properties, oat malt is mainly used in Stouts. A pretreatment of oat by steeping in water is necessary to remove undesirable flavouring substances.

h) *Rye* (Secale cereale *L.*): Rye has been used as an adjunct in brewing[28]. Steeped grains are cooked at 100°C for 20 min, then dried at about 50°C until the moisture level comes down to about 5 per cent or less. Then the rye grains are debranned and granulated before use. The foam characteristics of beer so prepared is reported to be good.

i) *Triticale:* This man-made cross between rye and wheat possesses the grain quality, productivity and disease resistance of wheat with the vigour and hardness of rye. Pomeranz *et al.*[27] reviewed the properties of various triticale crosses and the results of micromalting of 10 selections and beers from them were compared with beer from malted barley. Being rich in protein triticale malt samples yielded worts rich in nitrogen and beer with darker colour and high pH. Except for these drawbacks beer compared well with that of barley malt in other characters.

j) Sugars: Sucrose and invert sugar are used as adjuncts in brewing. Of the sugars, mostly sucrose comprises 17 per cent of the raw material used in Great Britain[23]. Both invert sugar and sugar candies, and solid and liquid glucose made from starch are used. Brewing sugars are added either prior to fermentation or as primings subsequent to fermentation. If added before pitching of yeast, they supplement the fermentable extracts of wort. They improve flavour, fullness and colour of beer. Primings in general consist of blends of cane sugar, sucrose inversion products and starch conversion products to give materials of the desired fermentability, flavour and colour. Sucrose syrup is convenient to handle, transport, store and use apart from being cheap. However the danger of microbial deterioration cannot be ruled out. The concentration at which sucrose syrups may be prepared is limited by its solubility at normal temperature which is 67 per cent (w/w). The addition of glucose or partial inversion allows the total solids concentration to be increased to the point at which viscosity becomes a limiting factor[24].

Attempts[25] have been made to use concentrated beet juice for the production of beer with little success. Beer with a low alcohol content (1. 93 per cent) was obtained using whey as an adjunct.

II. Addition of microbial enzymes with adjuncts

Detailed investigations in the study of mashing has advanced sufficient knowledge about enzymes and their activities during wort production. Attempts have been made to use microbial enzymes in the production of wort, when adjuncts are employed in large measures or when malt of low enzymatic activities is used. Utilisation of microbial protease and amylase to produce wort from barley, maize, etc., has to be adopted with caution. Traditional beer is a complex which has its own characteristics and any deviation from the conventional method should not have an adverse effect on the quality of beer.

Reports[29] on brewing with 100 per cent unmalted barley and added Nervanase 10×, a trade preparation containing bacterial amylase and protease, indicate that wort had a lesser content of fermentable carbohydrates. Undesirable flavouring compounds had been, however, removed from raw barley by heating to 70°C for 4 hr. Insufficient saccharification was also noticed by Klopper[30]. Addition of about 10 per cent barley malt gave a better beer but the diacetyl content was high. Satisfactory brew was obtained by Wieg[31,32] using 25 per cent barley malt along with 20 per cent maize grits and 55 per cent barley to which enzyme preparation containing amylase and protease was added externally. Addition of glucoamylase increased the fermentability of the wort and the resistance against the formation of chill-haze of beer increased. Bley *et al.*[33] claimed to have successfully brewed with an unmalted barley content of 65 per cent. A comparable brew was obtained when 40 per cent raw barley was employed with added external enzyme. When the proportion of barley varied the conditions of brewing had to be altered.

Process of dry milling is of particular advantage when unmalted barley is employed for brewing[34]. Since the ordinary milling rollers used for malt are not well adapted to the hard unmodified grain, pretreatment of barley is necessary. The method[34] adopted consisted of steeping barley under alkaline conditions, washing and neutralising the excess alkali by sulphur dioxide and drying at 50–55°C to about 10 per cent moisture. The presence of husk is advantageous during lautering.

Several workers[35–38] have suggested wet grinding of unmalted barley. The optimum conditions for steeping barley were 25–30 min at 50°C, when barley absorbed 30 per cent water and husks became soft and pliable. Beer made from wet-ground barley compared well with the traditional beer.

Barley has a higher specific gravity than malt and during mashing tends to settle down. Mashing with water to grain ratio of about 2.7 to 2.9:1 has been recommended. The other critical points include: an initial heating to 60°C (max.); saccharification at 65°C (max.); and conversion at 73°C (max.). The resulting wort has been reported to be good with greater fermentability.

Another important factor which should not be lost sight of is the high viscosity of barley worts. This is because of β-glucans content of barley which is about three times that of kilned malt. Hence any externally added enzyme preparation should have significantly high beta glucanase activity. It is claimed by Wieg[32] that barley brews filtered 20–30 min faster than malt brews. Normal fermentation was noticed, when pitched with yeast. The colour stability of barley beers was better than that of the control beer. The quality of beer compared with that of all-malt beer. The greatest utility of the enzymatic process is found when barley content is 50 per cent; the remaining being malt (30 per cent) and corn (20 per cent). Basarova and Bandova[34] have reported successful brewing using 50 per cent barley.

It was of interest to note the results of a taste test conducted at Interbrau Exposition in Munich. A pair of samples was presented to a total of 300 people from approximately 140 breweries. They were told that one beer was 70 per cent malt and 30 per cent corn and the other was 30 per cent malt, 20 per cent corn and 50 per cent feed barley. They were asked to give their comments. About 30 per cent said that they could not differentiate. Of the 70 per cent who reported a difference, about two thirds expressed preference to the feed barley beer.

Wort substitutes

Barley syrups: Recently 'Commercial Brewer's Concentrates' or 'Syrups' intended to provide a complete wort on dilution have been put in the market. Such liquids, therefore, must contain all the quantitative and qualitative components of wort. Barley has been used as a substrate for the preparation of syrups using microbial enzymes. Corn and other starchy cereals can as well be used for the preparation of syrups. Details of production of syrups and their use in brewing have been reported[27,31-46].

There are several advantages in using such syrups:

1. Beer of uniform consistency can be manufactured.
2. Characters of beer can be altered. For example, head retention of beer can be improved by using wheat flour.
3. Operation of a brew house is minimised.
4. Concentrates can be stored and used at will.
5. Low grade, sound and clear feed barley can be employed.
6. Uniform extract can be produced.
7. Capital investment per unit of output is less for an extract plant than for a malt plant.
8. The cost of brew house could be reduced.
9. Malting losses can be minimised.
10. Syrups from cheaper starchy materials can be used.

Malt adjuncts have been playing an important role in brewing.

A major break through in brewing is imminent with the increasing knowledge about enzymes, malt and fermentation.

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Amino Acid Composition of New Varieties of Cereals and Pulses and Nutritional Potential of Cereal–Pulse Combinations

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Manuscript Received: 20 *Feb.* 1975

The amino acid composition of some recently developed varieties of cereals and pulses are reported. It was observed that in wheat, the low protein lines had higher lysine content and, therefore, a higher chemical score of 60. The newly developed dwarf varieties of wheat did not contain lower levels of essential amino acids than the tall variety. The rice varieties, in general, had a balanced amino acid composition. The protein of the variety, 'Cauvery', had the higest chemical score of 66 per cent. In maize the chemical score of the protein was enhanced by the incorporation of the Opaque 2 gene, thus removing lysine as the most limiting amino acid, the increase was limited by the low amount of another essential amino acid *viz.* threonine. The recently identified barley strains, B_1 and 1098-2 showed a considerable improvement in the content of essential amino acids in their grain protein, with lysine content of 3.56 and 4.09 g/100 g protein, respectively. The content of lysine in the pulse species was considerably more than that present in the cereals. The chemical score of the pulse protein, however, was low owing to the limiting amino acids, methionine and tryptophan. Considerable variation in the chemical score was observed both at the inter—and intra-species level.

In a cereal-pulse combination, the proteins of the respective components complement each other. The maximum chemical score value of the protein in a wheat-pulse combination was obtained when the pulse content was around 10 per cent. With rice, maize or barley, the maximum score values were obtained when the content of pulse was around 20 per cent of the mixture. The chemical score of the protein in such combinations was limited by the content of threonine and methionine. Results have been discussed in relation to future strategy for grain protein improvement.

The nutritive value of dietary proteins is governed by the pattern and quantity of essential amino acids present in it. The presence of one or more of the essential amino acids in inadequate amounts would decrease the nutritive value of proteins[1–3]. To enhance the level of limiting amino acids in cereals and pulses, attempts are being made to identify genotypes with higher level of these amino acids. The effort is mainly directed towards development of strains which combine desirable agronomic characteristics with improved nutritional quality parameters[4–13].

In this communication the amino acid composition of some recently developed high yielding varieties/strains of wheat, rice, maize, various pulses and the high lysine strains of barley and maize are reported. Results of the amino acid spectra are discussed in view of the fact that cereals and pulses are consumed as a mixed diet and the problem of balance in the proportion of different limiting amino acids needs to be looked at in its totality rather than in isolation.

Materials and Methods

The materials used for the analysis were grown in the experimental plots of I.A.R.I., New Delhi, under normal recommended levels of fertilizers and other agronomic practices and were obtained from the various breeders. Representative amounts of the grains of different varieties/strains of cereals and pulses were ground to 60 mesh size in a hammer mill and thoroughly mixed before sampling.

Nitrogen percentage was determined by the micro-kjeldahl method[14], and the protein values computed by using the factor specific for each crop. Moisture in the flour was determined by drying known amounts of the samples to a constant weight in hot air oven at 105°C.

Amino acid analyses were done on the acid hydrolysates of the samples using Technicon Sequential Multi-sample Amino Acid Analyser[15].

Tryptophan was determined colorimetrically by a slight modification of the methods of Spies and Chambers[16], and Howe *et al*[17]. For cereals, 25 mg of the defatted sample meal ground to 100 mesh size were taken in a conical flask. Eight ml of a 23.5 N solution of sulphuric acid were added to it. Next, one ml of a 2 N solution of sulphuric acid containing 30 mg of p-dimethyl amino benzaldehyde was added, keeping the conical flask chilled in ice. One ml of distilled water was added to bring the normality of sulphuric acid to 19 N.

The flask was than kept for incubation for 15 hr at 32°C. Subsequently, 0.1 ml of a 0.12 per cent solution of sodium nitrite was added and the optical density was read at 590 mμ, after keeping it in the dark for 45 min. Estimations were made in triplicate. The values were calculated to hundred per cent recovery.

For pulse samples, the incubation period of 20 hr and a solution of 0.045 per cent sodium nitrite was found to be optimum.

Chemical score of the protein in the individual grain samples was calculated according to Block and Mitchell[18] by using the FAO reference pattern[19] as a standard. The chemical score of the protein in the various combinations of cereal and pulse was determined by computing the content of the amino acids in such a mixture on flour basis, and expressing the resultant values as percentage of the particular amino acid present in the FAO reference pattern, as above.

Results and Discussion

A. Amino acid composition of cereals: Amino acid composition of the grain protein of three recently developed dwarf strains of wheat (*Triticum aestivum*) viz. 'HD 2028', 'HD 1981' and 'HD 2009'. and a tall variety, 'C 306' are given in Table 1. The protein content in these strains varied from 10.4 to 14.4 per cent on moisture free basis. From the amino acid score values it was observed that the most limiting amino acid was lysine. It was of interest that low protein strains had a higher lysine content of 3.28 and 3.32 g/100 g protein which means a higher chemical score of 60 per cent, while the high protein strains, 'HD 2028' and 'C 306' showed lower lysine content with a concomitant decrease in the chemical score to the extent of 53 and 50 per cent, respectively (Tables 1 and 3).

That the dwarf strains did not contain lower levels of the essential amino acids than the tall variety was evident from the data. Similar results were reported by Sinha *et al.*[20] from their study of proteins of semi-dwarf and tall spring wheats.

The limiting amino acid in the grain protein of dehusked (brown) rice (*Oryza sativa*) was lysine (Table 3). The lysine content varied from 2.81 to 3.63 g/100 g protein. The content of other essential amino acids was fairly high (Table 1). The protein content of the varieties varied from 10.3 to 13.4 per cent. The grain protein of "Cauvery" had the highest chemical score of 66 per cent which was higher than that of the wheat varieties. This variety also combined a fairly high protein content of 13.4 per cent with a lysine content of 3.63 g/100 g protein. It was followed by the varieties "IET 1991" and "Pusa 221", with chemical score of 62 and 60 respectively (Tables 1 and 3).

That rice proteins have a balanced amino acid composition is evident from the low content of the prolamin fraction[21]. However, it needs to be mentioned that rice is milled before consumption thus resulting in losses of amino acids to varying degrees[8].

The amino acid composition of the varieties of maize (*Zea mays* L.) *viz.* "Ganga 5", "Vijay" and "Bassi" and one which contained the Opaque-2 gene[22] *i.e.* "Shakti" is given in Table 1. From an evaluation of the chemical score it was observed that the quality of the grain protein of the normal maize varieties was relatively poor as compared to that of wheat and rice (Table 3). Genetic upgrading of the content of lysine and tryptophan in the grain protein of "Shakti" resulted in an enhancement of its protein chemical score to 63 per cent. This association in the amino acid composition of maize proteins was reported by Mertz *et al*[22]. The morphological and biochemical changes, and the alternation in grain yield have also been reported[23].

In the normal maize varieties, the most limiting amino acid was lysine and in "Shakti" it was threonine (Table 3).

Barley (*Hordeum vulgare*) is used both as human food and animal feed. Small quantities are utilized for the preparation of malt for infant food and for brewing purposes. Thus, a study of the amino acid composition of its grain protein is of relevance. Pomeranz *et al.*[24] have reported the amino acid composition of some two row and six row barley cultivars. Protein percentage, amino acid content and the chemical score of the grain protein of some of the commercial varieties released for cultivation in India was reported from this Laboratory[11]. It was observed that the content of the essential amino acids was relatively low.

Recently, a number of barley strains obtained either by crossing with the high protein, high lysine genotype *viz.* "Hiproly"[25], or by treatment with chemical mutagens or obtained as segregant of "Hiproly" have been reported[12,15,26]. The protein and amino acid content of two such lines, *viz.* B_1 and 1098-2 are given in Table 1. Considerable improvement in their protein content over the values obtained for the commercial cultivars was observed. Lysine values in 'B_1', and '1098-2' were found to be 3.56 and 4.09 g/100 g protein, respectively. This resulted in an enhancement of the chemical score to 64 and 73, respectively, with threonine as the most limiting amino acid in both (Table 3).

B. Amino acid composition of pulses: The amino acid content of some of the new varieties of the commonly cultivated species of legumes like cowpea (*Vigna sinensis*), var. 'C 10' and 'C 13'; lentil (*Lens esculenta*), var. 'Pusa 1,' 'Pusa 2' and 'Pusa 4'; mung (*Phaseolus aureus*) var. 'Pusa Baisakhi', 'PS 6' and 'PS 7'; pigeon pea (*Cajanus cajan*), var. 'Sharda,' 'Mukta' and 'Pusa ageti'; pea (*Pisum sativum*) var. 'T 6113' and 'T 163';

TABLE 1. AMINO ACID COMPOSITION AND PROTEIN CONTENT OF THE GRAINS OF WHEAT, RICE, MAIZE AND BARLEY VARIETIES AND STRAINS

Amino acid content (g/100g protein)*

Amino acid	HD 2028	HD 1981	HD 2009	C306	Jaya	Pusa 221	Jamuna	Improved Sabar-mati	Cauvery	J349	IET 1991	Ganga 5	Vijay	Basi	Shakti	B_1	1098-2
Lysine	2.93	3.28	3.32	2.75	3.25	3.28	3.16	3.06	3.63	2.92	3.39	2.84	2.70	2.56	3.98	3.56	4.09
Histidine	2.48	2.76	2.83	2.43	2.14	2.55	1.72	2.53	2.49	2.39	2.40	2.89	2.57	2.60	3.35	2.69	2.37
Arginine	4.14	5.46	5.26	4.42	7.57	8.29	7.28	8.70	7.82	8.27	7.11	4.34	4.20	3.43	5.01	4.70	4.65
Aspartic acid	4.68	5.31	5.37	5.21	7.66	8.53	8.25	8.37	7.85	8.01	7.77	6.02	5.06	5.12	8.43	4.97	6.80
Threonine	2.43	2.93	2.87	2.48	2.81	2.99	2.89	2.86	3.02	2.93	3.02	2.79	2.56	2.70	2.53	2.54	2.91
Serine	4.62	5.20	5.22	4.78	4.36	4.72	5.18	4.69	4.60	4.86	4.74	4.53	4.16	4.33	3.60	4.53	4.29
Glutamic acid	28.11	34.87	30.58	28.82	16.04	15.88	15.36	15.95	16.16	16.25	16.59	17.29	17.08	17.41	17.41	26.80	23.48
Proline	8.33	9.03	9.16	9.05	4.36	4.35	4.23	3.62	4.66	4.00	4.22	7.45	6.38	6.55	6.35	10.44	9.31
Glycine	4.12	5.39	4.92	3.89	4.72	4.84	4.41	4.14	4.68	4.48	4.73	3.76	3.36	3.23	4.28	3.88	4.20
Alanine	3.88	4.72	4.94	4.13	6.08	6.19	5.77	6.05	6.43	5.99	5.88	7.76	8.02	7.63	5.37	4.38	5.13
Cystine	2.54	3.48	2.75	2.50	2.32	2.37	2.73	2.64	2.51	2.58	2.56	2.00	1.98	1.80	2.44	2.18	2.13
Valine	4.43	5.53	5.27	4.87	5.16	5.53	4.86	5.40	4.89	5.39	4.90	4.11	4.16	4.42	4.75	5.53	4.78
Methionine	1.63	1.92	1.81	1.73	2.92	2.21	2.44	2.20	2.20	2.16	2.71	1.86	1.78	1.44	1.82	1.81	1.72
Isoleucine	3.72	4.22	4.14	3.81	4.53	4.06	3.95	3.87	4.56	4.08	4.03	3.44	3.54	3.27	3.25	4.31	3.84
Leucine	6.75	8.58	7.89	7.33	7.47	7.95	7.45	7.79	8.02	7.86	8.91	11.20	11.45	13.64	7.57	7.58	7.02
Tyrosine	2.64	2.42	2.43	2.46	5.36	4.55	4.37	4.66	4.39	4.77	4.21	2.61	2.80	2.19	2.82	2.40	3.28
Phenylalanine	4.48	4.73	5.08	4.39	5.49	4.90	4.63	5.41	4.70	5.23	4.63	4.12	4.36	5.00	3.17	5.34	5.46
Tryptophan	1.08	1.10	1.10	1.12	1.28	1.26	1.26	1.20	1.22	1.19	1.25	0.78	0.80	0.70	1.28	—	1.18
Protein %	14.4	11.1	10.4	13.1	10.3	12.5	11.8	10.8	13.4	13.4	10.8	12.0	10.6	13.6	12.0	18.1	16.7

The multiplication factor for converting to protein is 5.7, 5.95, 6.25 and 6.25 for Wheat, Rice Maize and Barley respectively.
*Amino acid values are based on actual recoveries and are averages of duplicate estimations. Protein values are presented on dry weight basis.

TABLE 2. AMINO ACID COMPOSITION AND PROTEIN CONTENT OF THE GRAINS OF PULSE VARIETIES/STRAINS*

Amino acid content (g/100g protein)

Amino acid	Cow pea		Lentil			Mung			Pigeon pea				Pea		Chick Pea	
	C 10	C 13	Pusa 1	Pusa 2	Pusa 4	P B	PS 6	PS 7	Sharda	Mukta	Pusa Ageti	T 6113	T 163	T 3	BG 1	C 235
Lysine	5.57	5.59	6.71	7.09	6.18	5.24	6.48	6.53	6.17	5.84	7.27	6.09	6.31	5.91	6.09	6.83
Histidine	3.71	3.47	2.76	2.47	2.55	2.56	2.72	3.12	3.66	3.39	3.99	2.69	2.44	2.24	2.57	3.06
Arginine	7.91	7.31	7.75	8.55	8.76	6.49	7.20	7.63	7.13	7.42	7.27	10.09	11.43	12.01	9.04	12.20
Aspartic acid	10.62	9.98	10.94	11.01	9.80	9.15	9.88	9.81	8.75	9.14	8.46	9.95	10.80	10.20	9.16	9.54
Threonine	4.17	2.99	3.35	3.07	2.92	2.69	2.70	3.21	3.06	3.14	3.36	3.27	3.49	2.80	2.54	2.98
Serine	5.01	4.78	5.20	4.88	4.40	4.49	5.30	4.72	4.63	4.00	4.98	4.15	4.99	4.57	4.33	4.88
Glutamic acid	15.24	16.18	16.07	16.10	15.77	14.84	17.53	17.80	18.28	17.66	19.54	15.92	18.33	15.12	13.65	16.05
Proline	3.64	4.24	4.02	3.74	3.37	3.42	4.46	4.49	4.13	4.62	3.95	4.14	4.66	3.88	3.64	4.03
Glycine	3.36	3.90	4.25	3.46	4.17	4.11	4.12	4.43	3.66	3.67	3.85	4.26	4.81	3.78	3.39	4.12
Alanine	4.80	4.35	4.83	4.52	4.39	4.41	4.70	5.01	4.60	5.05	4.83	4.64	4.94	4.19	4.02	4.40
Cystine	2.49	2.42	1.89	1.87	1.72	1.37	1.65	1.92	2.29	1.98	1.94	1.82	1.86	2.09	1.39	2.19
Valine	4.87	5.21	5.18	5.32	5.60	5.52	5.92	6.09	4.88	5.08	5.05	5.07	5.51	4.69	4.29	4.82
Methionine	1.44	1.61	0.70	0.82	0.76	1.04	1.49	1.58	1.67	1.58	1.82	1.11	1.26	1.12	0.80	1.21
Isoleucine	3.79	3.91	4.17	4.40	4.45	4.29	4.45	4.52	3.44	4.45	4.18	3.85	3.99	4.23	4.14	4.02
Leucine	7.14	7.50	6.79	7.38	6.74	7.13	8.07	7.89	6.48	7.24	7.21	6.84	7.30	7.17	6.98	7.03
Tyrosine	2.83	3.35	2.95	3.24	2.61	2.84	3.14	3.22	2.74	2.77	3.45	3.60	3.79	2.69	2.41	2.81
Phenylalanine	4.48	4.77	4.31	4.96	4.42	6.85	6.11	5.52	7.91	8.34	8.58	4.69	5.00	5.54	4.11	5.42
Tryptophan	0.75	0.69	0.64	0.72	0.74	0.73	0.75	0.75	0.54	0.75	0.64	0.66	0.65	0.63	0.64	0.72
Protein %(Nx6.25)	28.8	27.3	30.5	32.1	34.9	27.6	27.2	26.3	22.8	23.4	22.6	23.2	23.9	28.2	28.5	27.6

*Amino acid values are based on actual recoveries and are averages of duplicate estimations. Protein values are presented on dry weight basis. P.B.: Pusa Baishaki.

TABLE 3. CHEMICAL SCORE AND THE MOST LIMITING AMINO ACID OF SOME CEREAL VARIETIES/STRAINS

Cereal	Variety/Strain	Chemical score	Most limiting amino acid
Wheat	HD 2028	53	Lys
	HD 1981	60	Lys
	HD 2009	60	Lys
	C 306	50	Lys
Rice	Jaya	59	Lys
	Pusa 221	60	Lys
	Jamuna	57	Lys
	Improved Sabramati	57	Lys
	Cauvery	66	Lys
	J 349	53	Lys
	IET 1991	62	Lys
Maize	Ganga 5	52	Lys
	Vijay	49	Lys
	Basi	47	Lys
	Shakti	63	Thr
Barley	B_1	64	Thr
	1098-2	73	Thr

and chick pea (*Cicer arietinum*), var. 'T 3,' 'BG 1' and 'S 231', are given in Table 2. Evidently, methionine and tryptophan are present in least amounts. The contents of the amino acids in the various species of legumes studied were: methionine, 0.70 to 1.82 and tryptophan, 0.54 to 0.75 g/100 g protein. The content of lysine was considerably more as compared to its content in the cereals, and varied from 5.57 to 7.27 g/100 g protein. The content of glutamic acid, an amino acid usually present far in excess of its nutritional requirements was lower and varied from 14.84 to 19.54 g/100 g protein. The protein content ranged from 22.6 to 32.1 per cent.

TABLE 4. CHEMICAL SCORE AND THE MOST LIMITING AMINO ACID OF SOME PULSE VARIETIES/STRAINS

Pulse	Variety/Strain	Chemical score	Most limiting amino acid
Cow pea	C 10	65	Met
	C 13	73	Try
Lentil	Pusa 1	32	Met
	Pusa 2	37	Met
	Pusa 4	35	Met
	Pusa Baisakhi	47	Met
Mung	PS 6	68	Met
	PS 7	72	Met
Pigeon pea	Sharda	54	Try
	Mukta	72	Met
	Pusa Ageti	64	Try
Pea	T 163	57	Met
	T 6113	50	Met
	T 3	51	Met
Chick pea	BG 1	36	Met
	C 235	55	Met

The chemical score of the pulse protein was relatively low. Inter- and intra-species variation was evident; the chemical score varied in the case of mung from 47 to 72, in the case of pigeon pea from 54 to 72, and in the case of chick pea from 36 to 55 (Table 4). It was earlier reported that there was a large variation also in the biological values of legume grains both at the inter-and intra-species level[27,28].

The content of cystine and methionine in some of the legume species was relatively high. This may be attributable to the status of sulphur in the soil of some of the experimental plots at the I.A.R.I. In this context the report that methionine content was increased from 1.29 to 2.18 g/100 g protein in peas[28] and the study of the effect on the protein quality of cereals[29] due to sulphur fertilization is of some significance.

From the above studies it is obvious that some of the amino acids are deficient in cereals and pulses, while some others are in far excess of the dietary requirements. At a species level one can conclude that rice protein is relatively better balanced in its amino acid composition as compared to that of others. Barley and maize proteins have low levels of essential amino acids. In this context the use of the hilly and the Opaque-2 genes in breeding programmes which has resulted in the isolation of genotypes with better amino acid balance is of great value[12,22].

In the calculation of chemical score, the choice of the reference protein is of importance. Bender reported that whole egg protein contained a surplus of all essential amino acids for the rat[30]. In an earlier study from this laboratory[11] it was felt that the use of egg protein as a reference led to an underestimation of the nutritional quality of the protein. In the present study, therefore, the reference pattern suggested recently by FAO[19] has been used. That there is a good degree of correlation between the chemical score of protein quality and its biological value has been shown by several workers[18,31,36]. Bender[37] and Fisher[38] postulated a theoretical relationship between chemical score and biological value as a straight line from zero to 100 per cent. After extensive studies Bender concluded that there was a 1:1 relationship between chemical score and biological value for those values between 50 and 100 per cent. At lower values the relation was dependent upon the limiting amino acid. However, for proteins limited by valine and sulphur amino acids the relation was still 1 : 1. Thus, from the present study, except for the varieties of normal maize where the chemical score is limited by lysine, a very good correlation between the chemical score and biological value is envisaged.

It needs to be mentioned that for human consumption cereals and pulses are processed in various ways which have an effect on the digestibility of amino acids. Thus,

rice is parboiled (*i.e.* steam cooked) prior to consumption while wheat, maize and barley grains are ground into whole meal and baked into *chapatties*. However, Bender has indicated that lysine, threonine, methionine and valine were 100 per cent available[35]. Similarly, the lysine of cheese and processed corned beef was fully available. Also, it has been recently reported that baking or parboiling of wheat did not result in the loss of tryptophan in the resulting product[39].

Pulse grains are known to contain anti-nutritional factors. However, it has been reported that in pulse grains which are normally soaked overnight in water and then cooked in boiling water for 2-3 hr, the toxic factors were usually destroyed[40-42]. Also, the true digestibility was considerably enhanced. The special case of lathyrism[43] and of favism[44] is not of concern here.

An important factor is that of flatulence induced by legumes[45], on account of which it is usually not possible to consume large amount of a pulse.

C. Nutritional potential of cereal-pulse combinations: Since cereals and pulses are invariably consumed together, it was felt necessary by the investigators to look at the amino acid levels of such combinations where the two ingredients are present together in different proportions. Usually, the amount of pulse consumed is much less than the corresponding cereal, being about 10 to 20 per cent of the diet. The amount recommended for consumption by the I.C.M.R. is 400 g of cereal and 85 g of pulse[46]. In the present analysis the proportion of pulse has been varied from 10 to 40 per cent in some of the cases.

Mixing whole wheat meal (var. 'HD 2009') with mung (var. 'Pusa Baisakhi') in the proportion of 90:10 resulted in an enhancement of the chemical score of the protein (71) in the combination over that of either of the components alone (Table 3,4 and 5). This was mainly due to an increased supply of lysine from the grain protein of mung. A similar trend was observed when rice (var. improved 'Sabramati') was taken in combination with mung (var. "Pusa Baisakhi"). But unlike the wheat-mung combination, the proportion of rice to mung for optimum chemical score (70) was found to be 80:20. Threonine was found to be the most limiting amino acid in these combinations. With increases in the proportion of the pulse, the chemical score of the protein in the cereal-pulse combination decreased.

When the common cultivars of maize and barley *viz.* 'Ganga 5' and 'Jyoti' are combined with mung, there is an enhancement in the chemical score of the protein in the mixture which reached an optimum value when the ratio of the cereal to the pulse was 80:20. From animal feeding experiments using rats Bressani *et al*[47] found that the maximum nutritive value of various mixtures of lime treated corn and cooked black beans (*Phaseolus vulgaris*) was in the combination where the ratio of maize to beans was 72 to 28 per cent.

When the flour of the genotypes of maize and barley *viz.* 'Shakti;' and 'B_1' and '1098–2,' wherein a genetic upgrading of the lysine content in the grain protein has been effected, were mixed with mung there was little or no change in the chemical score of the protein in the mixture over that of the cereal component taken individually (Tables 3 and 6).

TABLE 5. EFFECT OF VARIATION IN THE PROPORTION OF WHEAT, RICE AND A PULSE ON PROTEIN QUALITY OF CEREAL-PULSE COMBINATION

Protein source		Protein %	Chemical score	Most limiting amino acid
Wheat + Mung				
90%	10%	12.1	71	Thr
80%	20%	13.8	68	Met
70%	30%	15.5	64	Met
60%	40%	17.3	60	Met
Rice + Mung				
90%	10%	12.5	68	Lys
80%	20%	14.2	70	Thr
70%	30%	15.9	69	Thr
60%	40%	17.5	67	Met

TABLE 6. EFFECT OF OPAQUE 2 AND HILLY GENE ON THE PROTEIN QUALITY OF CEREAL-PULSE COMBINATION

Protein source		Protein (%)	Chemical score (%)	Most limiting amino acid
Maize[1] (Ganga 51)	+ Mung			
90%	+ 10%	13.6	64	Lys
80%	+ 20%	15.2	69	Thr
Maize[2] (Shakti)	+ Mung			
90%	+ 10%	13.6	64	Thr
80%	+ 20%	15.1	65	Thr
Barley[3] (Jyoti)	+ Mung			
90%	+ 10%	15.1	65	Lys
80%	+ 20%	16.5	67	Thr
Barley[4] (B_1)	+ Mung			
90%	+ 10%	19.0	64	Thr
80%	+ 20%	20.0	65	Thr
Barley[5] (1098-2)	+ Mung			
90%	+ 10%	17.8	72	Thr
80%	+ 20%	18.9	69	Met

[1]Normal maize variety; [2]Maize variety containing opaque-2 genes; [3]Normal barley variety; [4]a mutant; [5]isolated from the progeny of 'Hiproly' X 'Jyoti'. Mung variety used: "Pusa Baisakhi"

Thus, it would appear from Tables 5 and 6 that the nutritional value of a cereal-pulse mixture would be limited by the content of threonine and methionine in the mixture. Carpenter arrived at a similar conclusion from his study on the calculated chemical score of whole wheat and *Phaseolus* bean proteins in different proportions[48]. These theoretical calculations were in complete agreement with Bressani's findings for diets of Guatemalan children[49]. This suggests that fortification of *chapatties* with lysine as advocated by some investigators[50] may not be necessary. To a cereal wherin its lysine content has been genetically upgraded thus enhancing its nutritional quality, the addition of a pulse may not further increase the nutritional quality of the protein in the combination.

In conclusion, a few relevant points that emerge from the above study may be mentioned: firstly, that cereal and pulse proteins complement each other; secondly, that while planning a strategy for combating malnutrition, particularly from the point of view of agricultural research, one has to look for an improvement in the content of limiting amino acids in the diet as a whole and consider the possibility of increasing their content in any of the components, for example, methionine may be increased in the cereals; thirdly, that lysine, threonine and methionine belong to the asparate family of amino acids-an understanding of their regulation at the biosynthetic level[51] may elucidate as to which of the amino acids could be enhanced in which of the species; fourthly, that our present availability of pulses is at a level of 6-7 per cent of the cereals and bearing in mind that pulses are the major sources of proteins in the Indian diet, an increase in their production so as to bring them around to 15–20 per cent of the level of cereal production in the country is suggested; and fifthly, that identification of genotypes which are able to utilize nitrogen fertilizer better and accumulate higher protein content in the grain, thus resulting in more availability of the amino acids even though all of them may not show corresponding increases, may be worth pursuing.

Acknowledgement

The authors wish to thank Dr A. B. Joshi, Director, IARI and Dr B. R. Murty, Project Director, Nuclear Research Laboratory, IARI, for providing the facilities. Thanks are also due to the various plant breeders for the supply of the grain material.

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Uptake and Metabolism of Carbofuran in Maize Plants

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Manuscript Received: 24 *March* 1975

The uptake and metabolism of carbofuran was studied in maize crop (*Zea mays* L.) raised from treated seeds. Plants at different stages of growth and the grains at harvest stage were sampled and analysed. In plants, the major metabolite constituting about 50 to 80 per cent of the total carbamates was found to be 3-hydroxy carbofuran. Indications of the presence of 7-hydroxy phenols of 3-hydroxy carbofuran, carbofuran and 3-oxo carbofuran as conjugates were also obtained. Maximum level of carbamates in the plants reached after 11 days of germination. The combined residues of carbofuran and 3-hydroxy carbofuran reached below the tolerance level of 0.5 ppm after 50 and 57 days of sowing in the crop raised from seeds treated with 5 and 10 g of the insecticide per 100 g of seeds. Matured grains contained residues lower than the tolerance in both the treatments.

Carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl N-methyl carbamate), is a broad spectrum systemic and contact insecticide, is gaining popularity to replace the persistent insecticides like DDT and endrin for the control of various crop pests[1,2]. This insecticide is registered for use on corn, pea nuts and alfalfa in USA[3]. Sandhu (1972, unpublished) found that the application of carbofuran to maize seeds before sowing gave effective control of maize pests, particularly the maize borer *Chilo partellus* (Swinhoe).

Several studies have shown that the principal metabolic pathways of carbofuran in plants include hydrolysis oxidation and conjugation[4,5]. Carbamate residues that have been identified in plant materials include the parent carbofuran, 3-hydroxy carbofuran, 3-oxo-carbofuran and the conjugated glycosides of the 3-substituted compounds. Hydrolysis of the 7-position has also been reported to occur producing non insecticidal phenols. Two of the metabolites namely, 3-hydroxy carbofuran and 3-oxo carbofuran are toxic. Tolerances have, therefore, been established for combined residues of carbofuran and its toxic metabolites[3,7]. In contrast to

metabolism studies, there is not much information available on the uptake and dissipation of carbofuran in plants. Recently, Turner and Caro[8] reported the uptake and distribution of carbofuran and its metabolites in field grown *Zea mays* L. as a result of soil application of the insecticide. The present communication summarises the results of the investigations on the uptake and metabolism of carbofuran in *Zea mays* L. raised from the insecticide treated seeds. Additional effect of soil application was also studied.

Material and Methods

Field treatment: Seeds of *Zea mays* L. ("Vijay" variety) were treated with the requisite amounts of Furadan 50 per cent S.P. using water as a sticker to have the dosages of 5 and 10 g of active ingredient per 100 g of seeds. These were then sown in the Entomological Experimental Farm, Punjab Agricultural University, Ludhiana and the crop was raised during March-June, 1973 following the recommended agronomic practices. After 29 days of germination, carbofuran granules (Furadan 3G) were also applied to the soil at the rate of 1.5 kg a.i./ha in some of the plots.

Sample collection: Samples of plants raised from treated seeds were taken at different intervals from 4 to 70 days of germination. In the plots which received seed and soil treatment, the samples were taken at different intervals starting from 1 to 41 days of application of the insecticide to soil. At harvest stage, the samples of grains were taken. About 1 kg of plant and grain samples were taken from each treatment. These were chopped/crushed and a representative sample of 100 g was taken for analysis.

Extraction: Free and conjugated carbamates were extracted separately from the plant/grain samples. For free carbamates, the method described by Gupta and Dewan[9] was followed. The plant samples were extracted by blending with acetone whereas the grain samples were extracted in Soxhlet. The acetone extract after evaporation of the solvent was diluted with water and partitioned with methylene chloride to extract carbamates present in the free form. The aqueous phase thus obtained after partitioning was combined with the plant/grain residue left after acetone extraction and processed following the method described by Cook *et al.*[10] for extraction of water-soluble conjugates.

Clean up: The methylene chloride extract of the plant samples was treated with charcoal as recommended by Gupta and Dewan[9] for clean-up. Additional treatment with coagulating solution was, however, found necessary. In the case of grains, no clean-up with charcoal was done. However, treatment with acetonitrile and hexane as recommended by Cook *et al.*[10] was done to remove the interfering oil.

Analysis: Thin layer chromatographic[4,11], colorimetric[9] and enzymatic[12] methods were used for analysis. The extracts were chromatographed with known standards of carbofuran and metabolites on silica gel-G coated plates. Chromatograms were developed in ether-hexane (3:1, v/v) and ethyl acetate-hexane (1:1, v/v) and visualized by spraying with p-nitrobenzene diazonium flouroborate to produce reddish colour with carbofuran metabolites having free hydroxyl group. Metabolites without free hydroxyl group were visualized by treatment with ethanolic NaOH (1N) to free hydroxyl group prior to treatment with dye. The clean-up extracts of the plant samples obtained at different intervals were estimated quantitatively both by colorimetric and enzymatic methods. The clean-up extracts of the grain samples was estimated by enzymatic and TLC methods only.

Results

Thin layer chromatographic analysis of the organo soluble extracts of the plants raised from treated seeds using ethyl acetate-hexane (1:1, v/v) indicated the presence of carbofuran and 3-hydroxy carbofuran. By using ether-hexane (3:1, v/v) as the developing solvent again the two spots having the Rf value of carbofuran (0.42) and 3-hydroxy carbofuran (0.13) were observed (Table 1). The organosoluble plant extract on hydrolysis gave rise to the 7-hydroxy phenols of carbofuran and 3-hydroxy carbofuran thereby confirming their presence. The aglycone forms of the water-soluble conjugates when analysed by TLC showed the presence of 3-hydroxy carbofuran and 7-hydroxy phenols of carbofuran and 3-hydroxy carbofuran. Indications of the presence of another metabolite, probably 7-hydroxy phenol of 3-oxo carbofuran, was also obtained. However, its identity could not be established due to the lack of the reference material. The results obtained therefore, indicated the presence of only two toxic compounds, carbofuran and 3-hydroxycarbofuran. Based on the determination of carbamates in the organosoluble plant extracts by colorimetric and enzymatic methods, the individual amounts of these compounds were estimated as per the procedure outlined under Fig. 1. For obtaining the quantitative estimate of conjugated 3-hydroxy carbofuran, the silica gel corresponding to the standard was scrapped from the chromato plate, eluted with acetone and estimated colorimetrically. The results obtained (Table 2) indicated significant amount of 3-hydroxy carbofuran even 4 days after germination. About 62 per cent was present at 4 days which increased to 82 per cent at the end of 42 days of germination. Furthermore, quite a significant portion of 3-hydroxy carbofuran was present in free form. Similar pattern was observed in the case of other treatments.

TABLE 1. RF VALUES OF CARBOFURAN AND ITS METABOLITES BY TLC

Compound	Developing solvents a	Developing solvents b
Carbofuran	0.42	0.58
3-hydroxy carbofuran	0.13	0.29
7-hydroxy phenol of carbofuran	0.70	0.80
7-hydroxy phenol of 3-hydroxy carbofuran	0.46	0.56

a-Ether-hexane (1:1, v/v), b-Ethyl acetate-hexane (1:1, v/v)

The combined levels of carbofuran and 3-hydroxy carbofuran at different stages of plant growth as a result of various treatments are presented in Fig. 1. In the case of crop raised from seeds treated at the rate of 5 g of carbofuran per 100 g of seeds, the amount of carbamates was 2.64 ppm at 4 days of germination which increased upto 5.8 ppm after 11 days of germination. The insecticide degraded to 0.34 ppm and below detectable level (0.1 ppm) after 42 and 50 days of germination respectively. In the case of seeds treated at the rate of 10 g per 100 g of seeds, the levels of carbamates were 3.6 and 8.2 ppm at 4 and 11 days of germination respectively. The insecticide was degraded to below the tolerance level of 0.5 ppm in 50 days. In the case of plots where the soil application at the rate of 1.5 kg a.i./ha was given in addition to the seed treatment, the level of carbamates came below the tolerance level of 0.5 ppm in plants after 50 days of germination. The level of residues in the grains in all the treatments were below the tolerance of 0.1 ppm.

Discussion

The metabolites of carbofuran in maize plants were found to be 3-hydroxy carbofuran and 7-hydroxy phenols of carbofuran and 3-hydroxy carbofuran. Indications of the presence of 3-oxocarbofuran phenol were also obtained. Major portion of carbamates ranging from 50 to 60 per cent was, however, associated with 3-hydroxy carbofuran. The results did not reveal the

TABLE 2. AMOUNTS OF CARBOFURAN AND 3-HYDROXY CARBOFURAN IN MAIZE PLANTS AT DIFFERENT STAGES OF GROWTH*

Days after germination	Carbofuran	3-hydroxy carbofuran Free	3-hydroxy carbofuran Conjugate	3-hydroxy carbofuran %
4	1.00	1.14	0.50	62.12
11	2.67	2.92	0.30	54.67
18	1.11	2.29	0.35	70.40
26	0.29	0.92	0.72	84.97
34	0.11	0.45	—	80.35
42	0.06	0.28	BDL	82.47
50	BDL	BDL	,,	—
Grain	,,	,,	,,	—

—Not done
BDL-Below detectable limit of 0.1 ppm.
*Seed treatment 5% (active ingredient).

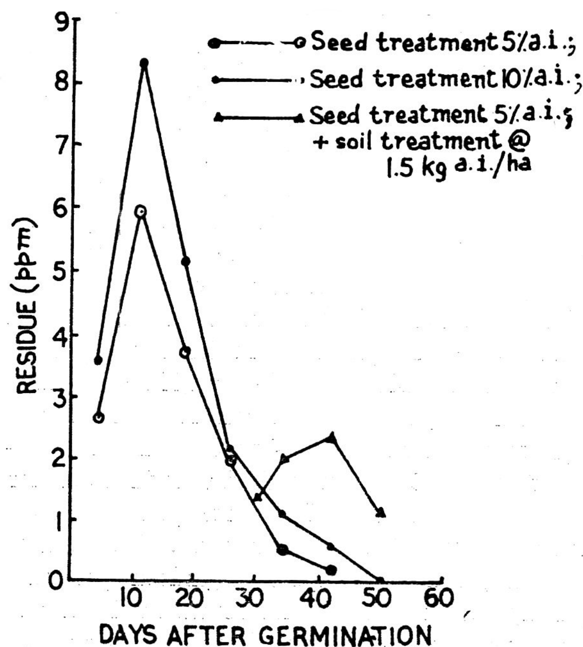

Fig. 1: Combined residues of carbofuran and 3-hydroxy carbofuran in maize plants after treatment of seeds and soil application.

Following procedure was adopted for computation of carbofuran and 3-hydroxy carbofuran from the estimates of carbamates obtained through colorimetric and enzymatic methods.

Standard curves of carbofuran and 3-hydroxy carbofuran were obtained by colorimetric and enzymatic methods. By colorimetric method, one unit of 3-hydroxy carbofuran can be expressed as one unit of carbofuran because both compounds gave the same optical density-concentration curve. However, the concentration of 3-hydroxy carbofuran required was 4 times than that of carbofuran to cause the same level of inhibition of acetyl cholinesterase. The amount of 3-hydroxy carbofuran can, therefore, be expressed on $\frac{1}{4}$ unit of carbofuran when estimated by enzymatic method. The estimates of carbamates present as carbofuran and 3-hydroxy carbofuran in the organo-soluble plant extracts obtained through the use of these methods were expressed in terms of carbofuran. The relative amounts of carbofuran and 3-hydroxy carbofuran were then computed using the following linear relationship between the two methods:

Estimate of carbamates in terms of carbofuran by colorimetric method be M and by enzymatic method be N. Let the amount of carbofuran be X and the amount of 3-hydroxy carbofuran be Y.

Then,

$$M = X + Y$$
$$N = X + Y/4$$
$$M - N = 3Y/4$$
$$Y \text{ (amt. of 3-OH carbofuran)} = (M-N)\tfrac{4}{3}$$
$$X \text{ (amt. of carbofuran)} = M - Y.$$

presence of 3-oxo carbofuran and 2,2-dimethyl 2-3 dihydrobezofuranyl-7-N-hydroxy methyl carbamate. Metcalf *et al.*[4] also found 3-hydroxy carbofuran and 7-hydroxy phenol of 3-oxo carbofuran in the corn raised from seeds treated topically with carbofuran. The toxic carbamates in corn raised from treated seeds were only carbofuran and 3-hydroxy carbofuran. Ashworth and Sheets[11] and Van Middelem and Peplow[13] also found that the major metabolite was 3-hydroxy carbofuran in tobacco and cabbage respectively. Butler and Mc Donough[14] found the presence of negligible amounts of 3-oxo carbofuran in cucumbers, lettuce, potatoes and tomatoes raised in soil treated with carbofuran. Turner and Caro[8] conclusively demonstrated the presence of 3-oxo carbofuran in addition to 3-hydroxy carbofuran in maize plants raised in soil treated with carbofuran. The absence of 3-oxo carbofuran in the present studies may be due to the difference in the methods of application of the insecticide. Knaak *et al.*[15] and Knaak[6] considered that plants receiving carbofuran metabcilsed it to 3-hydroxy carbofuran and 7-hydroxy phenols of 3-oxo carbofuran and 3-hydroxy carbofuran which were largely stored as conjugates. In maize plants also, these metabolites were found as conjugates. It was, however, interesting to note that quite a significant portion of 3-hydroxy carbofuran was also found in the free form. The absence of 3-oxo carbofuran and its presence as phenol indicated this compound to be hydrolytically unstable as has already been suggested by Metcalf *et al.*[4].

The results further revealed that the uptake of carbofuran depended upon the dosage applied on the seeds. The degradation was found to be quite rapid. In the plants raised from seeds treated with 5 and 10 per cent active ingredient the level of carbamates was reduced by 50 per cent between the sampling done at 11 and 18 days of germination. About 94 to 96 per cent was degraded at the end of 42 days. It appears that in addition to the metabolic degradation the dilution factors due to growth of the might have also substantially contributed in bringing down the level of residues. Fairly rapid break down of carbofuran has been reported by various other workers in different plant species[4,11,16,17]. On the basis of combined residue of carbofuran and 3-hydroxy carbofuran, the levels less than tolerance of 0.5 ppm was reached after 42 days of germination at the recommended treatment of seeds with 5 per cent carbofuran. The combined level of carbamates was below the tolerance of 0.1 ppm in the matured grains even when applied at twice the recommended concentration.

Acknowledgement

The authors are thankful to Dr O.S. Bindra, Professor and Head of the Department of Entomology for providing the necessary facilities and to Dr C.C. Cassil of Niagara chemicals U.S.A. for supplying samples of carbofuran.

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Regional Variations in the Farinographic and Other Quality Characteristics of Kalyan Sona Variety of Wheat

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Manuscript Received: 2 *April* 1975

The physico-chemical and farinographic characteristics of 15 samples belonging to 1970–71 crop of 'Kalyan sona', a new high yielding variety of wheat, collected from different wheat producing areas in India were studied with a view to find out the best end use to which this variety of wheat can be successfully utilised. The study revealed that these samples though seemed hard and amber coloured remarkably differed in their quality characteristics such as thousand kernel weight, hecto litre weight, moisture content, total yield of flour, gluten content, sedimentation value and gluten strength in the grains obtained from different places.

The availability of systematic data on the quality characteristics of some of the promising high yielding varieties of wheat such as 'Kalyan sona' will not only help in formulating a comprehensive price policy that will enable the farmers to get a fair return for their produce but also will facilitate the marketing agencies in procuring the wheat for different purposes. Besides, such a compilation of data will enable the plant breeders to evolve better quality of this variety by inducing desirable characteristics in them. While the physico-chemical characteristics of wheat and the effect of environment as well as the varying levels of nitrogen application, etc. have been extensively studied[1–7], very little has so far been studied on the rheological characteristics of Indian wheats[8–11]. The present study was undertaken to observe the variations in different quality characteristics within a variety and also to find out the suitability of this variety for various usages. It may be noted that the present system of grading wheat and its flour, does not indicate the end uses to which they are better suited.

Materials and Methods

Out of the 15 samples of 1970–71 crop of wheat, 11 were from different areas in Maharashtra (Nagpur, Raver, Amalner, Barshi, Bombay, Pune, Dhulia, Amaravathi Buldana and Nasik) 2 from Rajasthan (Begum and Bhilwara) and one each from Madhya Pradesh (Jabalpur) and Gujarat (Anand). After removing the various defectives, the samples were thoroughly cleaned with a clipper in the laboratory before analysis.

Physical and chemical characteristics: The total yield of flour was determined in duplicate by milling 500g of the sample for each determination on 'as is basis' in the Brabender experimental mill (Quadrumant Junior).

The relavant methods prescribed by the Indian Standards Institution were adopted for the determination of the following factors: (*a*) Thousand kernel weight[12]; (*b*) hecto litre weight[13]; (*c*) moisture; (*d*) gluten content[14]; and (*e*) sedimentation value[15].

Farinographic characteristics: The method followed was in essence that of the American Association of Cereal Chemists[16].

Results and Discussion

The results obtained are given in Table 1 and 2. Photographs of two typical farinograms of the same variety procured from Dhulia and Nagpur (both in Maharashtra) are also given.

Out of the 15 samples only one sample collected from Nagpur area weighed below 30g while determining 1000 kernel weight. With the exception of 3 samples, one each from Nagpur, Jabalpur and Gujarat, all the remaining samples gave hecto litre weight above 80 kg. Only in one sample procured from Bombay the percentage of moisture exceeded 12 but for milling purposes the moisture content shown by the sample was well within the prescribed limits. The total flour yield of 5 samples was above 70 per cent. In two samples procured from Chittor and Bhilwara areas in Rajasthan, the gluten content was below 11 per cent. In majority of samples the sedimentation value was around 25.0 units.

The ratio between sedimentation value and gluten content may be considered as a rough index of gluten quality. In two samples the ratio between these two factors was 1:2.5 and 1:2.9 while in others it was below 1:2.5. The samples from Dhulia which had 12 per cent gluten and 35.0 units of sedimentation value showed highest gluten/sedimentation ratio i.e. 1:2.9.

The higher percentage of water absorption of the

ห้องสมุด กรมวิทยาศาสตร์

TABLE 1. PHYSICAL, CHEMICAL AND MILLING CHARACTERISTICS OF KALYAN SONA WHEATS

	Physical characteristics			Milling characteristics	
Place of production	1000 Kernel wt (g)	H. L. wt (Kg.)	Moisture content (%)	Flour yield (%)	Bran content (%)
Anand	38.0	77	9.2	70	30
Jabalpur	39.7	78	9.1	72	28
Nagpur	29.9	79	11.2	71	29
Nagpur	35.0	81	10.1	72	28
Raver Dist. Jalgaon	34.9	82	10.9	67	33
Amalner Dist. Dhulia	38.2	83	8.8	71	29
Barshi Dist. Sholapur	33.4	80	9.1	67	33
Bombay	37.0	82	12.6	67	33
Baramati Dist. Pune	39.0	83	11.8	67	33
Dhulia	34.0	84	10.4	67	33
Morshi Dist. Amarvathi	36.0	84	10.3	69	31
Mehekar Dist. Buldana	35.0	84	11.9	67	33
Satna Dist. Nasik	35.0	82	11.9	67	33
Begun Dist. Chittor	39.0	81	10.9	67	33
Bhilwara	40.0	82	11.1	68	32
Mean	36.3	81	10.6	68.6	31.4
Range	29.9 to 40.0	77 to 84	8.8 to 12.6	67 to 72	28 to 33

TABLE 2. FARINOGRAPHIC AND CHEMICAL CHARACTERISTICS OF KALYAN SONA WHEATS

	Farinographic characteristics					Chemical characteristics		
Place of production	W.A. (%)	DDT (Min)	DST (Min)	DRT (Min)	D.S. (B/U)	Gluten content %	S.V.	S.V. ratio
Anand	67.0	3.4	3.1	6.5	40	11.0	25	2.3
Jabalpur	67.5	3.5	3.0	6.5	64	12.2	28	2.3
Nagpur	66.4	3.9	0.8	4.7	80	12.3	26	2.1
Nagpur	66.0	3.0	1.1	4.1	70	11.1	19	1.7
Raver	69.3	3.7	0.7	4.4	70	12.4	19	1.5
Amalner	67.0	3.0	1.0	4.0	100	14.0	26	1.9
Barshi	67.5	3.5	0.8	4.3	60	12.0	24	2.0
Bombay	67.3	3.9	2.9	6.8	40	11.1	27	2.5
Baramati	66.0	3.0	3.2	6.2	30	12.0	27	2.3
Dhulia	66.5	5.6	3.1	8.7	10	12.0	35	2.9
Morshi	67.0	4.5	2.4	6.9	20	13.4	29	2.2
Mehekar	64.9	4.0	4.0	8.0	20	12.4	29	2.3
Satna	62.5	2.5	1.5	4.0	5	11.8	23	1.9
Begun	66.0	4.5	3.1	7.6	15	1.08	25	2.3
Bhilwara	64.0	2.5	3.0	5.5	40	10.6	25	2.4
Mean	66.3	3.6	2.2	5.9	44	11.9	25.8	2.2
Range	62.5 to 69.3	2.5 to 5.6	0.7 to 4.0	4.0 to 8.7	5 to 100	10.6 to 14.0	19 to 35	1.5 to 2.9

samples (generally above 60 per cent) showed good hydrophylic nature of the gluten, the strength of which may be considered as from medium strong to strong. In four samples from Dhulia, Buldana, Morshi and Chittor, the dough development time was 4 min and above. This may be considered to be the characteristic of strong gluten. The dough stability time in six samples viz. from Nagpur, Jalgaon, Dhulia, Sholapur and Nasik, was very short and this indicated lack of mixing tolerance. The dough resistance time varying from 7.6 to 8.7 min in the three samples from Chittor, Buldana and Dhulia indicated greater fermentation tolerance. In the five samples procured respectively from Anand, Jabalpur Bombay, Pune and Amaravati, the dough resistance time was above 6.0 but below 7.0 min exhibiting thereby medium tolerance. In the remaining samples the dough resistance time was below 6.0 min and this indicated poor tolerance.

The following inferences could also be drawn from the farinographic studies:

(*i*) The samples showing a dough resistance time above 6.0 min and with an average gluten content of 11.9 per cent and an average of 27.2 units of sedimentation value were suitable for blending with strong flour or for preparing *chapatties*.

(*ii*) The flour of the remaining samples could be used as all purpose flour.

Finally it may be added, that wheat of one and the same variety when cultivated under different agro-climatic conditions may not yield a produce of uniform quality, but may differ in quality characteristics, on account of various reasons some of which are still difficult to explain.

Acknowledgement

Thanks are due to late Shri A. C. Sinha and the Agricultural Marketing Adviser to the Government of India for readily granting permission to carry out this work.

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Application of Polarisation Technique for Studying the Corrosion Behaviour of Tinplate with Some Fruit Products

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Manuscript Received: 30 *May* 1975

Corrosion behaviour of tinplate with some fruit products has been studied using polarisation technique. Among different fractions of mango pulp and orange juice, organic acid fraction was more corrosive. Among different organic acids studied, malic acid was found to be least corrosive, while oxalic acid was highly corrosive. Corrosion current increased with decrease in the thickness of tincoating and grain size structure in presence of mango nectar and orange juice. Polarisation technique is useful in can manufacturing units or in research work connected with corrosion studies and evaluation of tinplates or cans.

Several methods are being followed to determine the degree and nature of corrosion of tinplate. A number of rapid corrosivity test methods have been developed in recent years utilizing the electrochemical methods. A corrosivity tester has been developed by Daly[1]. Reznik and Manheim[2] developed an electrochemical method for following the corrosion of a can containing the product after normal industrial processing by fitting a calomel electrode hermatically into the container. A method for predicting the occurrence of a type of pitting corrosion in plain tinplate cans of food has been described by Board *et al.*[3]. Other known tests striving for correlation with test pack method are crystal size, porosity, pickle lag and iron solution value tests[4]. Kamm *et al.*[5], developed an accelerated test for tinplate using grape fruit and other juices. The galvanodynamic and Potentio-dynamic methods including the appropriate equipment have been described by Reid[6] and Johnson[7]. Recently Sherlock *et al.*[8] applied the polarisation measurements to find the rate of dissolution of tin from tinplate in oxygen free citrate solutions.

In India, at present, the practice to express the extent of corrosion in canned food products is the actual canning of the product and determination of tin and iron contents in canned product and observing the appearance of the can interior periodically. The possibility of application of polarisation studies for the corrosion behaviour of tinplate with some fruit and vegetable products has been initiated and the results of these experiments are presented in this paper.

Materials and Methods

Raw material: Mango nectar (15°Brix and 0.5 per cent acidity) prepared from 'Badami' ('Alphonso') variety and Coorg Mandarin orange juice (15°Brix and 0.5 per cent acidity) were used in these experiments.

Tinplate: Tinplates with varying thickness of tincoating and different grain size structure manufactured by M/s. Hindustan Steel Ltd., Rourkela, India were used in these experiments.

Separation of different fractions: Organic acid, amino acid and sugar fractions from mango pulp and orange juice were separated by passing through cationic and anionic exchange resins and eluted separately following the method described by Hussain *et al*[9].

Tin content: Tin content from the composite sample of six cans was determined in duplicate by the volumetric method described by Mckenzie[10].

Polarisation cell: The rate of corrosion was measured by polarisation technique under nitrogen atmosphere as described by Fontana and Greene[11].

The polarisation cell consisted of a 250 ml round bottom flask fitted with ground glass joints with provision for the admission of electrode, solution and gas. A platinum electrode served as auxiliary electrode and the reference electrode was saturated calomel electrode (SCE). A high voltage d.c. source in combination with variable resistance was used as a constant current source for polarising its electrolytes. 200 ml of the test solution was taken in the polarisation cell and one sq cm (1 cm $\times$ 1 cm) of the tinplate strip was immersed in this solution. To measure the potential in the cathodic region current of 0-100 micro amp and in the anodic region 0-2 milli amp was passed through the solution in the cell between the auxiliary electrode and specimen. The electrode potential in millivolts (Emv)

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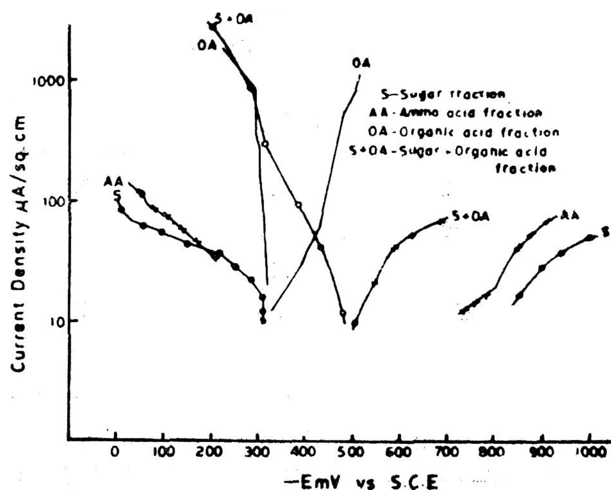

Fig. 1. Polarisation studies with different fractions of Badami mango pulp

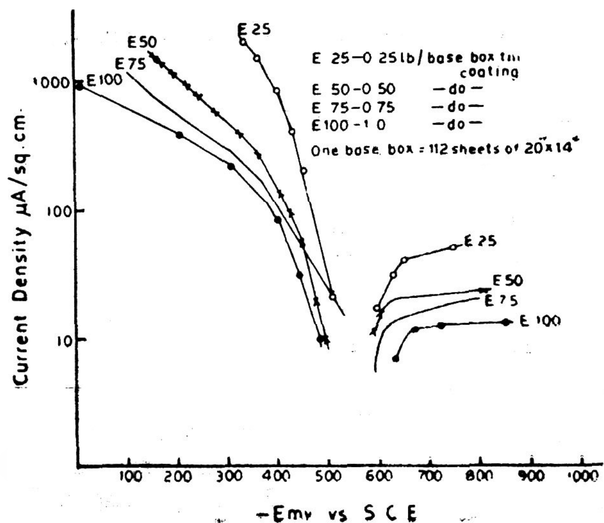

Fig. 4. Influence of thickness of tincoating on corrosion with orange juice

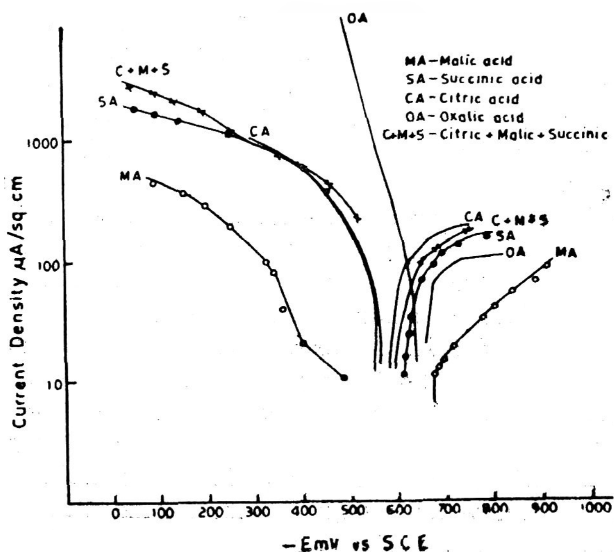

Fig. 2. Influence of organic acids on corrosion

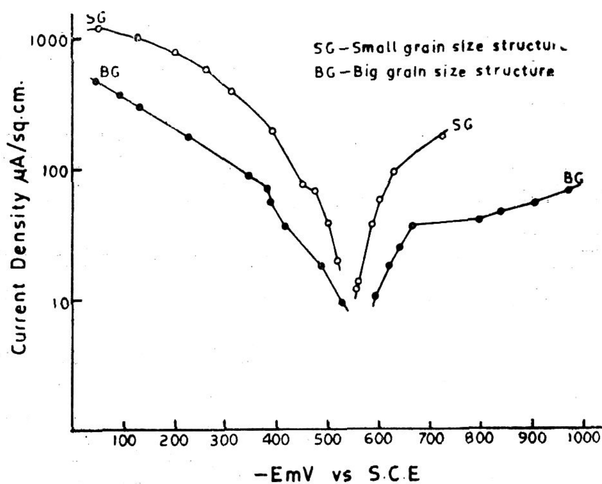

Fig. 5. Effect of grain structure of tincoating with mango nectar

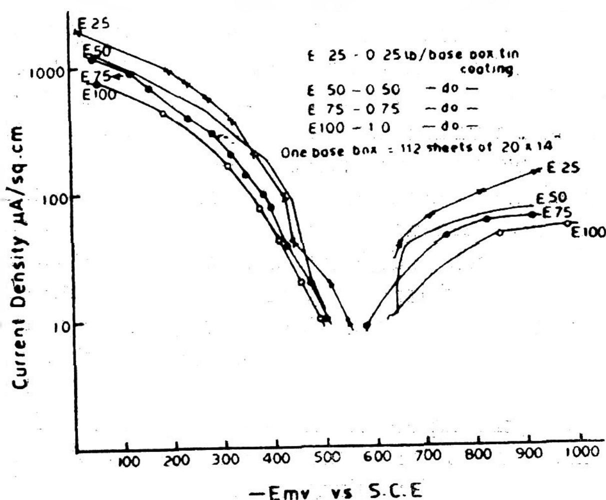

Fig. 3. Effect of thickness of tin coating on corrosion with mango nectar

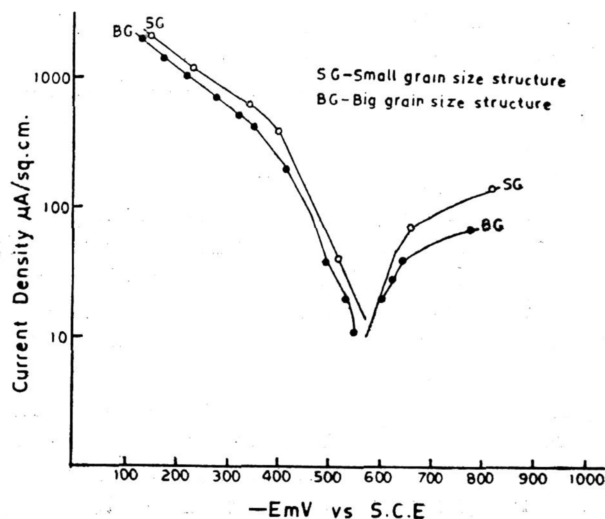

Fig. 6. Effect of grain structure of tincoating with orange juice

TABLE 1. TIN CONTENT (PPM) IN CANNED SUGAR SYRUP ALONE AND IN COMBINATION WITH DIFFERENT ORGANIC ACIDS

Storage period (months at 37°C)	Sugar syrup	Oxalic acid	Citric acid	Succinic acid	Malic acid	Citric +malic +succinic acid
Initial	Traces	65	35	32	30	29
3	,,	265	125	130	123	128
6	25	385	258	246	219	224
9	32	456	356	293	250	265
12	38	576	406	347	324	328

was measured using a vacuum tube voltmeter. Values of the applied current and the potential measured were plotted on a semi log paper. By extrapolating the Tafel lines of cathodic and anodic polarisation curves to the corrosion potential, the corrosion current of the specimen was determined.

Influence of different fractions of mango pulp: Organic acid, amino acid, sugar, sugar+organic acid fractions were separated from 250 g of 'Badami' mango pulp. Eluates of different fractions were diluted with distilled water (15 ml of the eluate made upto 100 ml). After dilution, the pH of different fractions were: organic acid 1.6; amino acid 8.8; sugar 5.7; and sugar+organic acid 2.6.

The potentials of the tinplate strips, having 1 lb of tin coating per base box in the diluted fractions were measured.

Influence of organic acids: Oxalic, citric, malic and succinic acids (all 0.3 per cent) were adjusted to pH 4 with sodium hydroxide and mixture of citric, malic and succinic acids (0.1 per cent each adjusted to pH 4).

Effect of varying thickness and grain size structure of tin coating: Tinplates with tin coating of 0.25, 0.5, 0.75 and 1.0 lb per base box and grain size structure equivalent to ASTM[12] No. 10 (small grain size) and No. 9 (big grain size) were used to determine the corrosion behaviour in presence of mango juice and orange juice.

Results and Discussion

Influence of different fractions: It may be seen from Fig. 1, in which the polarization curves of tinplate in mango pulp are represented that in most cases it is difficult to isolate the Tafel region from the overall polarization curve for making use of the same for directly estimating the corrosion rate. However, it is evident that the overall polarisation increases in the order: sugar(S), amino acid (AA), sugar+organic acid (S+OA), organic acid (OA) indicating thereby that the corrosion of tinplate is maximum in organic acid fraction and least in sugar fractions. These results are broadly in agreement with the direct weight loss measurements for different periods and also with the corrosion tests based on the estimation of tin contents in the respective media[13].

Influence of organic acids: The polarisation behaviour in the pure acids as well as in a mixture of acids is shown in Fig. 2. The Tafelian behaviour is observed in a few cases as far as anodic polarisation behaviour is concerned. The cathodic polarisation masked to some extent by concentration polarisation in all the cases. A qualitative comparison of the polarisation behaviour indicates that the corrosivity of organic acid decreases in the order oxalic, citric, succinic, citric+malic+succinic and malic acid. The measurements of corrosion rates by the method of estimation of tin content in the organic acids given in Table 1, confirms the above results.

Influence of thickness of tin coating: It may be observed from Fig. 3 and 4 that in both the mango nectar and orange juice, the corrosion resistance increases with increase in weight of tin coating, due to better coverage of the steel surface by tin coating. The above results are in agreement with the weight loss measurements carried out for periods upto 12 months.

Influence of grain size of tin coating: The effect of grain size on the polarisation behaviour of tinplate in mango nectar and orange juice has been brought out in Fig 5 and 6 from which it is clear that the coatings with bigger grains are more corrosion resistant than those with a smaller size, a fact which is also confirmed by direct weight loss measurements[12].

Conclusion: Steady state galvanostatic polarisation studies have been made use of for evaluating the degree of aggressiveness of different acids, and also to evaluate the relative performance of the coatings of different thickness and grain sizes. The agreement of the polarisation data with direct corrosion tests indicates that this method can be made use of as a rapid method for evaluating the tinplates in different food products. This technique is only of a qualitative nature and it takes about an hour for taking reading of one sample after the preparation of solutions and setting up of the apparatus.

Acknowledgement

The authors are grateful to Dr B. L. Amla, Director Central Food Technological Research Institute, Mysore for his keen interest and encouragement in these investigations. They are also thankful to the Director, Central Electrochemical Research Institute, Karaikudi for providing the facilities to conduct these experiments.

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The Influence of Ambient Temperature on the Biological Activity of Juvenile Hormone Analogue on Khapra Beetle (*Trogoderma granarium* Everts) Larvae

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Manuscript Received: 3 *June* 1975

Effect of ambient temperature on the pesticidal activity of a juvenile hormone analogue (JHA) ethyl trans-7. 11-dichloro-3, 7. 11-trimethyl-2-dodecenoate on khapra beetle (*Trogoderma granarium* Everts) larvae was studied. It was observed that about 16 per cent larvae entered facultative diapause when reared at 44°C on semolina. Incorporation of 15 to 100 ppm of JHA in the diet resulted in a progressive increase in the percentage of diapausing larvae from 25 to 90 per cent. Diapausing larvae survived at 44°C for over two months, but lowering of the temperature to 25°, 29° or 34°C resulted in termination of diapause and resumption of normal development which was interrupted in the presence of JHA. It is suggested that JHA brings about a feed-back inhibition of the neurosecretory system, which induces diapause and that khapra beetle larvae can adapt themselves to counteract the pesticidal action of JHA.

Slama *et al*[1] have recently reviewed the potential of juvenile hormone analogues (JHA) as pesticides. Our earlier studies[2-4] have shown that small concentrations of methyl or ethyl farnesoate dihydrochloride could control several serious coleopteran pests including the khapra beetle (*Trogoderma granarium* Everts) under laboratory conditions. Studies of several other investigators[5-7] have also established the potential use of JHA as pesticides for *Trogoderma* sp. which is a major pest of cereals in the tropics. All the studies reported above were conducted at 27 to 34°C. In tropical countries like India however, the day temperature may go upto as high as 45°C. Since the activity of enzymes involved in the metabolism of JHA is likely to be influenced by the change in external temperature, the effectiveness of JHA as a pesticide can be expected to be influenced by temperature fluctuations. Studies are reported in this paper which suggest that the biological activity of JHA ethyl-trans-7.11-dichloro-3, 7.11-trimethyl-2-dodecenoate (ETDD) on *Trogoderma granarium* Everts is

dependent to a great degree on the fluctuations in the environmental temperature.

Material and Methods

Juvenile hormone analogue ETDD was a generous gift from Hoffman-La Roche (Switzerland). All the experiments were performed at 44±2°C and 60±5 per cent RH using 16 to 18 day old khapra beetle larvae, reared on semolina under identical conditions according to the procedure described earlier[8]. Pesticidal action of ETDD was tested by rearing the larvae on semolina treated with 15 to 100 ppm of JHA according to the method of Thomas and Bhatnagar-Thomas[2]. Each treatment dose was replicated four times with 100 larvae in each replicate, and appropriate control was set up under identical conditions. To ascertain the morphogenetic effects of JHA, observations were generally made every 24 hr. Any deviation from the above experimental conditions is described in the text.

Results

Effect of JHA on larva at 44°*C*: Observations on the effect of JHA on larvae, at 44±2°C have been compared on 20th day of experiment and are presented in Fig. 3 and the larval-pupal, pupal-adult intermediates as well as the abnormal adults observed are shown in Fig. 1 and 2. The larvae which failed to pupate by 20th day of experiment are regarded as diapausing larvae as they undergo several supernumerary moults and show an average gain in weight of about 2 mg over a period of 2-3 months.

From Fig. 3 it can be seen that in the control about 84 per cent larvae had completed development upto the adult stage and 16 per cent larvae entered into facultative diapause. In the experimental group the percentage of larvae entering into diapause was dependent upon the concentration of the hormonal analogue in their diet and increased from 25 to 90 with increase in concentration of ETDD from 15 to 100 ppm, while the percentage of adults declined from 52 at 15 ppm to 1 at 100 ppm. Similar decrease in the percentage of intermediates from 23 to 8 was also recorded at these concentrations. However, it was interesting to note that at 15 to 30 ppm of JHA only larval-pupal intermediates were recorded (Fig. 1, 1a and 1b) while at higher concenerations mostly pupal-adult intermediates (Fig. 2, No. 3 and 4) were obtained. Except at 15 ppm of JHA, where the adults were normal in appearance, at all the other test concentrations, they were larger and abnormal (Fig. 2, No. 2).

Effect of reducing the temperature below 44°*C*: To examine if diapauses introduced in the presence of JHA by high temperature (Fig. 3) could be broken by lowering the treatment temperature, the larvae which were in diapause for 65 days on treated as well as untreated semolina were divided into three equal lots along with their respective diets. Two sets of such larvae, consisting of control as well as those containing 15 to 100 ppm of JHA were transferred to incubators maintained at 34±1°C and 29±1°C respectively, while the third set was retained at 44±2°C for another 25 days and then exposed to 25±2°C for 6 hr and retransferred to 44°C to see the effect of short exposure to low temperature. All the three sets of experiments were examined daily for any morphogenetic effects of JHA on the insect and the results are presented in Fig. 4. Larval diapause was considered to be terminated upon the pupation of the larvae.

When the larvae were transferred from 44° to 34±1°C (Fig. 4 B) diapause was terminated on both treated as well as untreated semolina within 4 to 5 days, as all the diapausing larvae pupated during this period. In control and samples containing 15 ppm of JHA, all the larvae developed upto the adult stage within 9 days of transfer. While in the presence of 20 and 50 ppm of JHA, 22 and 2 per cent of larvae developed into abnormal adults (Fig 2, 2) and remaining 78 and 97 per cent into larval-pupal intermediates (Fig 1, 1a and 1b). At 100 ppm of ETDD all the larvae developed into larval-pupal

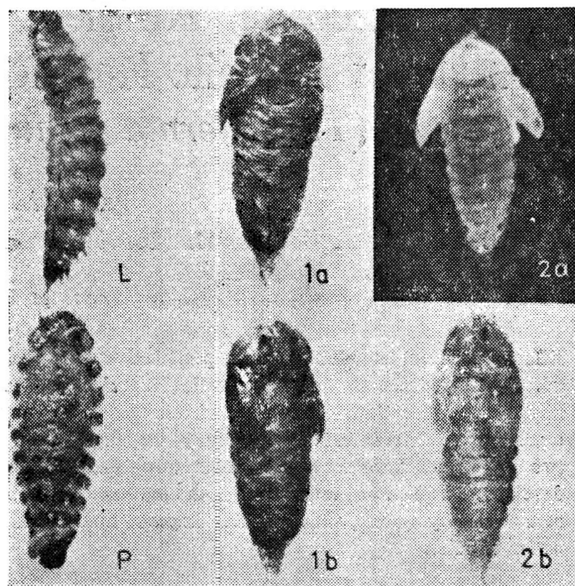

Fig. 1. Larval-pupal transformations of *Trogoderma granarium* Everts larva in the presence of juvenile hormone analogue at various temperatures.

L, normal larva; P, normal pupa; 1a and 1b, dorsal and ventral view of intermediate recorded at 44°C and 34°C, sclerotization confined to everted wings, brown in colour, excessive dorsal pubescence while ventral region comparatively free of hair, abdomen larval; 2a and 2b, dorsal and ventral view of intermediate mostly recorded at 29°C, wings everted opaque like onion skins, soft pale white body with short larval setae preserved on abdomen.

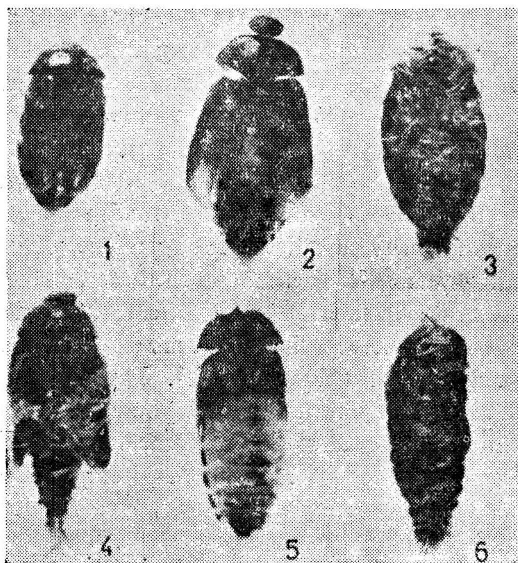

Fig. 2. Pupal-adult transformations of *Trogoderma granarium* Everts larva in the presence of juvenile hormone analogue at various temperatures.

1, normal adult; 2, abnormal adult, one-and-a-half times the size of normal adult, wings with partial sclerotization and held at an angle to the abdomen, mouth parts and genitalia abnormal; 3, intermediate recorded at 44°C, adult-like shape, short wings, body covered with excessive pubescence, mouth parts and genitalia abnormal; 4, intermediate form recorded at 44°C, anterior normal adult-like with normal mouth parts and head capsule, abdomen with crescent shaped pupal cuticle, genitalia arrested with localized pupal cuticle; 5, intermediate produced upon lowering the treatment temperature from 44 to 25°C for 5 hr, head capsule, mouth parts adult in nature, short sclerotized wings, abdomen pupal with mid-dorsal sclerotized patches, while ventral region fully sclerotized; 6, same as 5 except both dorsal and ventral parts completely sclerotized.

intermediates and no pupal-adult intermediates or abnormal adults were observed.

Within 7 to 8 days of transfer of the larvae from 44°C to 29±1°C the diapause was terminated and all the larvae pupated in the control group and developed into adults by 12th day (Fig. 4C). Except at 15 ppm of ETDD where 70 per cent normal adults were recorded, the rest of the larvae at 15 ppm and higher concentrations of JHA developed into larval-pupal intermediates (Fig 1, 2a and 2b).

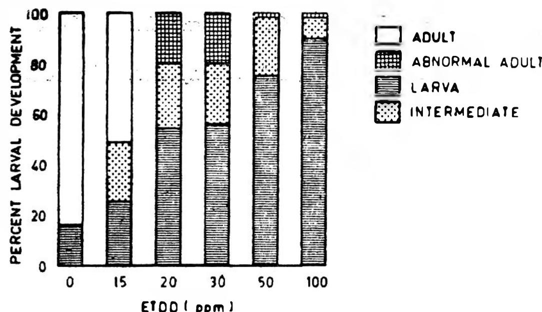

FIG 3 EFFECT OF ETDD ON THE METAMORPHOSIS OF TROGODERMA GRANARIUM EVERTS LARVAE AT HIGH TEMPERATURE (44 ± 2°).

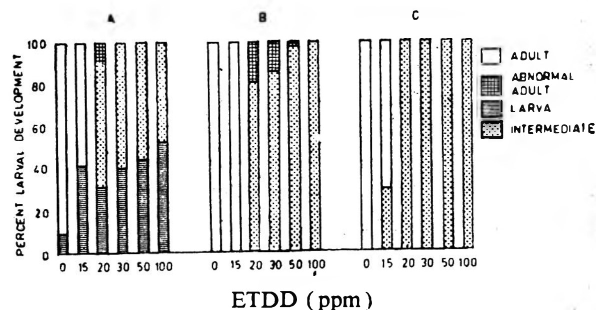

Fig. 4. Modification of the Effect of ETDD on the metamorphosis of *Trogoderma granarium* Everts by Temperature. A : 44° → 25° → 44°; B : 44° → 34°; C : 44° → 29°.

By lowering the temperature from 44° to 25±2°C for 6 hr the diapause of khapra beetle larvae was broken to various extent (Fig. 4A). In the control about 90 per cent larvae developed into adults in comparison to 58 per cent at 15 ppm and 10 per cent at 20 ppm and the latter were abnormal. In addition to this about 58 and 47 per cent pupal-adult intermediates (Fig 2, 5 and 6) were recorded at 20 to 100 ppm of JHA. These intermediates differed markedly from those observed at 44°C. The percentage of larvae unable to terminate diapause state varied from 10 in the control to 8 and 53 at 15 ppm and 100 ppm of JHA. These larvae continued to be in diapause for another 25 days when the experiments were discontinued. These studies indicate that without exposure to the low temperature the larvae probably would have continued in diapause state at least for 30 to 35 days.

Discussion

Diapause in khapra beetle larvae has been described by Burges[9,10] and is known to occur at or below 30°C and in the presence of faecal matter in the diet at optimum temperature of 34°C. However, this is the first instance when diapause has been detected in these larvae at higher temperatures. Author's previous studies[3] have shown that diapause can be induced in these insects at 34°C with 300 to 1200 ppm of JHA. The level of JHA required to induce 90 per cent diapause at 44°C is now found to be only 100 ppm of JHA. A recent report by Nair[11] indicates that at 30°C a much higher level of JHA (1 per cent) is required for inducing diapause in khapra beetle larvae. As discussed earlier[3] induction of diapause in khapra beetle larvae in the presence of 300 to 1200 ppm of JHA was possibly due to insufficient food consumption. Since the concentrations of 15 to 100 ppm of JHA employed in the present studies were too low to affect food consumption, insufficient food intake is unlikely to be a major factor responsible for diapause. However, induction of diapause in 16 per cent control larvae together with the dose dependent relationship between externally administered JHA and percentage of

diapausing larvae suggests that both temperature and JHA are causative factors in induction as well as maintenance of larval diapause. Termination of larval diapause upon lowering of temperature from 44°C as well as the differences in the morphogenetic effects of JHA at various temperatures further suggest that the rate of metabolism of JHA may be different at various temperatures.

Although the present studies do not give any information on the physiological mechanism(s) involved in diapause at various temperatures, the termination of diapause in about 50 per cent larvae upon a short exposure (6 hr) to 25°C suggests that the mechanism(s) involved in induction as well as termination of diapause are reversible. It is conceivable that the high temperature inhibits synthesis or inactivates the enzymes involved in JH/JHA degradation resulting in the increased titre of the hormone and the analogue in the haemocoel of the larva. The increased titre of the hormone probably induces diapause by feed-back inhibition of the neuroendocrine system, thereby postponing the "critical period" essential for the action of exogenous JHA as a pesticide. Termination of diapause upon exposure to lower temperatures may similarly be attributed to the acceleration of synthesis of JH/JHA degrading enzymes or their stability, thus facilitating the action of low concentrations of exogenous JHA to exert its pesticidal effect.

Present observations on the termination of diapause in about 50 per cent larvae on short exposure (6 hr) to low temperature, further indicate that the chances of khapra beetle larvae entering and continuing in diapause in tropics are remote, since the night temperature is generally found to be about 25°C during summer. However, temperature is only one of the several factors known to induce diapause in khapra beetle. It is known that khapra beetle larvae can survive conditions of high population density, insufficient food, presence of excreta, etc., upto 4 years[9,10]. These factors have to be taken into account while considering the practical development of pesticides based on JH, since JHA mixed with the diet is stable only for about three months[3]. Thus the ability of khapra beetle larvae to undergo diapause at high temperature in the presence of low concentrations of JHA or high concentrations of JHA at low temperatures[3,11], together with the ability of such larvae to develop into normal fertile adults in the absence of JHA[3,11] suggest possible limitations on the use of JHA as pesticides. It is likely that larvae of khapra beetle as well as other insects, where JH/JHA has been shown to be the principle agent for larval diapause[3,11-14] can employ their inherent capacity to enter diapause as a means of overcoming the pesticidal activity of JHA and may, therefore, be able to develop resistance to JHA in due course of time.

Acknowledgement

The author is grateful to Shri N. S. Rao, Head of Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay for providing space and facilities for the present work.

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Studies on the Formulation of Egg Substitutes from Milk Protein Complexes for Use in Cake Making

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Manuscript Received: 3 *June* 1975

An acceptable egg substitute has been formulated using whey protein isolate in place of egg in cake mix. Loaf volume was found to be maximum in the whey protein based cakes.
Foam stability was studied at different levels of sodium caseinate, calcium caseinate and lecithin. Sodium caseinate did not improve the foam stability. Calcium caseinate was found to have a favourable effect on the stability of the foam. Addition of lecithin to the formulation had an adverse effect on the foam stabilising properties.

A material that could substitute egg in the manufacture of bakery and confectionary products has been long sought by the food industry. Development of suitable materials, with milk proteins as base, possessing the functional characteristics of egg, would be of special interest in a country like India where a majority of population have religious reservations on the consumption of egg as such or with other food products.

It has been well known that whey proteins, contribute substantially towards the foam forming characteristics of milk[1]. Pavcek[2] and later on Lindewald and Gruben[3] have reported preparations of egg substitutes using a blend of skim milk and whey mixture. In recent years, methods have been developed for the isolation of whey proteins by cold precipitation technique using ionic-hydrocolloids, thereby preserving the functional characteristics of whey proteins[4]. The present work was undertaken to evaluate the foam forming and whipping abilities of whey protein complexes with carboxymethyl-cellulose and emulsifiers. The efficacy of use of such combination for cake making was also experimentaly ascertained.

Materials and Methods

Whey protein-CMC complex was prepared from casein whey according to the methods of Hidalgo and Hansen[4]. Sodium caseinate and calcium caseinate were obtained from the Experimental Dairy, National Dairy Research Institute, Karnal.

Measurement of whipping ability: Whipping ability, specific volume of the foam and foam stability were determined according to the method of Hansen and Black[5]. Specific volume of foam was expressed as ml per g. The rate of free volume released from the foam was taken as an index for foam stability.

Manufacture of cake: For the evaluation of suitability of formulated egg substitute for cake making the following recipe was used.

	Percentage
Sweetened condensed milk	51.0
Butter	14.6
Maida (wheat flour)	32.0
Baking powder	1.2
Vanilla	0.6
Baking soda	0.6

The egg, egg substitute and water for egg substitute added were 12, 9 and 8.9 per cent respectively of the above mixture.

Preparation of cake: The butter and *maida* (wheat flour) were beaten to a creamy consistency. Sweetened condensed milk, baking powder and baking soda were mixed. Egg substitute was dispersed in the water required for cake making and aged over-night before mixing with other ingredients of cake mix. The mixture was then transferred to a mechanical kneader and the beaten mixture was placed in a mould and baked in an electrically heated oven at 110°C for 90 min.

Determination of rise in volume during baking of cake: This was determined by modifying the method of American Institute of Baking[6]. 250 ml (V_1) of cake mix was poured into the cylindrical moulds (1000 ml capacity). After baking, the void volume above the cake surface in the mould was filled with fine ground sugar and levelled upto the brim. The volume of this sugar was determined (V_2). Likewise the volume of the sugar in the empty moulds was determinded (V_3). Volume ratio was determined as follows:

$$\frac{(V_3-V_2)-V_1}{V_1}$$

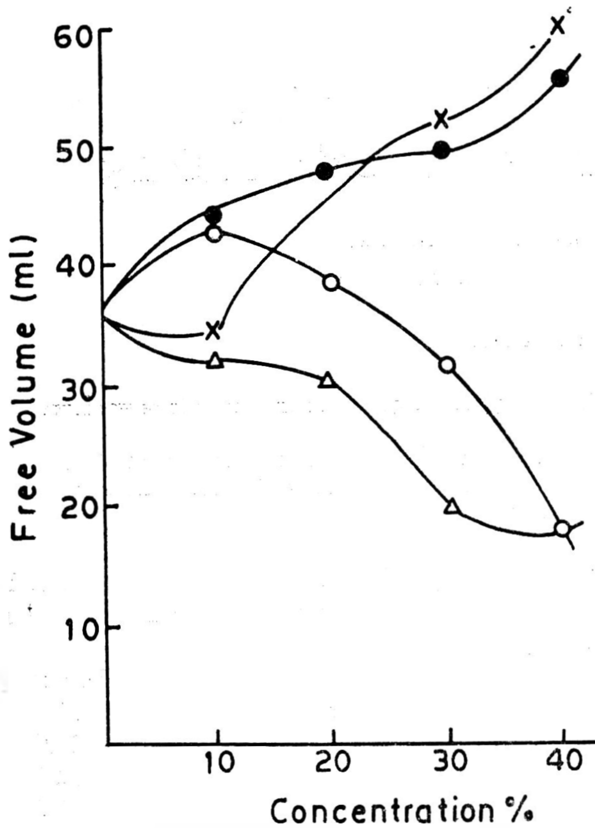

Fig. 1. Effect of additives (sodium caseinate, calcium caseinate & lecithin) on the foam stability of CMC-complexes

×—×, Sodium caseinate; ●—●, Sodium caseinate + 0.1% lecithin; △—△, Calcium caseinate + 0.1% lecithin; O—O, Calcium caseinate.

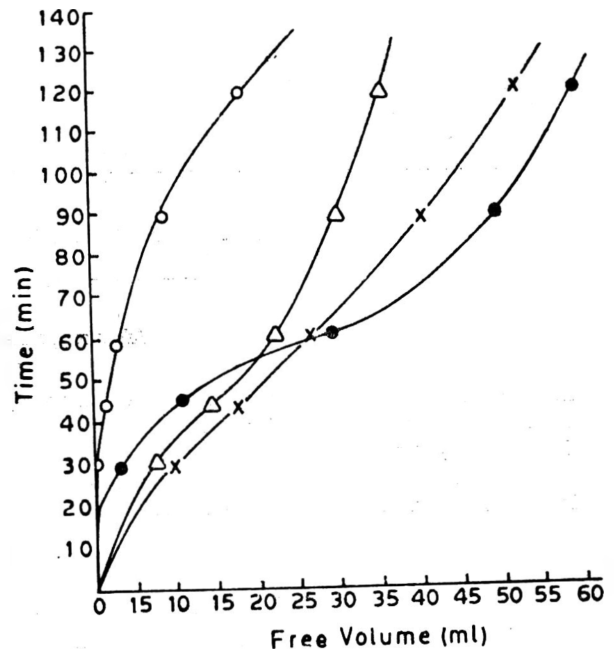

Fig. 3. Comparative foam stability of whey protein–CMC complex with egg and formulated egg substitute.

O—O, 60% Whey protein carboxymethyl cellulose + 40% calcium caseinate; ×—×, Egg; △—△, Whey protein–CMC complex; ●—●, 60% Whey protein–CMC complex+40% sodium caseinate.

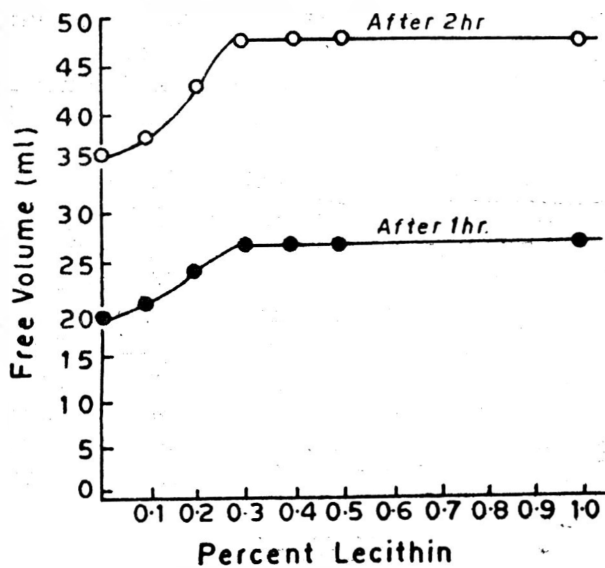

Fig. 2. Foam stability of whey protein carboxy methyl cellulose complex with different levels of lecithin.

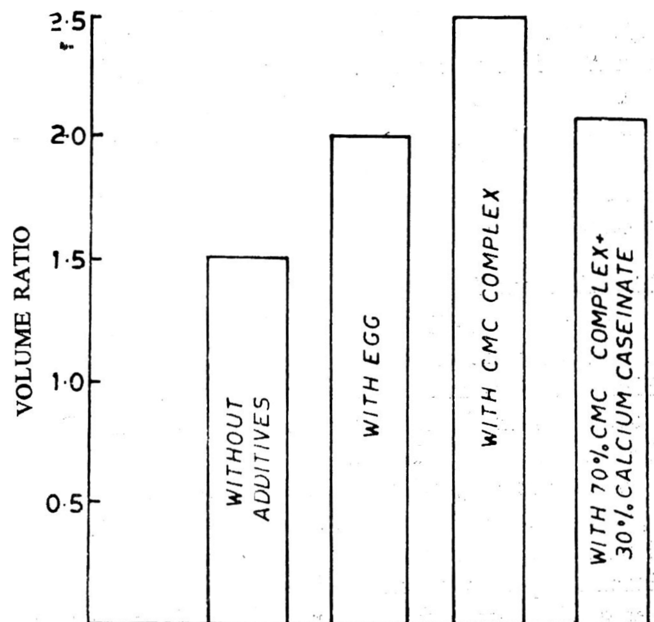

Fig. 4. Effect of additives in the cake mix on the increase in cake volume during baking

Sensory evaluation of cake: Subjective examination of cake was done in accordance with the method recommended by the American Institute of Baking for cake scoring. External characteristics of cake were examined for the colour of crust, symmetry of form, body and texture. Internal characteristics were examined for the crumb structure, size and uniformity of cells, uniformity of baking, crumb colour and structure, flavour and mastication qualities.

Results and Discussion

With a view to determine the optimum level of whey protein-CMC complex for the study of foam stability in these trials, the spray dried material was dispersed in water at 2.5, 5.0, 7.5 and 10 per cent level using a mechanical stirrer and soaked overnight. At 7.5 and 10 per cent levels there was considerable difficulty in wetting and dispersing of powder. The specific volume at 2.5 and 5 per cent levels was observed to be 2.7 and 2.08 respectively. From these trials it was concluded that the spray dried materials at 5 per cent level in water could be suitably utilised for the formulation studies.

It was observed that spray dried whey protein-CMC complex exhibited superior whipping properties compared to egg, producing a stiff foam. Since whipping of solutions containing only CMC do not produce comparable foams, Hansen and Black[5] attributed this property to the whey proteins.

The effect of addition of sodium and calcium caseinates to spray dried whey protein-CMC complex on the foam stability was studied at 10, 20, 30 and 40 per cent levels. The resulting mixtures were dispersed to give 5 per cent whey protein-CMC complex in water. Foam stability of these mixtures was measured and results are expressed in Fig. 1. Addition of sodium caseinate does not show a favourable effect on the foam stability. Addition of sodium caseinate for the formulation of 'egg substitute' was, therefore, not considered worthwhile in the trials on cake making. However calcium caseinate exhibited a very favourable effect on the stability of the foam. It may be seen from the Fig. 1 that addition of calcium caseinate at concentrations upto 30 per cent in the whey protein-CMC complex had substantial stabilising effect, but further addition did not prove to be of more advantage for improving the foam stability.

Comparison with egg: It was observed that the addition of lecithin to the formulation had slightly adverse effect on the foam stabilising properties of the whey protein-CMC complex as shown in Fig. 2. However above 0.3 per cent level, addition of lecithin does not further reduce the foam stability. It may be noted from the Fig. 3, that the CMC-whey protein formulations exhibited superior foam stabilities when compared to beaten egg, except in the case when sodium caseinate was used.

TABLE 1. AVERAGE SCORE REPORTS OF THE CAKES MADE WITH EGG WHITE AND FORMULATED EGG SUBSTITUTES

Characteristics	Perfect score	Average scores A	B	C	D
A. External					
Volume	10	9.0	10.0	9.5	9.5
Colour of crust	8	7.5	7.0	7.0	7.0
Symmetry of form	3	3.0	2.5	2.5	2.5
Evenness of bake	3	3.0	3.0	3.0	3.0
Character of crust	3	2.5	2.5	2.5	2.5
Break & shred	3	2.5	2.5	2.5	2.5
Total score	30	27.5	27.5	27.0	27.0
B. Internal					
Grain	10	9.5	9.0	8.5	9.0
Colour of crumb	10	9.5	9.5	9.5	9.5
Aroma	10	9.0	9.0	9.0	9.0
Taste	15	14.0	14.0	14.0	14.0
Mastication	15	14.0	13.0	12.5	12.5
Texture	70	65.0	63.0	61.0	62.5
Total score		92.5	90.5	88.0	89.5

A: Cake with egg white
B: Cake with whey protein-CMC complex
C: Cake with whey protein-CMC complex plus 30 per cent calcium caseinate
D: Cake with whey protein-CMC complex plus 30 per cent calcium caseinate plus 0.1 per cent lecithin.

The organoleptic evaluation of the cake prepared by various formulations are given in Table 1.

Based on the results of foam stability whey protein-CMC complex as such, and in combination of sodium caseinate, calcium caseinate and lecithin, it was considered desirable to evaluate the suitability of following mentioned formulations for the replacement of egg in cake making:

i) Whey protein-CMC complex
ii) Whey protein-CMC plus 30 per cent calcium caseinate.

In our trials, cakes made with whey protein-CMC complex (as substitute for egg) exhibited maximum swelling during baking. The volume ratio in this case was found to be 2.5 (Fig. 4). The increase in volume during baking when 30 per cent calcium caseinate was also added in the whey protein—CMC formulation was found to be 1.9 and was quite comparable with the values obtained with egg cake. The depressing effect of caseinate on the loaf volume during baking is also observed in bread making, when low heat milk powders

are used in the preparation of dough[8]. It appears that caseinate in the presence of undenatured whey proteins form such complexes during baking, which have a depressing effect on the increase in volume. It may be observed therefore, that although calcium caseinate improves the foam stability of the whey protein-CMC complexes, but in cake making, their presence slightly impairs the performance of whey proteins towards yielding higher cake volume.

These studies show that egg can be fully replaced by the whey protein-CMC formulation in the cake mix and with this it is possible to prepare cakes, which compare favourably with the conventional product containing egg.

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Studies on Meat Evaluation of Broiler Chickens

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Manuscript Received: 26 *June* 1975

Meat evaluation of four breeds of broiler chickens in terms of dark-light meat ratio and meat-bone ratio of raw and cooked carcasses for differences due to 16 genetic groups produced in a 4×4 diallel-system were studied. Significant genetic group differences for only meat-bone ratio of cooked meat were observed. Analysis for the effects of heterosis, purebreds, general combining ability, maternal ability, specific combining ability and reciprocal is also presented. Purebreds and reciprocal effects were significant ($P < 0.05$) for only cooked meat-bone ratio.

Broiler meat is liked by consumers because of its tenderness and juiciness, which determine to a large extent the palatability. Availability of actual meat and the relative proportion of the type of meat (dark or white) influence the choice of broiler strain among almost all meat consumers. Type of meat and meat-bone ratio are, therefore, the important characteristics contributing to the consumers preference. This study was thus undertaken to find out the difference in these poultry meat attributes (on raw and cooked meat) in different broiler strains and their crossbreds.

Material and Methods

Sixteen genetic groups involving white rock, White Cornish, New Hampshire and Austrolorp breeds in a 4×4 diallel system were produced for this investigation. At the age of 12 weeks, 2 birds of each of the 16 genetic group were taken for estimation of dark-white meat ratio and meat-bone ratio on raw as well as cooked carcasses. The cooking of the dressed carcasses was done in an electric hot air oven at 163°C for 1.5 hr. Sixty four carcasses used for this study were the random sample representing the population under test.

Analysis of the data was conducted using the following statistical model.

$$Yij = \mu + Gi + eij$$

where,

Yij = j-th observation of i-th genetic group.

μ = overall population mean

Gi = i-th genetic group ($i = 1, 2-p, p = 16$)

eij = random error peculiar to ijth observation, NID with mean zero and variance σ^2_e.

TABLE 1. MEANS OF RAW AND COOKED CARCASS CUTS IN DIFFERENT GENETIC GROUPS OF BROILERS

	Raw		Cooked	
	Dark light meat ratio	Meat bone ratio	Dark light meat ratio	Meat bone ratio
White Rock (WR)	1.045	3.085	1.010	3.135
Australorp (A)	1.090	2.905	0.985	3.585
White Cornish (WC)	1.070	2.920	1.020	3.600
New Hampshire (NH)	1.020	2.955	1.145	2.360
WR×A	1.045	3.170	1.125	2.580
WR×WC	1.160	3.050	1.110	3.590
WR×NH	1.055	2.710	1.055	2.685
A×WR	1.080	2.800	0.950	3.100
A×WC	1.050	2.845	1.140	2.910
A×NH	1.130	2.670	1.135	2.610
WC×WR	1.000	2.775	1.095	2.630
WC×A	1.025	3.170	0.990	3.340
WC×NH	1.150	3.020	1.175	2.440
NH×WR	0.995	2.910	1.085	2.875
NH×A	1.040	2.965	1.125	3.395
NH×WC	1.100	3.095	1.160	2.655
Overall	1.072 (32)	2.917 (32)	1.081 (32)	2.910 (32)

TABLE 2. ANALYSIS OF VARIANCE OF RAW AND COOKED CARCASS TRAITS FOR GENETIC GROUPS

		Raw		Cooked	
Sources of variation	d f	Dark light meat ratio	Meat bone ratio	Dark light meat ratio	Meat bone ratio
Between genetic groups	15	0.006	0.041	0.010	0.339*
Residual	16	0.008	0.111	0.017	0.069

*$P < 0.05$

TABLE 3. LEAST SQUARE ANALYSIS OF VARIANCE FOR RAW AND COOKED CARCASS CUT TRAITS

		Raw meat		Cooked meat	
Sources of variation	d f	Dark light meat ratio	Meat bone ratio	Dark light meat ratio	Meat bone ratio
Heterosis	1	0.08	2.53	1.76	0.08
Among purebreds	3	0.86	1.34	1.02	62.29*
General combining ability	3	0.03	3.67	0.45	2.05
Maternal ability	3	0.95	8.88	0.31	19.05
Specific combining ability	2	0.88	9.09	0.40	22.64
Reciprocal effects	3	0.43	2.67	1.20	42.99*
Error	14	0.79	10.11	2.08	9.28

*$P < 0.05$.

TABLE 4. LEAST SQUARE CONSTANTS FOR PUREBRED AND RECIPROCAL EFFECTS FOR COOKED CARCASSES

Effect	Cooked meat: Dark light meat ratio	Cooked meat: Meat-bone ratio
Purebred		
White Rock (P 11)	−0.300	2.150
White Cornish (P 22)	−0.200	6.800
New Hampshire (P 33)	1.050	−5.600
Australorp (P 44)	−0.550	−3.350
Reciprocal		
R 12	−0.313	3.644
R 13	−0.344	0.331
R 14	0.657	−3.975
R 21	0.313	−3.644
R 23	0.269	1.337
R 24	−0.582	2.307
R 31	0.344	−0.331
R 32	−0.269	−1.337
R 34	−0.075	1.668
R 41	−0.657	3.975
R 42	0.582	−2.307
R 43	0.075	−1.668
Overall mean	1.067	2.914

Since the genetic groups were significant in some cases, detailed analysis as to the effects due to heterosis, purebred, general and specific combining abilities, maternal ability and reciprocal was carried out using model 8 suggested by Harvey[1].

Results and Discussion

Average of dark light meat ratio and meat bone ratio of raw and cooked carcasses of 16 genetic groups are presented in Table I.

Dark-light meat ratio: This ratio ranged from 0.995 to 1.10 with an average value of 1.072 in raw meat, while the corresponding values for cooked meat were 0.985, 1.175 and 1.081 respectively. Differences among genetic groups for this trait were statistically non-significant both for raw and cooked carcasses (Table 2). This indicated that the proportion of dark light meat is not variable among the broiler breeds and breed crosses studied. This ratio remained almost constant with slightly higher weight of light meat than dark meat. Improvement of the dark: light meat ratio through involving these broilers breeds in crossbreeding is, therefore, hardly possible.

Meat bone ratio: Values for this ratio ranged between 2.670 to 3.085 and 2.360 to 3.600 for raw and cooked meat respectively (Table 1) with their overall average as 2.917 and 2.910. Although the genetic group differences were non-significant for raw meat, yet the differences were significant at $P < 0.05$ for cooked meat. Statistically significant differences for the latter may be

indicative of the fact that the varying water losses in cooking introduced variation due to the genetic groups under study. Every possible care was taken to cook the carcasses under same temperature and time. The increase in the variability of meat-bone ratio may probably be due to carcass differences within genetic groups for raw and cooked meat and also due to differential water loss response from raw to cooking stage among genetic groups. The overall meat-bone ratio in raw and cooked were apparently similar but still the differences for this ratio of individual genetic groups in most cases were quite marked. This may indicate that besides differential water losses from the musculature, similar losses could have occurred from bone marrow after cooking. Some genetic groups may have heavier, thicker bones at the same age than others, eventually affecting the available edible poultry meat. This needs to be studied on a larger scale for selection of only those genetic groups which make available more of edible meat.

Combining abilities: Analysis of variance for the effects of heterosis, purebreds, general combining ability, maternal ability, specific combining ability and reciprocal for dark-light meat ratio and meat-bone ratio is presented in Table 3. Significant differences at $P < 0.05$ were observed due to purebred and reciprocal effects for cooked meat-bone ratio only. All other effects in case of raw and cooked meat were non-significant. This indicated that the four breeds and also their reciprocal combinations differed in their meat-bone ratio, which direct towards making selection of broiler breeds for more meat bone ratio. For cooked meat the least square constant for White Cornish was maximum followed by White Rock, Australorp and New Hampshire in descending order (Table 4). Crossbred of Australorp male with White Rock female, White Rock male with White Cornish female, White Cornish male with Australorp female were the top three combiners and excelled over their reciprocals.

Acknowledgement

The authors gratefully acknowledge the facilities provided for this work to the Professor & Head, Deptt. of Animal Breeding.

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Isolation of Salmonellae from the Market Meat—A Field Study

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Manuscript Received: 26 *June* 1975

Describes the use of two abridged methods for Salmonella isolation from sheep/goat carcasses sold in the market. The various strains isolated during the course of the study consisted of *Salmonella bareilly, Aerobacter aerogenes, Aerobacter cloacae, Proteus* and *Citrobacter freundii.*

In food analysis laboratories for routine screening of large number of samples rapidly, quick methods become a necessity. This will facilitate the isolation and identification of maximum number of diversified serotypes of *Salmonella* as its presence in food is not allowed[1]. The methods also have to satisfy the criteria of sensitivity combined with simplicity and rapidity.

Different laboratories in the world follow different methods[2,3]. The methods recommended by American Public Health Association[2] (APHA) and that described by Thatcher and Clark[4] (TC) are some of the procedures adopted for routine working. The present communication deals with the efficacy of these two methods for revealing the presence of *Salmonella* from sheep/goat carcasses.

Materials and Methods

The media and reagents used were prepared according to Thatcher and Clark[4] and Difco Manual[5]. The polyvalent 'O' and polyvalent 'H' antisera used in these

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studies were obtained from Haffkine Institute, Bombay. The meat samples were derived from different parts of sheep and goat caracasses sold in the local market. In each case 25 g of the sample was transferred to 225 ml tetrathionate broth and incubated at 37°C for 24 hr. These samples were processed as per APHA and TC procedure with slight modifications.

The modifications introduced in APHA procedure consisted of: (*i*) Discontinuation of the use of Salmonella-shigella agar as the growth of *Proteus* and *Pseudomonas* especially the former found invariably to mask the growth of the other; (*ii*) characteristic colonies (grey to black colonies which show sometimes metallic sheen in the case of Bismuth Sulphite Agar and pink to fuchsia or transparent to opaque colonies with the surrounding medium pink to red in the case of Brilliant Green Agar); were further screened and purified on MacConkey's Lactose Agar (MLA) instead of Tryptone Agar; and (*iii*) instead of SIM medium as suggested in the procedure, motility test and indole reaction were carried out independently in motility and tryptone water media respectively. In the case of TC procedure (*i*) Gillies 'B' medium used did not contain salicin. Absence of salicin did not matter much as the medium does not indicate the fermentation of sucrose and salicin independently; (*ii*) serological tests with polyvalent 'O' and polyvalent 'H' antisera[4] were not performed simultaneously as indicated in the procedure but the cultures were first tested with polyvalent 'O' antiserum. Agglutination test with polyvalent 'H' antiserum was performed only with those cultures which could not agglutinate with polyvalent 'O' antiserum, since the isolates positive for either of the two antisera are considered to be *Salmonella*. Tests with group 'O', individual 'O', 'H' and 'Vi' antisera as laid down in the procedure were not performed due to non-availability of the same.

Isolates suspected to be *Salmonella* on the basis of above tests as well as those isolates showing typical biochemical reactions (positive presumptive test) but negative by serological tests (confirmative test) were sent to National *Salmonella* and *Escherichia* Typing Center (NSEC) Kasauli, Simla for confirmation and typing.

Results and Discussion

A total of forty one isolates (which included 31 isolates biochemically and serologically typical and 10 isolates biochemically typical but negative serologically) were obtained from 45 sheep and goat carcasses using APHA and TC procedures. On the basis of our own study, as well as by NSEC, it was observed that only three isolates were confirmed as *Salmonella bareilly*. The remaining isolates were identified as *Aerobacter aerogenes* (5), *Aerobacter cloacae* (20), *Proteus* (2), *Citrobacter freundii* (9), *Aerobacter* sp. (1) and one unidentified isolate. Details of biochemical and serological characteristics of the strains isolated and their typing by NSEC are indicated in Table 1.

During the analysis of foods for *Salmonella*, certain difficulties are encountered which are not common with the clinical material. Firstly, *Salmonella* in foods usually comprise an exceedingly small number when compared to the total microbial population. Secondly they are invariably out-numbered by coliform bacteria thus necessitating the use of selective enrichment media. In the present study, tetrathionate broth in combination with Brilliant Green Agar and Bismuth Sulphite Agar were used for selective enrichment and differentiation of *Salmonella*. In spite of inhibitory and selective action of these media the isolates obtained in the present study simulating the growth and colony characters of *Salmonella* managed to pass through successfully and later on proved to be non *Salmonella*. These non *Salmonellae* belonged to *Citrobacter*, late lactose fermenting coliforms and *Proteus* species. Hormasche and Peluffo[6] have stated that colonies of *Proteus* and late lactose fermentors belonging to *Escherichia* group produce similar colonies on Brilliant Green Agar medium. Taylor and Silliker[7] have reported that on Bismuth Sulphite Agar medium the non-*Salmonella* organisms simulating the growth of *Salmonella* appear after 48 hr of incubation, the time invariably required to grow *Salmonella* colonies also.

Non fermentation of lactose by *Salmonella* is the principal differential biochemical reaction. All the 41 *Salmonella*-suspected isolates were unable to ferment lactose in MLA on primary isolation. On further studies 29 of these strains were identified as *Citrobacter* (9) and *A. cloacae* (20). *Citrobacter* and *A. cloacae* are reported to ferment lactose[8]. Experiments of Lapage and Jairaman,[9] on known lactose fermenting strains of different genera of Enterobacteriaceae and its revealation in various lactose containing media, showed MLA to be least sensitive to record lactose fermentation especially in case of *Citrobacter* and *A. cloacae*.

Of the 41 *Salmonella* suspected isolates, 23 failed to produce H_2S. *Salmonella* usually produces H_2S[10], with the exception of species like *S. paratyphi A*, *S. cholerasuis*, *S. typhi-suis*, *S. sendei* and *S. berts*. The isolation of *S. paratyphi A*, from the food as an external contaminant[11] and from the sheep carcass has been reported. Therefore, these 23 isolates though negative for H_2S production were tested against polyvalent "O" antiserum, which they agglutinated readily, excepting 3 isolates which were biochemically typical but serologically negative. But on further studies none of these isolates proved to be *Salmonella*. Incidentally these cultures also could ferment sucrose, a reaction rather rare with *Salmonella*. It, therefore, seems to be advisable that those cultures,

TABLE 1. BIOCHEMICAL AND SEROLOGICAL CHARACTERS OF ISOLATES FROM SHEEP AND GOAT CARCASSES

No. of isolates	Triple Sugar Iron Agar						Gillies 'A' medium				Gillies 'B' medium			Serological Tests		NSEC typing
	Butt	Slant	H_2S	Urea	Indole	Motility	Urea	Glucose	Mannitol	Sucrose Salicin	Indole	H_2S	Motility	Polyvalent 'O' anti-serum	Polyvalent 'H' anti-serum	
APHA Procedure																
1	YG	NC	—	—	—	±								+		*Aerobacter aerogenes*
1	YG	NC	—	—	—	+								+		*Aerobacter?*
4	YG	NC	—	—	—	+								+		*Aerobacter cloacae*
2	Y	NC	—	—	—	+								+		*Proteus*
1	Y	Y or NC	—	—	—	+								+		Strain under study
1**	YG	NC	+	—	—	+								—		*Citrobacter freundii*
TC Procedure																
1							—	YG	Y	—	—	+	+	+	Not done	*Salmonella bareilly*
6							—	YG	Y	+	—	+	+	+	,,	*Aerobacter cloacae*
2							—	YG	Y	—	—	+	+	+	,,	*Citrobacter freundii*
1							—	YG	Y	—	—	+	+	+	,,	*Salmonella bareilly*
7							—	YG	Y	+	—	—	+	+	,,	*Aerobacter cloacae*
1							—	YG	Y	+	—	—	+	+	,,	*Aerobacter aerogenes*
3							—	Y	Y	+	—	—	+	+	,,	*Aerobacter cloacae*
6**							—	YG	Y	—	—	+	+	—	—	*Citrobacter freundii*
1							—	YG	Y	—	—	+	+	+	Not done	*Salmonella bareilly*
3**							—	YG	Y	±	—	—	+	—	—	*Aerobacter aerogenes*

*NSEC: National *Salmonella* and *Escherichia* Typing Centre, Kasauli, Simla, U.P.

**Strains biochemically typical but serologically negative.

+Positive for given reaction YG = Acid and gas production NC = No change or alkaline reaction

—Negative for given reaction Y = Yellow (acid production)

though positive for agglutination test against polyvalent "O" antiserum, but are H_2S negative as well as sucrose positive, may be considered as doubtful *Salmonella*.

Agglutination test with polyvalent 'O' antiserum helps in screening the cultures but at the same time as it is apparent from this limited study, the reliance upon this reaction for identification is not without risk. Since cross agglutination with polyvalent 'O' antiserum by non-*Salmonellae* is not an uncommon phenomenon[12,14]. As seen in Table 1 many of non-*Salmonellae* agglutinated with polyvalent 'O' antiserum. Silliker and Greenberg[15] have also made observations that there is an ubiquitous distribution of non-*Salmonellae* which agglutinate promptly in commercially available polyvalent antiserum.

It is therefore felt that the cultures showing positive reaction with polyvalent 'O' antiserum should be subjected to at least one more biochemical test like lysine decarboxylation which will differentiate *Salmonella* from *Citrobacter* and *A. cloacae*.[8] More reliance on biochemical test may suit to our conditions as ready availability of the antiserum may prove to be a problem to us.

Acknowledgement

The authors wish to thank the Director, National *Salmonella* and *Escherichia* Centre, Kasauli, Simla for the help in identifying and typing *Salmonella* cultures.

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Chromatographic Identification of Skin Pigments of *Solanum melongena*

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Manuscript Received: 14 *July* 1975

The skin pigments of brinjal (*Solanum melongena*) were extracted with acidic methanol and separated on alumina column. The pigments were further resolved into 7 individual components by two-dimensional microcyrstalline cellulose TLC using n-butanol-HCl-water (5:2:1) and water-HCl-formic acid (8:4:1). The aglycone, sugars and acyl moieties of each component were characterized on the basis of paper chromatographic study. Delphinidin was found to be the only aglycone common to all the compounds. Sugars detected in their conjugates were glucose and rhamnose. The presence of coumaroyl and caffeoyl moieties was also noted. On the basis of these findings the pigments were tentatively characterized.

The anthocyanins in several fruits and vegetables have been studied earlier[1]. However, the anthocyanin composition of only a few fruits and vegetables is known in detail[2,3]. The bright red skins of radishes, the red skins of potatoes and the dark purple skins of egg plant are reported to contain anthocyanins[4].

Some pigments of egg plant were identified as glucosides, rhamnosides and coumaroyl glycosides of delphinidin[5]. Similarly, caffeoyl glycosides of delphinidin were also reported to be present in the peels of *Solanum melongena*[6]. The occurrence of violanin, a complex triglycoside of delphinidin with glucose, rhamnose and coumaric acid has been reported in the variety Burma Beauty of *S. melongena*[7]. Sakamura *et al.*[8] found one major anthocyanin in egg plant and identified it as delphinidin-3-(p-coumaroyl rutinoside)-5-glucoside.

The work reported here was undertaken to clarify the apparent discrepancy regarding the identity of the skin pigments of brinjal (*Solanum melongena*).

Materials and Methods

The anthocyanin rich peels of brinjal were used as pigment source. Alumina for column chromatography was obtained from Riedel, Germany; microcrystalline cellulose for TLC from Acme Synthetic Chemicals, India; galactose, glucose, arabinose, xylose and rhamnose from Nutritional Biochemical Corporation, Cleveland, Ohio; and chlorogenic, caffeic, ferulic, coumaric and gallic acids from Sigma Chemical Company, U.S.A.

Isolation of anthocyanins: Pigments were extracted from fresh brinjal peels with 1.6 lit of 1.5 N HCl-methanol (15:85) per kg. After keeping the mixture overnight it was filtered through layers of Whatman No. 1 filter paper[2]. The filtrate was vacuum concentrated to a lesser volume and passed through a 15×1 cm alumina column brought to pH 5 with 2N NaOH as employed by Birkofer *et al.*[9] The column was washed with methanol to complete the elution of non-complex-forming phenolics and related compounds. The complex-forming pigments were then eluted with 1 per cent HCl.

Estimation of anthocyanins: Total anthocyanin content of the eluate was determined by the method of single pH total anthocyanin determination of Fuleki and Francis[10].

Two ml of pigment solution was diluted to 100 ml with 0.1 per cent HCl in methanol and its optical density measured at 543 mμ using DK-2 Beckmann spectrophotometer. The total anthocyanin content in terms of delphinidin-3-glucoside was calculated in absolute quantities using the reported value of its extinction coefficient[11].

Thin layer chromatography: The acidic eluate from the column was further concentrated to 10 ml under vacuum and then subjected to two dimensional microcrystalline cellulose thin layer chromatography as employed by Nybom[12]. Glass plates of $10 \times 10''$ were coated with a suspension of microcrystalline cellulose to 0.35 mm thickness. The two solvent systems used were n-butanol-HCl-H_2O (5:2:1) and water-HCl-formic acid (8:4:1).

The chromatograms were examined visually and the numbering of spots was done on the basis of increasing R_f values in the first solvent system, n-butanol-HCl-H_2O (5:2:1). The tentative identification was made by comparing with the reported photocopied chromatograms of anthocyanins by Nybom[12].

Preparative thin layer chromatography: After application of the samples, five batches of three microcrystalline cellulose TLC plates were processed simultaneously under identical conditions as before. The individual

TABLE 1. CHROMATOGRAPHIC IDENTIFICATION OF AGLYCONES, SUGARS AND ACYL MOIETIES

Spots	Aglycone: R_f in water-Acetic acid-HCl (10:30:3) (v/v)	Aglycone: Tentative identification	Sugar moieties: R_f in BAW (4:1:5) system	Sugar moieties: Identification	Acyl moieties: R_f in BAW (4:1:5) system	Acyl moieties: Tentative identification
1	0.30	Delphinidin	—	—	—	—
2	0.31	,,	0.98	Glucose	—	—
3	0.315	,,	0.98	Glucose	—	—
4	0.31	,,	0.99,2.00	Glucose, Rhamnose	—	—
5	0.31	,,	0.99	Glucose	0.82	Caffeic acid
6	0.315	,,	0.99,2.00	Glucose, Rhamnose	0.91	Coumaric acid
7	0.30	,,	0.98	Glucose	0.91	,,

spots from all the plates were eluted with 50 ml of 1 per cent HCl in methanol and concentrated to 10 ml under vacuum.

Hydrolysis of anthocyanin glycosides: The hydrolysis of anthocyanin glycosides was carried out by the method employed by Levy and Zucher[13].

To 5 ml of solution of the conjugate, 5 ml of 10 N KOH was added and the hydrolysis was allowed to proceed for 45 min at room temperature. The hydrolyzate was acidified with 1 ml of 6 N HCl and extracted five times with double the volume of ethyl acetate. The combined ethyl acetate extracts were evaporated to lesser volume under vacuum.

Paper chromatography of aglycone, glycoside and acyl moieties of anthocyanins: Both ethyl acetate and aqueous fractions were co-chromatographed with authentic sample ascendingly on Whatman No. 1 paper using n-butanol-acetic acid-water (BAW) (4:1:5) for 18 hr in duplicate in order to check the acyl and sugar moieties of the anthocyanins respectively, whereas for aglycones the aqueous portion alone was used employing the same chromatographic technique using the Forestal solvent system—water-acetic acid-HCl (10:30:3) for 8 hr[14].

Results and Discussion

Isolation of anthocyanins: The crude acidic methanol extract containing anthocyanins along with other naturally occurring phenolics was subjected to a preliminary isolation by passing through an alumina column brought to pH 5. Excepting the complex forming anthocyanins, other less polar compounds were washed off from the column by methanol. No anthocyanin-type pigment was observed in the methanolic eluate because the absorption maximum of the eluent was not in the 510–580 mμ region[15]. Birkofer *et al.*[9] reported that Al_2O_3 brought to pH 5 with 2 N NaOH could be used to separate malvidin, peonidin and pelargonidin, which did not complex with metal ions, from the complexing pigments petunidin, delphinidin and cyanidin glycosides. The former could be eluted with methanol, the latter group with 1 per cent HCl.

Estimation of total anthocyanins: The method developed for total anthocyanin determination by Fuleki *et al.*[10] was used. In the present case "the single pH method (pH 1.0)" was preferred to "the pH differential method (pH 1-4.5)" assuming that there is no anthocyanin degradation products present in the acidic eluate from the column. A reported $E^{1\%}$ value of 559.3 for Dn-3-glu at 543 mμ[11] was used in determining the total anothcyanin content of brinjal peels. The value of total anthocyanin content was found to be equivalent to 9.12 mg of Dn-3-glu per 100 g peels.

Thin layer chromatography: Fig. 1 represents the anthocyanin composition of brinjal skin. When acidic eluate from the alumina column was chromatographed

TABLE 2. ANTHOCYANINS IN BRINJAL PEELS

Spot	R_f values: n-butanol-HCl-water (5:2:1)	R_f values: Water-HCl-formic acid (8:4:1)	Aglycone	Sugar moiety	Acyl moiety	Pigment*
1	0.08	0.05	Dn	—	—	Dn
2	0.09	0.15	Dn	Glu	—	Dn 3, 5-diGlu
3	0.10	0.05	Dn	Glu	—	Dn 3-Glu
4	0.19	0.50	Dn	Rham, Glu	—	Dn 3-Rut, 5-Glu
5	0.30	0.50	Dn	Glu	Caf	Dn(?)-Glu(?)-(Caf)
6	0.50	0.52	Dn	Rham, Glu	Coum	Dn 3-(Coum)-Rut, 5-Glu
7	0.61	0.28	Dn	Glu	Coum	Dn 3-(Coum)-Glu

*Dn = delphinidin, Glu = glucose, Rham = rhamnose, Rut = rutinose (glucose+rhamnose) Caf = caffeic acid, Coum = coumaric acid.

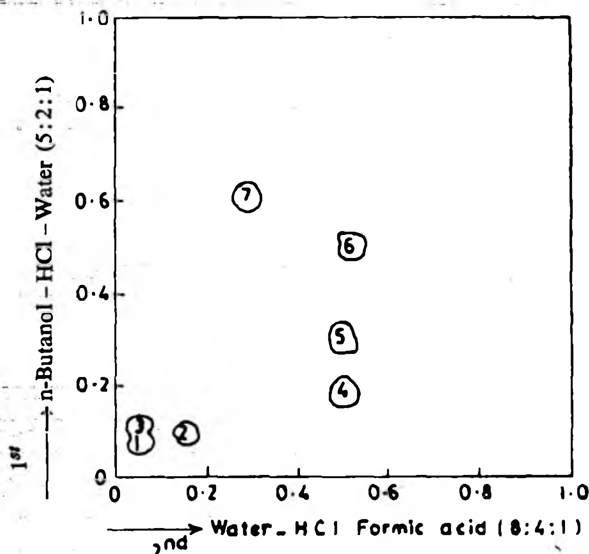

Fig. 1. TLC of anthocyanins of brinjal peels

on microcrystalline cellulose two-dimensional thin layer chromatography using n-butanol-HCl-water (5:2:1) and water-HCl-formic acid (8:4:1), seven distinct purple spots were visually detected. These spots were tentatively identified as delphinidin derivatives on the basis of findings of Nybom[12]. The low R_f value of spot 1 – 0.08 and 0.05 in n-butanol-HCl-water and water-HCl-formic acid respectively, indicates that it should be a less polar compound which could be delphinidin itself. The other spots were expected to be the glycosides of delphinidin due to their higher mobility in both the solvent systems.

The individual spots from as many as 15 plates were eluted with 1 per cent HCl in methanol and the concentrate obtained from this was subjected to alkaline hydrolysis and subsequent ethyl acetate extraction to detect the aglycones and sugar portions from aqueous layer, and acyl portion from the ethyl acetate layer. Neutralization and subsequent acidification with HCl after alkaline hydrolysis helped to minimize the degradation of anthocyanidin.

Identification of aglycone, sugar and acyl moieties of anthocyanin: Table I shows the R_f values of aglycones of all the seven spots on one-dimensional Whatman No. 1 paper chromatography in Forestal solvent system. The only one aglycone that was found common in all seven spots was probably delphinidin, when its R_f value (0.3 ± 0.015) was compared with that (0.31 of delphinidin) reported by Harborne[15]. It was far removed from cyanidin.

Table I also represents the R_f values of sugar moiety present in the aqueous portion obtained after alkaline hydrolysis of different spots of anthocyanins. When authentic samples were co-chromatographed and developed in n-butanol-acetic acid-water (4:1:5) system on Whatman No. 1 paper using aniline hydrogen phthalate[16] as spraying agent, all spots were found to have glucose (R_f value 0.98) except spot 1. Rhamnose (R_f value 2.0) was identified to be associated with spots 4 and 6. However, none of the sugars was found to be present in spot 1 which led to indicate that it could be the aglycone itself.

The acyl part of the anthocyanins present in the ethyl-acetate fraction after alkaline hydrolysis is also shown in Table I. When ethyl acetate fractions of different spots were co-chromatographed with authentic phenolic acids on Whatman No. 1 paper using BAW (4:1:5), spot 5 was found to be caffeic acid (R_f 0.82) whereas spots 6 and 7 were coumaric acid (R_f 0.91). Chlorogenic, ferulic and gallic acids (R_f 0.56, 0.89 and 0.68 respectively) were discounted.

The possible identification of the 7 compounds is shown in Table 2 taking into consideration the observations outlined above as well as the reported occurrence of delphinidin derivatives in plant materials. The exact configuration of these compounds, however, needs confirmation.

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Evaluation of Spices and Oleoresins. V. Estimation of Pungent Principles of Pepper

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Manuscript Received: 6 *August* 1975

Labruyere's alkaline hydrolysis method for estimating the total pungent principles of pepper has been adapted for use directly on pepper powder. In a first stage contolled hydrolysis the interfering constituents are eliminated and the pungent principles estimated in the second stage hydrolysis. This method obviates the time consuming step of oleoresin preparation required by all other methods, and is simplc requiring no special equipment.

Pepper (*Piper nigrum* L.), a major spice, is valued for its pungent taste and aroma. The major pungent principle is piperine, the trans-trans isomer of 1-piperoylpiperidine[1]. The other geometrical isomers, chavicine (cis-cis)[2], isopiperine (cis-trans)[3] and isochavicine (trans-cis)[3,4] and homologs, piperettine[5] with three conjugated and piperanine[6] with one ethylenic linkage in the side chain and pyrroperine,[7] the pyrrolidine homolog, have been shown by different workers to occur as minor components. The structural investigation of Staudinger and Schneider[8] has shown that all these compounds could be expected to contribute to the pungency. The correlation of the structure of pungent compounds of pepper, capsicum and ginger has shown that an acid amide bond in the side chain considerably enhances the pungency. There are, however, conflicting reports[2,9-11] about the occurrence and relative pungency of these different compounds in pepper.

In assessing the value of pepper as a spice, a method is necessary which assesses total pungency. Earlier methods proposed for estimating the pungent principles have been critically reviewed by Labruyere[12]. The methods are either too general as nitrogen estimation[13] or relied on chromogenic reactions[14-17] which were also rather general that much interference by other constituents could occur. The ultraviolet absorption at specific wavelength is specific for piperine, its isomers and piperettine being based on the typical system of conjugated double bonds in the molecules[18,19]. To meet the needs of laboratories having no sophisticated equipment, Labruyere[12] developed a new hydrolysis, distillation and titration method for the estimation. His method also introduced greater specificity to the estimation in attacking the amidic bond which grouping is common to all the isomers and homologs of piperine, isolated so far. This method has been slightly modified and converted to a colorimetric procedure[20] by the specific reaction of the piperidine, released on hydrolysis, with carbon disulfide and copper sulfate. Recently Wijesekara *et al.*[21] developed a TLC separation of pungent principles and quantification by measurement of spot area. This method did not give reproducible results in our hands.

All these methods, except that of Graham[15], require the preparation of the oleoresin as a preliminary step before estimation. This step is time consuming and also introduced a source of variation. Graham's phosphoric acid method, which could be applied directly to the pepper sample, suffers from the requirements that several experimental variables are critical[15] for quantitative determination.

The simplicity and specificity for estimating the total pungent principles by Labruyere's[12] hydrolysis method led us to study the application of the method for use directly on pepper without the time consuming step of oleoresin preparation. The results of the study and the developed method is given in this paper.

Materials and Methods

Specific cultivars and trade types of pepper were obtained from Pepper Experimental Stations and large export houses respectively and used as such and as oleoresins, extracted with ethylene dichloride by standard soxhlet procedures, for comparison.

Piperine isolated from Tellichery garbled pepper and recrystalized three times from ethyl acetate, m.p. 128 °C, was used as standard.

Residue—that remaining after exhaustive solvent extraction of whole pepper.

Potassium hydroxide solutions at required concentrations were prepared in water, alcohol or propylene glycol.

2.5N KOH in propylene glycol: 140 g KOH in 80 ml water and diluted to 1 litre with propylene glycol[20].

Acetone, (B.D.H.).

Mixed indicator: 0.1 g methyl red and 0.5 g bromocresol green in 100 ml of ethyl alcohol[13].

Standard acid: 0.01N HCl.

Soxhlet, refluxing, distillation equipment and flash-head were routine laboratory glassware with ground-glass joints.

U.V. spectrophotometric estimation of pungent principles was done on the oleoresins of samples at 345 nm according to Fagen *et al.*[18] except that the solutions were made in benzene as recommended by Tausig *et al.*[9].

Labruyere's[12] procedure was followed as a second standard procedure on the same oleoresin samples.

The development of the modified distillation method was effected by studying (*i*) the conditions for selective digestion and distillation for removing the non-pungent interfering constituents in the total pepper powder, and (*ii*) final digestion and distillation of the pungent principles according to Labruyere's[12] method as modified by Shankaranarayana *et al.*[20].

Recommended procedure: Reflux 1 g pepper, powdered to pass a 30 mesh sieve, with 20 ml of 0.25 N aqueous KOH for 30 min. Add a few drops of silicone antifoam to the flask at the beginning of the experiment and swirl the contents of the flask occasionally to prevent frothing, cake formation of the solids and bumping. Add 10 ml of distilled water to the flask from the top of the condenser. Convert the assembly for distillation and distill off 15 ml using a measuring jar as a receiver and discard the distillate (pH of the distillate in the end is nearly 8) representing non-pungent constituents.

For the second stage hydrolysis of piperine and related compounds, add 65 ml of 2.5 N KOH in propylene glycol to the same flask and reflux for 2 hr on a sand bath or heating mantle. Cool and add 100 ml of distilled water and distil using a flash-head till the distillate is neutral to indicator paper. Lead the distillate (about 100 ml) below the surface of 25 ml acetone containing 2 drops of mixed indicator in a flask. Titrate the distillate against standard 0.01N HCl to the original red colour. Calculate the content of the pungent principles as piperine using the equivalent, 1 ml of 0.1N HCl ≡ 0.0285 g piperine.

Results and Discussion

Labruyere's[12] digestion and distillation procedure was used with three samples of pepper, both on the extracted oleoresin and directly on the powder. The results given in Table 1 clearly show that very high fictitious values were obtained when the estimation was done directly on pepper powder. The difference in values for the whole powders over that of the oleoresins was contributed by non-pungent constituents was established by showing that the residues of exhaustive oleoresin extraction of these samples (and where absence of pungency was confirmed by taste testing also) gave significant amounts of alkaline volatiles when subjected to Labruyere's procedure (Table 1). While the nature of the interfering constituents in the residue has not been investigated, the conditions of selective digestion and elimination of the alkaline volatiles from the interfering non-pungent constituents in pepper powder was determined.

Labruyere[12] by studying different conditions of alkali concentration, volume and refluxing time selected the optimum conditions for hydrolysis of piperine as 2 N KOH in diethylene glycol and two hours refluxing time.

TABLE 1. LABRUYERE'S METHOD ON WHOLE PEPPER, OLEORESIN AND RESIDUE

Sample (Cultivar)	Whole pepper powder*	Oleoresin (pungent principles)	Residue (non-pungent constituents)
Balankotta	10.70**	4.68**	5.47**
Mumdi	10.40	5.06	4.79
Kuthiravally	10.33	5.47	4.24

*Includes pungent principles and non-pungent constitute

**All values are expressed as per cent piperine on sample, dry weight.

TABLE 2. EFFECT OF HYDROLYSIS CONDITIONS ON PEPPER, ITS RESIDUE AND PIPERINE

Sample: Kuthiravally—as in Table 1

Media	Concn. of KOH	Whole pepper powder (Pungent principles + Non-pungent constituents)	Residue (Non-pungent constituents)	Pure piperine
Alcohol	0.5N	5.95*	4.39*	8.1*
Propylene glycol	0.5N	6.54	4.48	12.2
Water	1.0N	7.24	4.46	—
Water	0.5N	6.00	4.34	10.8
Water	0.25N	4.36	4.24	3.7

*All values are expressed as per cent piperine on sample, dry weight.

TABLE 3. NON-PUNGENT CONSTITUENTS IN PEPPER SAMPLES

Sample	Whole pepper powder	Residue
Karimunda	5.54*	5.20*
Balankotta	5.75	5.47
Kuthiravally	4.36	4.24
Garbled	4.12	4.05
Ungarbled	3.01	3.28

Selective hydrolysis with 20 ml of 0.25N aqueous KOH for 30 min distilled off 15 ml after addition of 10 ml of distilled water and the distillate titrated against 0.01N HCl.

*All values are expressed as per cent piperine on sample, dry weight.

TABLE 4. PUNGENT PRINCIPLES IN PEPPER SAMPLES

Sample	Our method	Labruyere's method	U.V. spectro-photometric method
Karimunda	4.45*	4.51*	4.25*
Balankotta	4.65	4.68	4.25
Kuthiravally	5.61	5.47	5.10
Mumdi	4.99	5.06	4.80
Panniyur	5.40	5.80	5.30
Garbled	5.88	5.61	5.30
Ungarbled	5.33	5.54	5.10
Light	3.49	3.62	3.50
Pinhead	1.51	1.51	1.60
Piperine (locally prepared)	96.10	97.20	99.92

*All values are expressed as per cent piperine on sample, dry weight.

He found that in presence of glucose large excess of alkali is required. Lower concentrations and volumes of alkali resulted in varying extent of hydrolysis of piperine and with 0.5N alcoholic KOH and 60 min refluxing, there is very little hydrolysis of piperine. Results of our studies on the hydrolysis of pepper powder, the exhausted residue and pure piperine under the milder conditions are given in Table 2. At 0.5N KOH and 60 min refluxing, 8.1 per cent of piperine was hydrolysed in alcohol medium and 12.2 per cent of piperine in prophlene glycol medium. With pepper, even under these mild conditions, there was considerable volatile alkaline compounds produced, 25–45 per cent more than the value for the residue, indicating greater hydrolysis of piperine along with non-pungent constituents. This high value is possible due to critical increase in the concentration of alkali during the final stages of the first distillation. We therefore studied the hydrolysis in the aqueous medium at different alkali concentration (Table 2). It may be seen from these results that with 0.5 N KOH and 60 min refluxing, piperine was hydrolysed to 10.8 per cent and the whole pepper powder to a higher value than the residue similar to the other media. However at milder conditions, 0.25 N aqueous KOH at its boiling point for a period of 30 min piperine was hydrolysed only to 3.7 per cent while the hydrolysis of non-pungent constituents yielding volatile alkali was complete as seen from the value being nearly the same as that obtained for the oleoresin extracted residue. This has been tested on more pepper samples and their residues and the results are given in Table 3. It is clear from Tables 2 and 3 that all the non-pungent constituents could be completely removed without affecting piperine appreciably under the milder conditions selected.

These selected conditions were used for the first stage of initial hydrolysis and removal by distillation of the interfering non-pungent constituents in pepper samples and the true piperine and related pungent principles estimated in the second stage following the conditions (2N KOH and 2 hr refluxing) established by Labruyere[12]. The standardised procedure is given earlier.

The recommended procedure has been tested with pure piperine and on a number of cultivars and trade types of pepper. The results are presented in Table 4 along with the estimations by Labuyere's procedure and the U.V. spectrophotometric method. It may be seen that the values obtained agree well with those of Labruyere's method but is slightly higher than the U.V. spectrophotometric method. The ratios of the values of the pungent principles in a number of samples estimated by the two distillation procedures to the U.V. spectrophotometric method varied between 0.92 to 1.11 and show that the estimation on whole pepper powder by the method standardised by us is comparable to the other two standard methods.

The reproducibility of the method is good. Repeated estimations with a few varieties and pure piperine gave a variation of less than 5 per cent to the mean value. The recovery of added piperine to pepper powder has also been checked with a few samples recovery being 95–98 per cent.

Compared to the standard U.V. spectrophotometric method there is an error of about 5–10 per cent in the values obtained by both our and Laburyere's methods. This, we believe, is negligible in routine quality control work, as the pungent sensation between two pepper samples differing in piperine content upto 10 per cent is not perceivable at use level in soups, etc. as tested by a panel.

The advantage of this method is that the time consuming step for total oleoresin extraction (16–20 hr by the standard method of soxhlet extraction[20]), is eliminated

and estimation can be carried out directly on pepper powder. The method is also suitable for routine estimation in all the public analysts' laboratories.

Acknowledgement

The authors thank the Director of this Institute for permission to publish this paper.

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RESEARCH NOTES

STUDIES ON SOME IMPORTANT COMMERCIAL VARIETIES OF MANGO OF NORTH INDIA IN RELATION TO CANNING, FREEZING AND CHEMICAL PRESERVATION OF PULP

This relates to the evaluation of some commercial varieties of mango viz., 'Bombay-Green', 'Dashehari', 'Langra' and 'Chousa' of North India and 'Baneshan' of South India for canning, freezing and chemical preservation of pulp. The changes in total soluble solids, sugars, acids, ascorbic acid and carotenoid pigments were low in frozen pulp than those of canned and preserved ones. Among the different methods of preservation tried, canning was found to be better than freezing and chemical methods of preservation of pulp in all the varieties even after nine months of storage. The variety 'Dashehari' scored highest rating followed by 'Langra', 'Baneshan' ('Safeda'), 'Bombay Green' and 'Chousa' irrespective of methods of preservation.

Mango pulp is an important raw material for the preparation of different products like juice, squash, nectar, mango leather, mango cereal flakes, strained baby food, mango juice powder, mango toffee, etc. as reported by Bhatnagar and Subramanyam[1]. Mango nectar and juice are exported in large quantity. During the year 1972–73[2] India exported 9, 670, 453 kg of mango juice valued at Rs. 20, 364, 619. Mango pulp is prepared and preserved in order to manufacture a wide spectrum of mango products in off season. Various workers in the country have studied the varietal characteristics of mango for the preparation of different products. Roy *et al.*[3] assessed some important North Indian varieties of mango for processing into nectar. Suryaprakash Rao *et al.*[4,5] studied some South Indian varieties of mangoes for the preparation of juice and nectar. Siddappa and Bhatia[6] reported the chemical composition and retention of ascorbic acid of 'Kalpadi' and 'Rumani' varieties during storage at different temperatures. Satyavati *et al.*[7] have also assessed some varieties of mangoes grown in Kerala. Nanjundaswamy *et al.*[8] observed the storage behaviour of canned pulp. Jain and Subbhiah[9] tried to improve the nutritive value of mango pulp. Mathur *et al.*[10] have done work on freezing of mango pulp. Karim and Rehman[11] and Dhopheshwarkar and Magar[12] studied the effect of heating on the quality of canned pulp while Bose and Lodh[13] conducted studies on loss of SO_2 in the pulp of 'Langra' and 'Fazli' varieties. Very little work has been done on the suitability of mango varieties for canning, freezing and chemical preservation of pulp. An attempt has been made to find out the best variety for pulp making and observation has also been made on the changes during storage of canned, frozen and chemically preserved pulp.

Fully ripe, but firm and medium size fruits of commercial varieties of mango of North India viz., 'Bombay Green', 'Dashehari', 'Langra', 'Chousa' and 'Baneshan' of South India were taken for the present investigation. The quantity of fruits used was about 50 kg of each variety. After getting the pulp the titratable acidity was raised to 0.5 per cent by adding citric acid. For canning, the pulp was heated up to 85°C and filled in plain 1 lb Jam cans (301 × 309), sealed, processed for 15 min at 100°C and cooled. In case of freezing, the pulp was further fortified with *l*–ascorbic acid @ 50 mg/100g and ameliorated with sugar (sucrose) in the ratio of 20:1 (pulp: sugar) as per method of Mathur *et al.*[10]. The pulp was filled in 1 lb Jam cans, sealed and kept in deep freeze at-12.2°C (roughly for 6 hr) and the storage study was continued. For chemical preservation, the pulp was heated to 85°C, cooled and mixed with potassium metabisulphite (500 ppm SO_2). About 350g of pulp was then filled in glass bottles of 350 ml capacity and sealed immediately. The number of cans (canned and frozen) and bottles filled for the experiment was 25 in each case.

Total soluble solids (w/w) were recorded with a hand refractometer correcting the value to 20°C. Sugars were estimated by Lane and Eynon's method[14]. Titratable acidity was determined by titrating the sample against

TABLE 1. CUT OUT ANALYSIS OF CANNED PULP

Variety	Storage period at R.T. (months)	Vacuum (in inch)	Head space (cm)
Baneshan (Safeda)	0	18	0.8
	3	16	0.8
	6	11	1.0
	9	10	1.0
Bombay Green	0	12	0.6
	3	10	0.6
	6	8	0.8
	9	4	0.8
Dashehari	0	14	0.8
	3	14	0.8
	6	12	0.8
	9	10	1.0
Langra	0	14	0.8
	3	13	0.8
	6	11	0.9
	9	11	1.0
Chousa	0	13	0.8
	3	11	0.8
	6	10	0.9
	9	4	1.0

The internal condition of the can in all the varieties were same, They were: 0 month: Light feathering; 3 months: Light feathering with bluish faint staining; 6 months: Moderate feathering with faint staining and 9 months: Moderate feathering with moderate bluish faint staining.

TABLE 2. CHANGES IN CHEMICAL COMPOSITION OF CANNED (C) AND FROZEN PULP (F) DURING STORAGE

Variety	Storage period at R.T. (months)*	T.S.S. (°Brix)		Sugars %				Acidity % (as citric) (W/W)		Ascorbic acid retention %		Total carotenoids retention %	
				Reducing		Non-reducing							
		C	F	C	F	C	F	C	F	C	F	C	F
Baneshan	0	18.5	22.0	6.80	6.50	7.20	12.16	0.40	0.46	—	—	—	—
	3	18.0	22.0	7.60	5.90	6.10	11.60	0.49	0.61	78.6	82.4	90.5	93.4
	6	17.4	22.0	7.77	5.54	5.38	12.12	0.55	0.61	57.1	74.4	84.3	88.4
	9	16.5	22.0	7.73	5.50	4.08	12.73	0.61	0.65	40.0	60.3	84.3	88.4
Bombay Green	0	19.0	20.0	5.60	5.20	8.90	10.40	0.43	0.30	—	—	—	—
	3	18.3	20.0	6.90	5.11	7.00	10.39	0.49	0.30	74.3	87.3	95.7	91.3
	6	18.0	19.5	7.52	4.50	6.18	10.45	0.56	0.36	54.5	71.1	93.9	92.9
	9	17.0	19.5	7.92	4.28	4.88	10.65	0.60	0.40	34.1	53.6	86.7	88.3
Dashehari	0	21.5	23.0	3.30	2.34	13.70	17.16	0.37	0.44	—	—	—	—
	3	20.5	23.0	3.90	2.30	12.00	16.93	0.41	0.50	81.9	91.3	93.6	97.8
	6	19.4	23.0	4.57	2.13	10.58	17.08	0.45	0.50	69.1	83.2	89.7	96.0
	9	18.5	22.5	5.51	2.01	8.93	16.54	0.54	0.55	47.2	64.7	89.7	96.0
Langra	0	20.0	22.5	2.89	2.93	12.61	15.73	0.37	0.38	—	—	—	—
	3	19.6	22.0	3.92	2.70	11.23	15.80	0.42	0.41	86.2	94.2	95.0	96.8
	6	19.4	22.2	3.96	2.65	11.09	15.07	0.48	0.45	76.8	89.0	91.8	96.8
	9	18.4	21.5	4.33	2.65	9.85	14.79	0.50	0.48	61.9	83.6	90.3	94.1
Chousa	0	20.2	22.5	4.85	4.40	10.85	14.00	0.32	0.33	—	—	—	—
	3	19.0	22.0	6.80	4.20	8.20	13.90	0.41	0.37	82.3	87.2	92.8	92.9
	6	18.7	21.8	6.73	3.69	7.85	13.48	0.42	0.40	70.3	88.4	85.6	89.3
	9	17.1	21.5	6.63	3.54	6.24	13.65	0.44	0.40	86.7	67.8	85.6	89.3

*Storage temperature for frozen pulp was −12.2°C.

*Storage temperature for canned pulp was 32.2 to 37.8°C.

TABLE 3. CHANGES IN CHEMICAL COMPOSITION OF CHEMICALLY PRESERVED PULP (GLASS BOTTLES) DURING STORAGE

Variety	Storage period at R.T. (months)	T.S.S. (°Brix)	Sugars % Non-reducing	Reducin	Acidity % g(as citric) (W/W)	Ascorbic acid retention %	Total carotenoids retention %	Total SO_2 (PPM)
Baneshan	0	18.3	8.00	5.80	0.38	—	—	448
	3	17.3	9.00	3.75	0.44	78.1	90.5	352
	6	16.4	9.25	2.75	0.57	56.3	85.7	256
	9	16.0	9.25	2.05	0.62	31.2	81.8	224
Bombay Green	0	18.0	5.10	8.46	0.47	—	—	480
	3	17.7	7.35	5.96	0.54	73.9	95.7	374
	6	17.2	8.33	4.57	0.64	52.7	90.6	334
	9	16.8	8.33	4.17	0.70	30.3	84.5	312
Dashehari	0	21.3	3.40	13.40	0.45	—	—	448
	3	20.5	4.36	11.74	0.50	79.6	95.0	360
	6	20.1	5.90	9.85	0.54	64.8	88.4	316
	9	19.0	5.92	8.85	0.57	43.6	88.4	264
Langra	0	19.7	2.87	12.33	0.40	—	—	450
	3	19.4	3.90	11.10	0.45	89.1	95.0	—
	6	18.9	4.21	10.29	0.48	77.7	92.6	320
	9	18.2	4.49	9.26	0.55	63.0	89.6	258
Chousa	0	20.0	6.00	9.58	0.38	—	—	480
	3	18.9	7.10	7.46	0.43	81.9	91.84	366
	6	18.1	8.33	5.35	0.47	58.9	84.30	338
	9	17.4	8.71	4.16	0.51	43.8	84.30	308

standard NaOH solution; it was calculated and expressed as citric acid. Ascorbic acid was determined by the visual titration method as described by the Association of Vitamin Chemists[15]. The total carotenoid pigments were estimated by the method standardised by Roy[16]. Total sulphur dioxide was determined in chemically preserved pulp by the distillation method of Amerine and Cruess[17]. The mango nectar (20 per cent pulp, 20° Brix and 0.3 per cent acidity) was prepared from pulp before organoleptic evaluation. The organoleptic evaluation of the mango nectar was done by a panel of seven judges; the average score is presented in Fig. 1. Cut out analysis was followed as per F.P.O[18] specifications. Each time, 5 containers (cans and bottles) were opened for cut out, chemical and organoleptic analysis. Before analysis frozen pulp, was thawed by keeping the containers in running water and the time taken for thawing was 15 to 20 min. The vacuum in the canned pulp showed a downward trend with the increase in time of storage and the internal

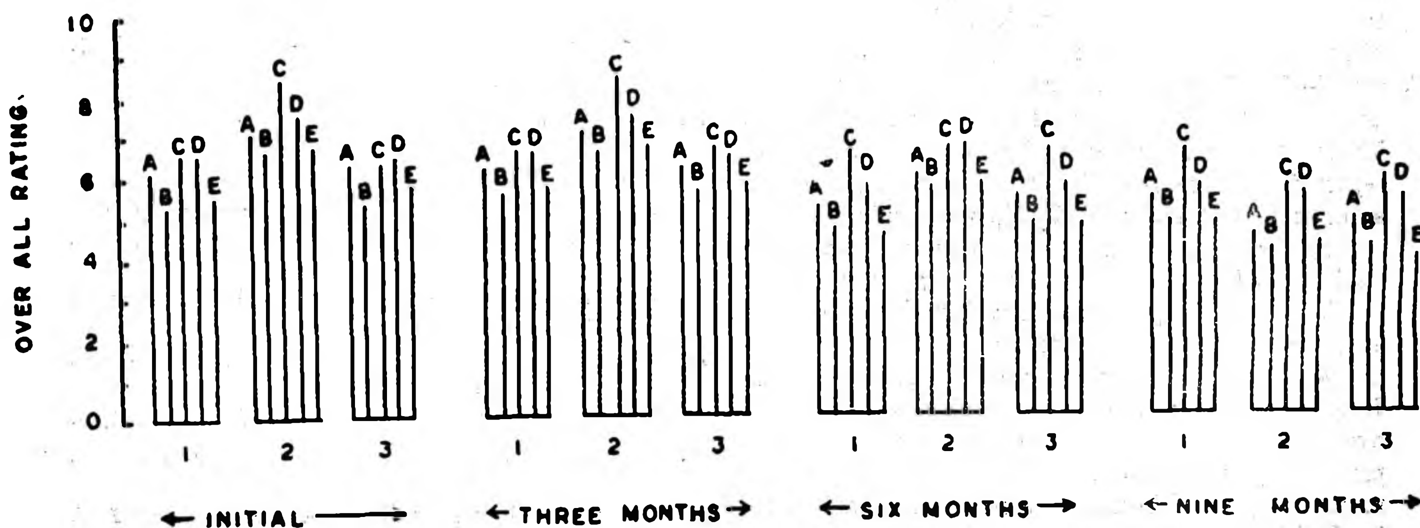

Fig. 1. Organoleptic evaluation of canned, frozen and chemically preserved pulp. A, Safeda; B, Bombay Green; C, Dashehari; D, Langra; E, Chousa. 1, Canned pulp; 2, Frozen pulp; 3, Chemically preserved pulp.

condition of can changed from light feathering to moderate feathering with the development of bluish faint staining in all the cases (Table 1). Tables 2 and 3 show decline in total soluble solids in canned and preserved pulp. This may be due to the breakdown of sugars into acids as the acidity of these products was found to have increased throughout the storage. This is in conformity with the findings of Andrabi *et al.*[19]. No significant change was noted in total soluble solids of frozen pulp, possibly due to storage at low temperature (-12.2°C). Initially the higher trend of total soluble solids in frozen pulp of all varieties was due to addition of extra sugar to it. The non-reducing sugar decreased throughout the storage period in case of canned and preserved pulp while reducing sugar increased. Increase in acidity was noted in preserved pulp as compared to the canned one. This could be due to formation of sulphurous acid by sulphur dioxide with water in pulp resulting in more inversion of non-reducing sugar than in canned ones as reported by Siddappa and Rao[20]. No significant change in total sugars was noted in frozen pulp. Ascorbic acid decreased in all the products during storage. Better retention was, however, noted in frozen pulp possibly due to addition of ascorbic acid. Canned pulp was found to have retained ascorbic acid better than preserved one, possibly due to the fact that tin acted as a protection to ascorbic acid as reported by Reister *et al.*[21] and Moore *et al.*[22]. Among the varieties, 'Langra' was found to have retained ascorbic acid better. Incidently, it also had high initial content of ascorbic acid. It appeared that higher the level of ascorbic acid, better was the retention. This is in conformity with the findings of Jain and Subbaiah[9]. The decline of carotenoid pigments was mainly due to the presence of residual oxygen and in case of bottled pulp it was coupled with exposure to light as reported by Lang[23]. Retention of carotenoid pigments was better in frozen pulp due to low temperature (-12.2°C) during storage. Total sulphur dioxide decreased with increase in time of storage (Table 3). The loss may be due in part to diffusion and oxidation as reported by Ingram[24]. The initial organoleptic evaluation of canned and bottled pulp was conducted after storing the product for 7 days at room temperature (32.2 to 37.8°C) while for frozen pulp by storing at -12.2°C. Organoleptic evaluation (Fig. 1) showed that at the end of six months the frozen pulp of all the varieties was slightly better than canned and preserved pulp. However, at the end of nine months there was decline in overall rating of frozen pulp as compared to canned and preserved pulp. This could possibly be due to development of slight off flavour as a result of power failure for 24 hr in between six and nine months, caused momentary thawing of frozen pulp. The present investigation revealed that the variety 'Dashehari' had highest overall rating followed by 'Langra', 'Safeda', 'Bombay Green' and 'Chousa' irrespective of the method of preservation.

The authors are grateful to Dr R. N. Singh, Head of the Division of Horticulture and Fruit Technology and Dr J. C. Anand, Fruit Preservation officer, for encouragement during the course of investigation.

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24 *March* 1975.

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A RAPID SCREENING METHOD FOR ORGANOCHLORINE INSECTICIDE RESIDUES ON VEGETABLES

A quick TLC method for determining the presence of organochlorine insecticide residues, in vegetables above tolerance levels.

Presence of residues of organochlorine insecticides in food materials have been reported from several parts of India[1]. Vegetables, along with other items of farm produce like cereals, eggs and milk contain such residues in varying quantities. A simple, quick and efficient method for detecting and estimating the organochlorine insecticides in vegetables has been developed.

The vegetable was extracted by shaking 25 g of a representative sample of the cut/grated material with 75 ml of a mixture of 1:3 acetone-hexane (v/v) for one hour in a stoppered flask on a mechanical shaker. The extract was filtered through a cotton plug into a bottle. Three ml of this was pipetted to a 10 ml beaker, concentrated to about 10–15 microliters and spotted on a silicagel plate with built in silver nitrate prepared according to the method of Moats[2], along with spots of standard insecticides. The spotted plate was developed in cyclohexane to a height of 10 cm, air dried and exposed to ultra violet light (250 nm) for 10 min. Insecticides appeared as brownish to black spots on a light white background. Steaming the plate and re-exposing to radiation increased the sensitivity of the plate.

Usually a clean-up is not necessary when 3 ml of the extract, equivalent to 1 g of the material is spotted for determination. Whether the residue present is exceeding the tolerance levels or not can be easily determined by spotting standards of insecticides in quantities equivalent to permissible level and comparing the area and intensity of the spots. The recovery of pesticides is very good as there are only two intermediate steps, extraction and concentration. When the insecticides aldrin (1, 2, 3, 4, 10, 10-Hexachloro-1, 4, 4a-5, 8, 8a-hexahydro-endo-1, 4-exo-5, 8-dimethanonaphthalene), dieldrin (1, 2, 3, 4, 10, 10-hexachloro-6,7- epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-endo, exo-5, 8-dimethanonaphthalene), endrin (1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-exo-1, 4-exo-5, 8-dimethanonaaphthalene), lindane (–1, 2, 3, 4, 5, 6-hexachlorocyclohexane) and DDT (1, 1, 1-trichloro 2, 2-bis (p-chlorophenyl ethane) were added at 1 ppm and BHC (1, 2, 3, 4, 5, 6-hexachlorocyclohexane) at 2 ppm levels to the vegetables, 90 to 95 per cent of the added insecticides were recovered by the above method. The co-extractives present were not a hindrance as no spots were produced by them that could be confused with an insecticide spot.

Highly coloured substances and those containing lipids require a preliminary clean-up before the determinative step. Simple clean-up methods based on thin-layer chromatography worked out by this laboratory[3,4], have been found very useful for this purpose.

The ease of operation and minimum equipment required makes this method an ideal for small laboratories which have to monitor food stuffs for insecticide residues. In the case of vegetables, the chemist will be able to quantitate the residue (s) present, within 3 to 4 hr.

Acknowledgement

We are thankful to Dr S. N. Banerjee, Plant Protection Adviser to the Government of India and Dr K. K. Nirula, Project Director, Central Plant Protection Training Institute, Hyderabad, for their keen interest and encouragement in the work.

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27 *May* 1975

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CHEMICAL EXAMINATION OF THE FRUITS OF *ZANTHOXYLUM ALATUM* ROXB

Zanthoxylum alatum Roxb. (syn. *Z. aromaticum* DC), popularly known as Timur or Baletimur in Nepal is an evergreen or sub-deciduous shrub, and sometimes a small tree upto 6 m high, found at the foot of Himalayas and in Nepal at altitudes of 1500-2400 m. Bark is pale brown, deeply furrowed and corky; leaves, 10-23 cm long, imparipinnate and leaflets 5-11, lanceolate, more or less serrate and each serrature with a pellucid gland; flowers, polygamous, yellow in dense pubescent lateral panicles[1]; fruits, 4-5 mm in diameter, ovoid, fruit carpels 1-3, pale red; seeds 2 mm in diameter, oval and dark[2].

The skin of the fruit is rough and possesses a strong aromatic smell. The carpels have a camphoraceous pungent odour and saltish taste. The fruits turn brownish black on ripening; on drying of the fruits, the carpels split into two halves and expose the seeds. Nearly half of the fruit comprises of seeds.

The carpels contain an essential oil which is reported to possess antiseptic, disinfectant and deodorant pro-

perties[3]. They are traditionally used in Nepal in pickles and meat preparations. The fruits and bark are used as tonic in fever and dyspepsia. Fruits, branches and thorns are used as remedy for toothache and are also considered to be carminative[2].

The present study was undertaken to collect basic data for the extraction of the essential oil and oleoresin from different parts of the plant.

Timur fruits were collected by the Food Research Section of Nepal from the market at Kathmandu. The carpels and seeds were separated by hand in the laboratory before analysis.

The separated carpels and seeds were analysed for proximate composition using standard methods[4]. The volatile oil was determined using 50 g by Clavenger distillation apparatus.

The extractives (oleoresin) were prepared using glass column with 30 g of crushed sample giving a contact of 1 hr at room temperature. Extraction was repeated separately using different solvents like alcohol, acetone, hexane and ethylene dichloride (EDC). After recovering the solvent by distillation and drying the extractives were weighed. Different ratios between the material and solvent were tried and optimum ratio for efficient extraction determined.

The physico-chemical properties of the essential oil were determined using standard methods. Results are given in Tables 1 and 2.

The oleoresin yield varied from 3 to 11 per cent, depending on the solvent used. The essential oil content was about 6 per cent in the carpels, the seed being devoid of it.

TABLE 1. APROXIMATE COMPOSITION OF CARPELS AND SEEDS

	Carpels	Seeds
Moisture (%) v/w ..	5.00	14.00
Essential oil (%) v/w ..	6.00	nil
Total ash (%) ..	5.95	7.24
Insoluble ash (%) ..	0.11	0.80
Crude fibre (%) ..	31.46	56.43
Crude protein (%) ..	11.00	9.36
Starch (%) ..	21.08	4.24

TABLE 2. YIELD OF OLEORESIN FROM CARPELS

Solvent used	Oleoresin (%)	Material to solvent ratio
Alcohol	11.41	1 : 5
Acetone	10.85	1 : 4
EDC	11.33	1 : 4
Hexane	3.15	1 : 6

Physico-chemical properties of essential oil: The volatile oil was clear and had a faint yellow colour, with characteristic spicy odour and biting, pungent taste. The oil was soluble in 80 per cent alcohol in the ratio of 1:1. Its other properties were as follows: sp. gr. (24°C)–0.8766; opt. rotn (24°C),+1.9; refr. index (29°C), 1.475; acid value, 1.954 mg of KOH/g of the sample; ester value, 38.77 mg of KOH/g of the sample; and saponification value, 40.72 mg of KOH/g of the sample.

Further studies on the nature of oil and oleoresin are in progress.

The author wishes to express her sincere thanks to Mr C. P. Natarajan, Project Co-Ordinator, Dr Y. S. Lewis, Mr E. S. Namboodiri, Scientists and Miss B. Sankari of CFTRI Mysore, India for their valuable guidance and suggestions in this work. I offer my sincere thanks to Dr E. H. Khan, Director of H. M. G. Food Research Section, Kathmandu for sending the necessary samples.

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18 *July* 1975.

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COMPARATIVE ASSESSMENT OF AROMATIC PRINCIPLES OF RIPE ALPHONSO AND LANGRA MANGO

A preliminary study on the aromatic principles of ripe Alphonso and Langra mangoes reported. Both these essences were isolated from the ripe mango pulp by high vacuum distillation and analysed by gas liquid chromatography followed by sniffing test of the separated components. Although there were similarities in aroma profile between these two varieties, some distinctive features among them were observable.

Among the several varieties of mangoes (*Mangifera indica* L), 'Alphonso' and 'Langra' are of commercial importance because of their characteristic pleasant and strong aroma in ripe stage. Although various physico-chemical characteristics of these mango varieties have been reported[1–3], very little information is available on their aromatic principles. Commercially available mango essence does not resemble the natural aroma of the fruit.

Pattabhiraman *et al*[4–5] have reported the presence of carbonyls and esters in 'Alphonso' mango. The concentrate of true aroma of this variety was isolated and aroma description of some major components being separated by gas liquid chromatography was initially reported.[6] Angelini *et al*[7] have characterised the aromatic principles of ripe 'Alphonso' mango as carbonyls, esters, alcohols, terpenes and lactones. Recently, Hunter *et al*[8] have also identified the volatile components of canned 'Alphonso' mango. Besides this variety of the fruit, no studies have so far been reported on other mango varieties. The present paper reports comparative evaluation of organoleptically important aromatic principles of ripe 'Alphonso' and 'Langra' mangoes. The procedure detailed elsewhere[6] for the preparation of aroma concentrate from the pulp of ripe 'Alphonso' and 'Langra' mango was essentially based on the isolation of the volatile components by high vacuum distillation followed by extraction of odourous principles from the distillate with diethyl ether and finally distillation of ether at low temperature under high vacuum. The aroma concentrates of the respective mango varieties were analysed by gas liquid chromatography (GLC) using a Backman GC-5 gas chromatograph equipped with a thermal conductivity detector and a dual column of stainless steel ($\frac{1}{8}$ in OD×6 ft). Each of GLC separated component of the respective mango essence was subjected to descriptive odour test[6] and the characteristic note of each component was recorded on the chromatogram.

GLC separation of the essence of ripe, 'Langra' mango along with the odour notes of the separated components is presented in Fig. 1. 'Langra' essence was resolved into 21 components, while in case of 'Alphonso' mango essence, a chromatogram almost similar to the earlier reported one[6] with prescribed odour notes of the separated components was obtained, none of the individual components represented the characteristic aroma of the variety suggesting that this was an effect of combination of all these components as observed earlier[6]. It is apparent that some notes are common to both varieties e.g. green mango, estery, burnt sugar and soily. 'Langra' essence also seems to have more soily note than 'Alphonso'. 'Alphonso' essence has its unique almond and coconut-like aroma notes, while 'Langra' has camphory, peach-like and above all woody notes. It is likely that many compounds having characteristic notes e.g. estery, soily, etc. are presumed to occur in both the varieties. However, the preponderance of coconut-like notes in 'Alphonso' mango[6] due to the presence of γ—C_6 to C_{10} lactones and δ-octalactone[7,8] are probably striking feature in comparison with 'Langra' mango essence, where these notes are virtually absent.

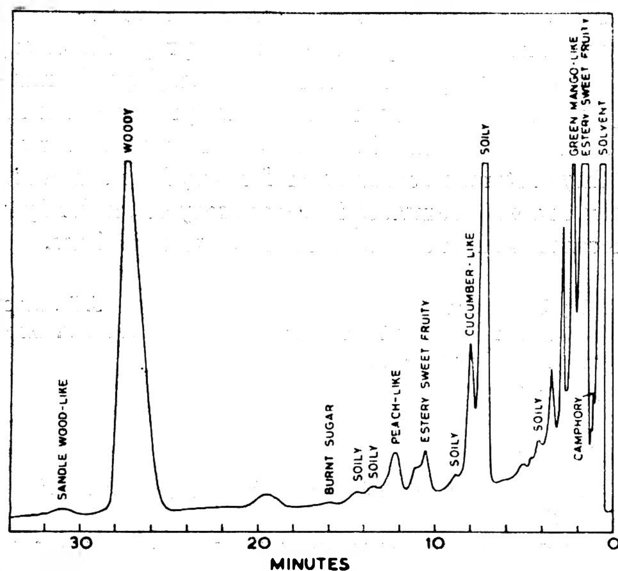

Fig. 1. Gas chromatogram of ripe Langra mango essence showing odour comments. Columns: 10% carbowax 20 M on 80/100 mesh chromsorb W (AW); carrier gas 20 ml helium/min; isothermal temperature, 130°C

The foregoing results, thus suggest that a combination of several individual odourous compounds in proper concentrations may contribute to characteristic aroma of mango varieties. The missing aroma notes, experienced in commercially available synthetic mango essence could be attributed to the absence of these components.

Acknowledgement

The authors wish to thank Dr G. B. Nadkarni for his helpful suggestions and criticism.

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RELATIONSHIP BETWEEN AMYLOSE CONTENT AND SETTING PROPERTY OF STARCH

Amylose component of the starch is responsible for the firm setting of concentrated starch-sugar mixtures on cooling.

In the manufacture of food products, starch finds applications as a thickener, filler, gelling agent or for imparting smooth velvety texture and consistency. The food industry uses starches from corn, sorghum, rice barley, rye, wheat, potato, sago, tapioca and arrow-root either in the native or in the modified form depending upon the needs of a particular product. In the present work, an attempt was made to exploit new indigenous sources of starch and to explore the possibility of substitution of these starches in typical food preparations.

Starches were isolated from three different sources viz. a legume like redgram (*Cajanus cajan*) a cereal like ragi (*Eleucine coracana*) and a seed like rajgeera (*Amaranthus paniculatus* Linn.) by the alkali steeping method[1]. Amylose and amylopectin contents of the starches were determined by colorimetric method of McCready and Hassid[2], as well as isolation by thymol precipitation[3]. Digestibility[4] and the physico-chemical properties of these starches were also studied[4]. Finally, it was attempted to study the possibility of using these starches in the preparation of a typical product like Badami (Bombay) Halwa, usually prepared from corn starch or wheat flour and which is a characteristic smooth textured solid mass set firmly. Bombay Halwa was prepared using the following ingredients (in g): Sugar 37, Starch 7, Ghee 15, citric acid, 0.5 g; water, 100 ml; colour-edicol egg yellow; avour-cardamom. A part of the sugar (25 g) was made into a syrup with a part of water and concentrated. Starch dispersed in remaining water was then added with stirring. To this was added fat and remaining sugar and the mixture was concentrated with continuous stirring. Heating was stopped when a ball of the mass cooled and pressed between fingers appeared to have been sufficiently thick and spongy and tended to set on cooling. The temperature at this stage was 110°C. The colour, flavour and citric acid were added immediately, mixed and the mass poured on a greased thali (plate), allowed to set at room temperature for 2 to 3 hr and then cut into square pieces. The products were evaluated for acceptability by comparing with a market sample prepared from corn starch using same recipe and procedure.

It was observed that the Halwa prepared from ragi and redgram starch was comparable in all respects with the standard preparation containing corn starch. Under the conditions mentioned above, both the starches could be set firmly within 2 to 3 hr to a solid, smooth firm elastic mass characteristic of Badami Halwa which can easily be cut with a knife to pieces of any desired shape.

TABLE 1. PERCENTAGE OF AMYLOSE AND AMYLOPECTIN IN STARCHES

Starch source	By fractionation			By colorimetric method		
	Amylose	Amylo-pectin	Sum	Amylose	Amylo-pectin	Sum
Ragi	13.8	63.3	77.1	16.0	84.0	100
Redgram	26.5	43.5	70.0	27.0	73.0	100
Corn	21.3	55.2	76.5	23.5	76.5	100
Rajgeera	—	79.3	79.3	—	—	—

However, when rajgeera starch was studied for the same purpose, even in spite of changing the receipes it always failed to get to a solid mass of characteristic smooth texture even with as long as 96 hr of setting period. It always gave a product like a paste which could be eaten with a spoon as against setting to a solid elastic mass which could be cut easily with a knife to give pieces of any desired shape.

As shown in Table 1, the basic difference in the ragi, redgram, corn and rajgeera starch seems to lie only in the amylose and amylopectin make up of these starches. It is observed that rajgeera starch which failed to set to a solid mass was entirely made up of amylopectin and was a waxy starch as against ragi, redgram and corn starches which contained 16, 27 and 23.5 per cent amylose as determined by the colorimetric method. Also by fractionation method, amylose contents of ragi, redgram and corn starches were found to be 13.8, 26.5 and 21.3 per cent respectively while from rajgeera starch, no amylose could be precipitated out. Lower values of the amylose and amylopectin contents of the starches obtained by fractionation method could be due to some losses during purification by repeated precipitation. Since ultimately the physico-chemical properties of any starch depend upon the amylose and amylopectin make up, from these observations, it seems probable that the amylose constituent of the starch is responsible for the ability of starch to set and therefore any flour containing starch in which amylose is present may be set firmly in presence of sugar and other appropriate ingredients.

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BOOK REVIEWS

Better Foods for Better Nutrition: Report of the Workshop conducted by the Protein Foods and Nutrition Development Association of India, Hyderabad Nov. 1973, pp. 134.

A Workshop on 'Better Foods for Better Nutrition' was organised by the PFNDAI at the Regional Research Laboratory, Hyderabad on November 24/25, 1973, the proceedings of which have been published in this report. As in the case of the earlier workshops held at New Delhi (1969) and Calcutta (1972) this Workshop provided the forum for discussion among the specialists and agencies dealing with the different facets of nutrition for the masses.

The report contains 10 chapters, the first two dealing with the backdrop and plan of the workshop and the inauguration. Chapter 3 summarises the results of the food habits and attitudes survey carried out in Andhra Pradesh, Kerala, Mysore (Karnataka) and Tamil Nadu during the period September 1971-January 1972 by the Operations Research Group, Baroda. Also included in this Chapter is a paper from the NIN, Hyderabad on the food habits and attitudes in some parts of Andhra Pradesh. Under the common title 'The role of government and industry in making better foods available', Chapter 4 has four papers on strategies for agriculture and Chapter 5 contains three papers on low cost prepared foods. An address to the Workshop by Dr. C. Gopalan on 'Some thoughts on nutritional strategies in India' is reproduced in Chapter 6. Problems connected with the mass feeding programmes and nutrition education are dealt with in Chapter 7 which has seven papers. Practices in the labelling and advertising of food products and the code of conduct for advertising of food products and the code of conduct for food advertising drawn by the PFNDAI Hyderabad Workshop are discussed in Chapter 8. Chapter 9 entitled 'Ideas Anyone' gives the report of a brain-storming session led by Dr S. Varadarajan. The concluding chapter gives data on national nutritional needs and food production as worked out by the Nutrition Expert Group of the ICMR and submitted to the NCSTin October 1972. Background notes prepared by the PFNDAI on the different topics and the discussions in the respective areas have also been included.

The food habits survey carried out in South India which formed the basis for the Hyderabad Workshop, has reemphasised the fact that the lowest economic strata of the society which constitutes 50–60 per cent of the total population are on a bare subsistence level and the greatest sufferers nutritionally are the infants, school age children, pregnant women and nursing mothers. The report as a whole is valuable as it provides not only new data on the food habits and attitudes of the people but highlights the need for an integrated systems approach for providing better nutrition to the vulnerable groups.

N. SUBRAMANIAN

Compressed Food Bars: Compiled by M. T. Gillies, Noyes Data Corporation, New Jersey and London, 1974, pp. IX+116; Price : $ 12.

Number 18, in the Food Technology Review Series, this publication covers the specialized food items of compacted dehydrated foods for military operational rations, explorers and the like. The information from 9 reports (1969–1972) made for by the U. S. Army Natick Laboratories and 8 U.S. patents (1967–1974) have been collected, giving essential details, recipes and quality data.

The material is given in 7 sections dealing with optimum processing conditions for compression and storage of freeze dried foods, evaluation of their quality through resorption, texture and sensory evaluation and the several forms in which these compressed bars could be made.

This compilation will be useful to those starting work in this area as a ready reference. Much of the information given appear to be only reproduction of preliminary reports. One expects that for a book on the subject a review of the industrial potential of the product type and some information on the transfer of the laboratory studies to industrial scale would have been given.

Some errors noted are: under Sensory Evaluation (p. 13) details given refer to conditions of compressing; in p. 43 Rahman (4) does not tally, with references given on p. 49; and in Reference (15) mentioned p. 45 is not seen on p. 50.

V. S. GOVINDARAJAN

Food Additives to Extend Shelflife: Nicholas D. Pintauro; Noyes Data Corporation, Park Ridge, New Jersey, 1974; pp. 402; Price, $ 36.

The present book is the seventeenth in the series of "Food Technology Reviews" publish by Noyes Data Corporation who are well known to publish patent literature in various fields of food technology. The U.S. Patent literature relating to chemical additives is the largest in the world. The information available on these patents is scattered and cannot be readily had at one place. "Food Additives to Extend Shelf Life" provides

practically the entire up-to-date patent technical literature in the field without the legal terminology, and can be used as a guide to U.S. patent literature. This information is of immense use to workers in the field and will indicate the trends of research going on in the field.

Food additives are used in small quantities to improve the appearance, flavour, texture and shelf-life of food. These may be broadly classified as preservatives, antioxidants, colour modifiers, stabilizers, flavouring agents, functional property modifiers, those which control moisture, pH, physiological functions, those used in processing viz., antifoaming agents, chelating agents, etc. The patent literature covering these various food additives are organized according to food category or food group. This approach is commendable and gives a better understanding of the subject rather than when the additives are discussed according to their functions in several food groups. Either way repetition is inevitable.

The number of patents in each food group and the years of coverage are: 20 in fats and oils (1963–70), 19 in meat products (1960–73), 10 in fish products (1960–71), 7 in egg and egg products (1960–71), 6 in dairy products (1960–71), 25 in cereal products (1961–73), 20 in fresh fruits and vegetables (1962–73), 9 in vitamins and natural colours (1961–72), 18 in salt, beverages and flavours (1961–73), 18 in the field of preservatives and antioxidants (1961–74). Total number of patents included are 152.

The book contains at the end three appendices. Appendix-I is a reproduction of the booklet "The use of Chemicals in Food Production, Processing, Storage, and Distribution" (1973) published by the National Academy of Sciences. It gives general information on food additives. Appendix-II provides "Selected Abstracts" from the Federal Food, Drug and Cosmetic Act and recent pronouncements on policy in the Federal Register by the Food and Drug Administration of the United States. The information given in the above two appendices is invaluable and enlighten the food manufacturers as to what constitute food additives, when to use them, their scope and limitations. Appendix-III contains "Selected Abstracts" from Federal Register viz., label declaration of chemical preservatives in foods and substances prohibited for use in foods. Besides, it also contains a list of general food categories and food ingredient functions. The general information provided in the appendices gives a bird's eye view of the various chemicals used in the food industry for several purposes,

The additive, company, inventor, and U.S. patent number index given at the end of the book provides quick reference to any one seeking further information from the US patent office.

The small types used in the book make the reading rather difficult, otherwise the get-up of the book is good. It serves as a useful reference book in food research laboratories and food manufacturing concerns.

S. RANGANNA

Current Trends in the Refrigerated Storage and Transport of Perishable Food Stuffs: by International Institute of Refrigeration Commission D_1, D_2 & D_3 Barcelonea Spain 1973. p. 245.

The book under review relates to the proceedings of the meeting of section D of International Institute of Refrigeration held at Barcelona during November 1973 to study statutory problems relating to the refrigerated storage, land transport and sea transport of perishable foodstuffs. The publication contains 29 papers of which 15 are in English and 14 in French, presented by well known specialists in the field from different countries at that meeting. All papers carry abstracts in the language other than the one used in the main text of the paper.

The papers presented are classified under four different sections, namely (1) general aspects of cold chain and its links (4 papers) (2) cold storage and cold storage equipments (8 papers) (3) refrigerated transport, cooling techniques and product quality maintenance (11 papers) and (4) refrigerated sea transport (6 papers).

In section I on general aspects, all the four articles are in French and from their English abstracts it is evident that these articles advocate the adoption of cold chain in developing countries taking perhaps the development of Spanish refrigeration net work as an illustration. The trends in the evolution of cold chain system and the factors that are responsible for this evolution, like increase in the world population, urbanization, change in food, habits, etc., the influence of cold chain on the economy of modern food trade such as development in the production and consumption of frozen foods, changes in the economy of fisheries, and the necessity of creating a well coordinated world-wide cold chain have been discussed. The effective role that organization like International Institute of Refrigeration and International aids, can play in the development of cold chain in developing countries has been focused. Current systems existing in refrigerated and sea transport of perishable food stuffs and trends in the future development have been pointed out.

Section II on cold storage and cold store equipment contains four articles each in English and French. Three of them deal with the technical aspects of the refrigeration equipments like problem of maintaining effective low temperature in the open topped retail display cabinets specially meant for display of thermo-labile frozen foods, and suggestion to over come these defects, the use

of reflective night blinds, corner cube reflective ceilings to the cabinets and the adoption of low emissive packaging materials have been advocated. The development of a new rotor quick freezing unit which satisfy the important requirements of freezing industry namely rapid freezing process providing a high quality product and a high level of process mechanisation and automation have been described. Experimental data on freezing of different varieties of fish are presented. The potential application of these units in various branches of the food industry is envisaged. The construction and operation details of the use of pallet-lift in cold stores having intermediary floor and the advantages of using these lifts have been enumerated. Four articles deal with the effect of the refrigeration environments and techniques (like temperature, velocity of air, etc.,) on chilling of pork; the technical improvements in the application of cold air for drying and storing grains; the influence of factors like the physico-geometrical characteristics of products, aerodynamic conditions in the fluidizing chamber and the thermal conditions of freezing on the dehydrations of foodstuff during fluidized freezing storage and transport; effect of temperature variations and the presence of air between the product and the package during storage and transport upon the weight loss of packaged frozen fish; have all been discussed. In one article utilization criteria for a cold store is described.

Section III comprises of eleven articles of which six are in English and rest are in French and they can be broadly classified into two groups, one relating to cooling techniques in refrigerated transport and other on the quality maintenance of the product in refrigerated transport. In the former the experimental data connected with thermal aspects of cargo transferring processes, the problems of air distribution inside loaded refrigerated containers and wagons, temperature rise in frozen products during transport, and development of a new designed F.G.R. insulated road trailer have been discussed. The articles in the latter are concerned with the influence of the different refrigerated transport systems (including nitrogen atmosphere and nitrogen cooled container) on the quality of varied food products like live fish, mussels, apricots, etc.

In Section IV, but for two articles the other four deal with refrigerated sea transport of perishable foods like oranges, fish, etc. Of the other two, one is on the use of screw compressor on board ships and the other on system analysis of scientific, technical information flows required for refrigerated ship design.

The paper on the technical aspects of establishing a cold chain on the whole have much value to the researchers who are engaged in setting up of a viable cold chain appropriate to the situation, be it in transportation or in cold storage. Most of these papers discuss the design details and the theoretical approach to the problems.

The papers on the application of cold chain to specific food products cover a wide range of perishables from frozen sea-foods to fruits. From the papers presented, the lines of research work in other countries are very much in evidence. Though the findings in research paper may not have immediate significance in Indian context the approach to the problem is of definite significance to the Indian research workers.

The reporting of question answer session of the Symposium adds to the value of the book, although one feels that the English/French translations of the questions and answers could have been provided.

On the whole, the volume should be welcomed by all, the refrigeration engineers, research workers in the field of food technology and authorities dealing in the transportation and storage of perishables.

B. ANANDASWAMY

Bakery Products: Edited by D. J. De Renzo, Noyes Data Corporation, Grand Avenue, Park Ridge N 707656, pp. 456; Price 36.

This Book is number 20 in the series of the food technology reviews produced by Noyes Data Corporation presenting an over-all view of most of the US Patents granted since 1960 that are concerned with improvements and modifications in the production of Yeast Leavened Bakery goods. The subject matter of this book will be of great interest and useful to the Bakery Technologists in India to whom such information is hardly available elsewhere.

The volume is divided into 10 major sections, each section comprehensively discussing the patent literature in a given area of processes.

The first major section deals with the continuous bread making process. Patents related to dough preparation deal with means of regulating mixing operations required for optimal dough development through the use of certain compounds like soybean protein additives, ascorbic acid, un-saturated alcohol or coated salt, the replacement of monoglycerides by wetting agents and elimination of fermentation. The section also covers methods for incorporation of non-fat milk solids, shortening compositions, flavour improvement and coated bread improvers.

The second major section deals with fermentation methods and features patents dealing with means of reducing fermentation time, yeast brews, sour dough preparation and other fermentation procedures.

The third major section covers the subject on emulsifiers comprehensively presenting the use of monoglycerides and polyoxylkylene derivatives with a final subsection devoted to other lesser known emulsifiers.

The fourth section deals with dough improvers like oxidising agents, composition based on whey or soy proteins, coated bread improvers and other dough improver's compositions not covered above.

Protection of baked products against staling and microbiological deterioration has been discussed in the fifth major section. Additives to preserve baked products against rope and mold and to prolong shelf-life are also discussed comprehensively.

The sixth major section deals with the enhancement of bread flavour by means of various additives and touches on flavouring compositions to impart cheese, garlic and sour-dough flavours to baked products.

The pre-treated flours, which include agglomerated flours and specialised flours, viz. high protein, granular, controlled bulk density flour and all purpose flour are discussed in the seventh major section.

The next major section deals with the glazes, coatings, fillings and dusting compositions.

Modified breads, which include non-fat dry milk substitute, high protein breads, low calorie breads, dietetic breads, and other modified breads are dealt in the ninth major section.

The final major section deals with the speciality bread products including sweet doughs, crackers, pizza dough, frozen bread products, home preparation and pretzels.

This book serves the double purpose, in that it supplies detailed technical information and can be used as a guide to US patent literature in this field. The patents have been put into readily understandable language and the procedural directions for use of ingredients covered in the patents are given in a great detail.

As the author points out in the Foreword, the US Patent literature contains the largest and most comprehensive collection of technical information which is often overlooked or ignored because of the difficulty encountered in understanding the language in the patents which has legal jargon and juristic phraseology. This book overcomes these problems and provides the Bakery Technologist with an invaluable tool in keeping up with the most recent advances in the commercial baking processes and affords a sound background for further researches in this field.

V. B. MITBANDER

Biology: Food and People: by Robert Barras, The English University Press Ltd., St. Paul's House, Warwick Lane, London, 1974, p. vi ± 246, Price: $ 2.25.

The book under review presents an integrated approach to the study of economic aspects of biology and advances made in biological sciences which have contributed to human welfare.

The entire subject area including the introduction is discussed in five chapters covering 18 sections.

Keeping in view the programmes initiated all over the world to constantly increase food production to meet the demands of rising population, the problems relating to production of plant and animal foods are reviewed in chapters II and III. The topics covered include: soils, soil nutrition, fertilisers; cropping patterns and crop rotation; land reclamation; cereal production and plant breeding; chemical regulation of plant growth; single cell protein; utilization of waste materials; animal breeding; hunting and destruction of forest fauna; and sea fisheries. A strong plea is made for conservation of forest fauna and flora in the face of increasing tempo of indiscriminate destruction of forests and rapid urbanisation programmes practised at present.

Post-harvest food losses are considerable, particularly in developing countries of the world. A detailed diagnosis of the nature of storage losses is presented and remedial measures suggested in Chapter IV.

Chapter V entitled production of people-outlines the basic requirements of human population for clean water and unpolluted air. Problems relating to sewage and effluent treatments; methods of supplying potable water and the increasing pollution problem are discussed in the earlier part of this chapter. The concluding part of chapter V discusses the methods of family planning practised and stresses the need for making the programme more realistic and pragmatic to achieve the desired goals.

An interesting post-script highlights the role of biology in human welfare and recommends its study not only by students, but by all enlightened citizens all over the world.

At the end of each section, literature for further reading is suggested. An exhaustive and useful bibliography and a subject index enhance the value of the book.

The book has a number of good illustrations. Written in a popular and attractive style, it covers a wide field and at best serves as an excellent introductory text book to students of general biology and as a reference book to scientists, administrators, sociologists, demographers and others interested in programmes that contribute to human welfare. It will be a useful addition to libraries in schools, colleges, research laboratories and Institutions engaged in higher learning and mass education programmes.

B. S. NARAHARI RAO

Evaluation of Certain Food Additives: 18th Report of the Joint FAO/WHO Expert Committee on Food Additives. Technical Report Series 557, published by World Health Organization, Geneva, 1974. pp. 38; Price SW. $ 5.

This is a compilation of the Report of the Expert Committee on Food Additives which met in Rome from 4th to 13th June 1974. The terms of reference of this meeting being—

1. to review the report of the 3rd Joint FAO/WHO Conference on Food Additives and Contaminants;
2. to prepare specifications and carry out the toxicological evaluation of certain food additives;
3. to revise the specifications for certain food additives; and
4. to re-evaluate certain food additives.

The booklet includes a Chapter on 'General Considerations' which gives valuable information on acceptable daily intake of ascorbic acid, benzoic acid and nitrates. For those working in the food industries this Bulletin provides valuable guidelines on the levels of food additives that could be used in food materials without contravening the recommendations of the W.H.O. The specifications as well as the current status in respect of food colours, such as, anato extracts, turmeric, carotenes, iron oxides, lycopene, orange colours, etc., are extremely useful in the selection of suitable food colours.

Similarly the information provided on the thickening agents and food enzymes commonly used in the food industries is also extremely useful. With the increasing use of synthetic sweeteners the guidelines provided in the Bulletin on the use of saccharin and cyclamates are extremely useful.

As Annexure to the Bulletin, acceptable daily intakes and information on specifications with regard to various food additives has been compiled in a tabular form. These serve a very useful purpose for the Food Technologists.

Annexure–III includes several items on which further toxicological studies for obtaining information required or desirable have to be carried out. This is useful for those engaged in certifying different food additives and also for those who would like to establish the suitability or otherwise of different food additives.

With all this information, this Bulletin is a very useful guide to Food Processors, Food Technologists as well as Food Analysts.

V. Sreenivasa Murthy

NOTES AND NEWS

Indian Standards Institution Publications

Following Standards have been published by the Indian Standards Institution, New Delhi.

Protein Efficiency Ratio (PER)	Rs. 5.00	IS: 7481–1974
High Protein Mixes for Food	Rs. 6.00	IS: 3137–1974
Protein Based Beverages	Rs. 5.00	IS: 7482–1974
Desiccated Coconut	Rs. 6.00	IS: 996–1975
Terminology for Spices and Condiments	Rs. 2.50	IS: 1877–1973
Glossary of Terms for Coffee and its Products	Rs. 4.00	IS: 7236–1974
Ethyl Ester of Beta-Apo-8'-Carotenoic Acid, Food Grade	Rs. 3.00	IS: 7260–1974
Wheat Flour for Use in Bread Industry	Rs. 5.00	IS: 7464–1974
Method for Estimation of Pyridoxine (Vitamin B_6) in Food Stuffs	Rs. 5.00	IS: 7530–1975
Method for Estimation of Vitamin B_1 in Food Stuffs	Rs. 5.00	IS: 7529–1975
Erythrosine, Food Grade	Rs. 5.00	IS: 1697–1974
Tartrazine, Food Grade	Rs. 5.00	IS: 1694–1974
Determination of Protein in Foods and Feeds	Rs. 5.00	IS: 7219–1973
Ponceau 4R, Food Grade	Rs. 5.00	IS: 2558–1974

Tests for Meat and Meat Products:

Determination of Nitrate Content–Part VII	Rs. 5.00	IS: 5960–1974
Determination of Nitrate Content–Part VIII	Rs. 6.00	IS: 5960–1974
Coaltar Food Colour Preparations and Mixtures	Rs. 5.00	IS: 5346–1975
Nomenclature of Vegetables	Rs. 10.00	IS: 7470–1974

Copies of the standard are available from the offices of the Indian Standards Institution located at New Delhi, Ahmedabad, Bangalore, Bombay, Calcutta, Chandigarh, Hyderabad, Kanpur, Madras and Patna.

International Institute of Refrigeration—forthcoming meeting on food science, refrigeration and air conditionning

JOINT MEETING OF COMMISSIONS C2, D1, D2, D3 & E1

Melbourne, Australia, 6th—10th September, 1976

The Australian National Committee for the I.I.R. is organizing a joint meeting of Commissions in Melbourne in September 1976.

The aim of the meeting is to bring Australian research and development in the relevant fields to the notice of an international audience and to acquaint Australian science and industry with current work being carried out in other countries.

A call for Papers for the meeting will be issued shortly setting out the selected theme and a list of preferred topics. Papers will however also be invited in the general areas of interest of the five Commissions. Limited funds will be available to assist selected authors with travel costs.

The organizer of the meeting is Mr. F. G. Hogg, I.I.R. Liaison Officer in Australia, from whom further details can be obtained. His address is P.O. Box 26, Highett, Victoria, 3190, Australia.

Annual General Body Meeting of the Eastern Regional Branch, held on 25th July 1975 at Calcutta.

The business session covered

(*i*) The confirmation of the minutes of the 14th Annual General Body Meeting.

(*ii*) The Annual Report presented by the Honorary General Secretary for the year 1974–75 and its approval.

(*iii*) Audited accounts for the period January 1973 to December 1973 and also January 1974 to December 1974 and its approval.

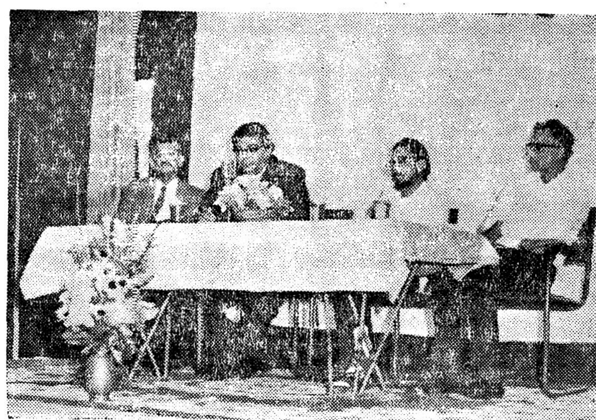
Following are the Office bearers elected for the year:

1.	Mr. K. C. Dé	*President*
2.	Prof. Sunit Mukherjee	*Vice-President*
3.	Mr. S. K. Das Gupta	*Honorary General Secretary*
4.	Mr. A. K. Banik	*Honorary Joint Secretary*
5.	Mr. D. Roy Choudhury	*Honorary Treasurer*
6.	Dr. D. N. Bose	*Members*
7.	Mr. B. S. Narayana	–do–
8.	Dr. B. R. Roy	–do–
9.	Mr. R. N. Ghosh	–do–
10.	Dr. R. N. Dutta	–do–
11.	Dr. G. C. Bhattacharjee	–do–
12.	Mr. A. K. Sen	–do–
13.	Mr. R. K. Rao	–do–
14.	Mr. P. Chattopadhyay	–do–
15.	Mr. Subrata Basu	–do–

Talk on Protein Food Industry in India by Dr K. T. Achaya

Dr. K. T. Achaya, Executive Director, Protein Foods Association of India spoke on "Protein Food Industry in India" and was followed by a discussion. Sri Santanu Chowdhury, Managing Director, The United Cereal Products, Calcutta was the chief guest.

The speaker made an attempt to give an exhaustive list of all protein-rich foods, and discussed their nature and characteristics. An estimate of the dimensions of the industry indicates that over one lakh tonne of such products appeared to be made in the country. A large proportion consists of foods for children of various ages, and by far the largest protein component in these foods is skimmed milk powder. Recent Indian Standard Specifications for such foods have attempted to provide for the use of high-quality vegetable proteins in certain products. In these standards the concepts



Chief guest (Managing Director, The United Cereal Products) addresses the gathering at the Annual General Meeting of AFST, Eastern Regional Branch held on 25th July 1975 at Calcutta.

From left: **Dr. K. T. Achaya,** Guest Speaker, (Executive Director, Protein Foods & Nutrition Development Association of India).
Sri Santanu Chowdury, Chief Guest.
Sri K. C. Dé, President of AFST, Eastern Regional Branch, Calcutta.

of protein quantity and quality have been incorporated. Future product possibilities depend mostly on the availability of oilseed proteins at reasonable prices. Availability of equipment is not a constraint on oilseed protein manufacture. There is much interest in the use of extruded technology, which is basically a simple, one-shot procedure for preparing ready-to-eat cooked foods. Innovations relating to low-cost distribution and promotion will be essentialif processed foods are to be more widely used.

"Role of Modere Bread in India"—talk delivered by Mr. K. S. Chaturvedi, Chief Manager, Modern Bakeries (I) Ltd., Calcutta on 23rd August 1975 at the Eastern Regional Branch

The specific role assigned to Modern Bakeries is to manufacture good quality bakery products in most hygienic conditions and to supply them to the general public and institutions, in the form of nutritious bread. Besides bread the bakery products supplied include Nan, Chappatti and Buns. It is the intention to supply them at very reasonable prices with a view to improving the dietary standard of people and to popularize wheat in rice eating areas. We are proud to say that at present all the units set up at different predominently rice-eating areas are working to full capacity.

Today there are 12 plants operating in 9 different centres in the country. Two more plants are ready for operation at Chandigarh and Ranchi.

Modern Bread is fortified with Vitamin A, Vitamin B_1, Vitamin B_2, iron and lysine. For the first time in the

world, such an extensively enriched bread is being manufactured on a large scale.

Modern Bakeries have been assisting the Central as well as many state Governments in the implementation of their programmes of feeding children, nursing mothers and other vulnerable sections of the community by supplying special nutritious bread for various Social Welfare Programmes like Slum and School Feeding. Smaller size loaves are supplied in Jhuggi-Jhumpri areas to make it available to the poorer class of people. Particularly in Calcutta, 200-g loaves are manufactured. Plans are there to prepare smaller packets containing 100g loaves each. The breads are supplied from Calcutta to Asansol and Bokaro on one side, Haldia on the other side and we are putting our efforts to supply to Siliguri in North Bengal. To make the bread available in fresh condition, regular fleet plys on road long distance covering about 450 km per trip. Besides this we are supplying regularly to army personnel and hospitals.

For the future expansion to meet the growing demand of the product it is necessary to promote the development of manufacture of bakery machineries and plants, manufacture of high quality yeast and production of standard quality of flour from indigeneous wheat. The feasibility of producing some of these products is being undertaken by the company, besides the possibilities of setting up wrapper plant.

In order to diversify the products recently, Modern Bakeries have introduced high protein 'Peanut Butter', for the first time in the market. This is gaining popularity in the country. Further this year 'Nan' is also introduced in the market. Packed 'Nan' will relieve thousands of working house wives from the hazards of making *Roti* or Nan at home.

Our Research and Development Division is experimenting with different food items which can conveniently be packed and marketed to the benefit of mass consumer in India.

Nutrition Policy and Planning: Need for a Systems Approach—Talk given by Sri S. Rajagopalan, Officer on Special Duty, Tamil Nadu Nutrition Project, Madras at the Southern Regional Branch, Madras on 11th July 1975.

Article 47 under the Directive Principles of the Constitution of India lays down that the 'State shall regard the raising the level of nutrition, the standard of living of its people and the improvement of public health among its primary duties'. All the three aspects namely nutrition, standard of living, public health are interconnected and requires simultaneous attention. Failure in any one area will have equally serious consequences in other areas. Policies and programmes to fulfil this objective have to be planned andim plemented in an integrated way. The aim of this paper is to know what are the logistics of such an integrated approach? How far the present programme helps in releasing this objective enshrined in the constitution? How are they planned? What are the shortcomings if any in planning and implementation of these programmes? It may be possible to produce theorietically an excellent model for integrated planning. Most often the process of planning ends up to the interests of existing administrative and technical entities.

After giving the definition of nutrition the speaker dealt at length the relationship existing between nutrition and infection, the necessity of providing nutritious food to children and the economic benifit of good nutrition.

The nutritional problem must be assessed from the nutritional profile of a region, the food balance sheet of a region, the food consumption and clinical surveys, nutrition related morbidity studies and the anthropometric measurements.

These revealed that the major nutrition problems in India are (1) protein calorie malnutrition; (2) vitamin A deficiency; and (3) nutritional anemia. The intensity of these varies with age, sex and reproductive status of the population exposed to the different levels of infection, infestation and environmental sanitation. In the critical phase of mental and physical growth among children these disorders have great importance. Incidence of severe forms of protein calorie malnutrition namely kwashiorkor and marasmus has been estimated to be about 1-2 per cent of children in the age group 1-5 yr. Mild and moderate forms of malnutrition manifests itself as a retarted growth and or clinical signs such as moon face, edema, discoloured hair, sparce hair, etc., Anthropometric measurements indicate that 17 per cent of the children were suffering from 3rd degree malnutrition (weight for age is deficit by 30 per cent and more) 65 per cent of children were suffering from grade 1 and 2 of malnutrition (weight of age deficit 10-25 per cent and 25-40 per cent respectively). The task force on nutrition set up by the planning commission estimates that nearly one million children die in our country as a result of severe malnutrition.

Besides protein calorie malnutrition, Vitamin A is another major nutritional problem and in India alone there may be one million cases of blindness arising from Vitamin A deficiency.

Nutritional anemia is a major problem among pregnant mothers. Many women of poor communities live on a very megre diet.

The socio-economic factors which influence nutrition status of the community may be classified as.

(*i*) Ecology; (*ii*) economy; (*iii*) public health & environmental sanitation; (*iv*) culture; and (*v*) taboos—religious or otherwise.

The ecology limits the production and availability.

The economy limits the purchasing power of the individual. Infection and environmental sanitation influence the prevalence of malnutrition by limiting biological utilisation. The culture of the region has its own overbearing influence on the nutritional intake or otherwise. Within this culture there are other religious taboos and customs which also influence in nutritional intake.

Summing of child's nutritional status is determined by food intake and health. Six determinants which influence food intake and health which lend themselves to interventions unlike, religion, caste and genetic traits are: (*i*) nutritional content of foods consumed; (*ii*) presence or absence of non-family feeding programmes; (*iii*) family's purchasing power; (*iv*) nutrition and health beliefs of mother; (*v*) health care and (*vi*) environmental and social factors.

Programme Planning

Programme for combating malnutrition consisted of school lunch programmes under the Education Ministry, preschool feeding programmes and urban crash programme under Social Welfare Ministry, applied nutrition under Rural Development Ministry, nutrition rehabilitation programmes under Health Ministry. A sum of Rs. 405 crores have been provided in the Fifth Plan both by the States and Centre who have committed to the nutrition improvement. These isolated endeavours of the different ministries have not had the desired impact on the vulnerable section of the population as evidenced by many isolated evaluation studies. No organised evaluation of these programmes were attempted and most of these programmes have no well defined objectives and goals in terms of nutritional improvement.

Augmenting Nutrient Availability

Some of the strategies for augmenting nutrient availability both in terms of quantity and qualityare: (*i*) use of high yielding varieties; (*ii*) use of improved methods of processing; (*iii*) genetic improvement; (*iv*) processing; (*v*) fortification; (*vi*) quality control and (*vii*) improved storage facility.

Thanks to green revolution, the grain production increased from 82 million tonnes in 1961 to 110 million tonnes in 1971. But the green revolution has been largely a wheat revolution. There is some progress in rice, but very little with other crops. The green revolution has also tilted the cereal-pulse ratio which is not very conducive from the nutrition point of view. Progress has been tardy regarding production of other food crops and very poor in the case of fruits and vegetables.

Genetic improvement of nutrient quality of basic stables is another strategy augmenting nutrients at no extra cost. Research and development efforts to improve genetically amino acid balance and nutrient quality of crops like maize, barley, jowar, wheat and rice have been successful.

Fortification of food is another technology available to augment and raise the quality of nutrients. Fortification of atta with lysine, thiamine and riboflavin, iron fortification of salt, fortification of milk with Vit A, idoine for fortification of salt, fortification of sago with proteins and vitamins, fortification of tea with Vit A etc., are some of the technology available for augmenting the nutrient quality or add a new nutrient for enrichment.

Nutrient availability can also be increased by reducing nutrient loss in processing using modern technology. Toxins in food like ergot in Bajra, lathyrus in kesari dhal, aflatoxin in groundnut, etc., require careful attention while processing.

The present cropping pattern is so phased that the needed sector of nutrient are obtained at lowest cost. This demands full knowledge about the existing policy levers and their impact on the cropping and production pattern.

The various distribution channels reaching the consumer may be studied in order to develop a consistent distribution system which will minimise transportation and other marginal cost. The trading, transport movement restriction, storage and structures of the organisation involved in these activities at various levels require a thorough study. Public distribution in urban areas and the different nutrition programmes may have to be examined with reference to quantum of nutrient delivered per unit cost. An efficient and economic distribution system has to be developed taking into consideration the buffer stock needed to control price mechanism.

Consumer Behaviour (Demand)

The main variables which influence the consumer behaviour are: (*i*) income; (*ii*) relative price of commodities/nutrients; (*iii*) availability; (*iv*) education; (*v*) demographic and social characteristics; and (*vi*) nutrition education.

Income and price are the primary components of purchasing power. No other single factor has such a major effect on the nutritional status as income. Diet surveys reveal highly significant differences in consumption along the income spectrum. Three trends appear to be universal. First commonly referred to as Engle's law, find that as income raises the percentage of

income allotted to food decreases, but absolute expenditure increases. This is not true with very low income group. Though the percentage of income spent on food is as high as 80 per cent the absolute expenditure are still low, that is why income increases in the beginning result in still higher percentage spent on food. Second trend is the proportion of calories supplied by cereals is replaced by more expensive calories from animal and vegetable sources, as income increases. Third trend is within the food group, there is shift towards processed, more expensive convenience foods. The speaker further worked out an example to show how much income redistribution is necessary to increase the consumption expenditure and thereby improve nutritional status under Indian conditions. The speaker stressed that the socio-economic programmes attempting to income redistribution may be necessary to have free or subsidised distribution of nutrients to these object poor. In fact these programmes of distribution if done properly can serve as instrument of income redistribution in addition to what is attempted through other economic programmes. The three distinct types of policies which the government can adopt are:

(*i*) redistribution through fiscal pricing and other policies; (*ii*) it can phase the base of growth in the economy and pull up the poor and the weaker; and (*iii*) specific policy measures to improve the productivity of the weaker units.

Nutritionally visible demand and their estimation under varied level of purchase price and income distribution policy may have to be attempted. The very nature of the problem require dis-aggregative estimate in terms of various socio-economic group and also for different demographic groups based on age, sex and reproductive status. Food taboos, cultural factors, health and environmental sanitation play a vital role in the consumer behaviour regarding demand for nutrients.

Systems Approach

It is seen that a set of co-ordinated programmes all along the line from production to consumption and biological utilisation is a pre-requisite for improving nutritional status of the population. These programmes may have to be examined with respect to, nutritional effectiveness, cost, acceptability, administrative viability, time frame, focus, multiplier and technical feasibility.

Systems approach helps in getting overview of all the variables influencing the nutritional system, from production to consumption. The basic difference between the system approach and the conventional approach of planning is in its focus. Instead of concentrating only on the immediate determinants of the problems, the systems approach looks into the complex, interacting forces of the environment within which the problem of malnutrition arises. Such a comprehensive approach will help in finding a lasting solution at a reasonable cost and will also help in preventing the recurrence of the same problem.

The elimination of poverty and thereby hunger no longer serves as a moral bestitude, but in the context of growth imperative. Dr. A. M. Boerma, Chief of F.A.O. in one of his addresses figuratively compared the need for hard and rational decision in the elimination of poverty. He said "Indeed we are discovering that a hard head and warm heart belong more comfortably together in the same body than had previously imagined". May I not say that the self same warm heart which motivates us to do something for the hungry millions, have brought all of us together in this hall to do some hard thinking.

Technical meeting held at the Eastern Regional Branch

a) Shri M. R. Bhattacharjee, Manager, Chemical Engineering Project and Marketing Division, the A.P.V. Engineering Co. Ltd., Calcutta, delivered a talk on "APV Paraflow Plate Type Heat Exchanger" on 17th August, 1974. Arranged in collaboration with Indian Institute of Chemical Engineers, Calcutta Regional Centre.

b) A group discussion was arranged on "Standardization and Quality Control in Processed Fruit and Vegetable Products" in collaboration with the Institute of Standard Engineers, Calcutta, on 11th September 1974. A large number of members and representatives from the food processing industries, attended and participated in the group discussion.

c) Dr B. R. Roy, Director, Central Food Laboratory, Calcutta, delivered a talk on "Food Adulteration" on 21st September 1974.

d) Shri B. S. Narayana, Technical Manager, Rechitt & Colman of India Ltd. Calcutta, delivered a talk on "Concept of Quality in Consumer Products with Special Reference to Foods" on 8th January 1975. Arranged in collaboration with the Institute of Standards Engineers, Calcutta.

e) Dr P. K. Kymal, Executive Director, Food & Nutrition Board, Department of Food, Ministry of Agriculture, delivered a talk on "Plan for Development of Food Processing Industries" on 10th July 1975.

Short Term Course

A short tern course of about 2 weeks duration on Quality Control in Fruit and Vegetable Processing Industry was organised (7-19 December 1974). The venue of the course was the Food Technology and

Biochemical Engineering Department of Jadavpur University. The course was inaugurated by the past President of AFST and Vice-chancellor, Jadavpur University, Dr. A. N. Bose. There were 7 participants, all from processing industries, viz., M/s The Metal Box Co. of India Ltd., M/s Hindustan Condiment Products., M/s Ramakrishna Laboratories Pvt. Ltd., M/s King's Fruit Products., M/s Daw Sen & Co., Ltd., M/s Alpha Food Products, M/s Poly Food (P) Ltd. The course comprised of both lectures and practicals.

The faculty members for the course consisted of Prof. Sunit Mukherjee, Sri S. N. Mitra, formarly Director, Central Food Laboratory and Dr. P. K. Dutta of the All India Institute of Hygiene and Public Health. The response to the course was very favourable and it is proposed to organise similar courses in future.

Bangalore Chapter—Inauguration of training programme in prevention and detection of food adulteration on 27th Aug. 75.

Food Minister of Karnataka State Smt. E. E. Vaz, inaugurating stressed the need to educate the people particularly women, on the method of combating food adulteration. She called for measures to inculcate an ethical sense in the people so that they would realise that food adulteration would be worse than a criminal offence. She pointed out that food adulteration in India dated back 2,000 years or still earlier and hence it was as old as India's cultural, religious and ethical traditions. India had to follow the Socialistic countries of the West in maintaining the food standards. The standard of food in these countries was the same both in roadside restaurants and in posh hotels and western countries were absolutely free from food adulteration.

Mr. M. K. Panduranga Setty, who presided, wanted suitable provisions in the new Act to prevent food adulteration for differentiating between sub-standard and adulteration. He said that the Centre had agreed to aid the training courses of the Association if the Association could run courses on a regular basis and for the benefit of the people from other states. Miss M. C. Madhura, Association Chapter Secretary, said that the training course conducted for the students of catering technology and food craft institutes, would enable them to combat the menace with a scientific strategy and methods. The Association had plans to develop a small useful kit for detection of food adulteration. It had also plans to conduct full time short term courses, besides separate courses for hotel industries, she said. Mr. Charles Wesley, Principal of the Food Craft Institute, propose vote of thanks.

South Regional Centre, Madras

The South Regional Centre of AFST at Madras is organising a seminar-cum-industrial clinic on food and Agro-based Industrial possibilities in Tamilnadu & Pondicherry on 14th November 1975. People interested may please contact Shri K. S. Krishnamurthy, Honorary Secretary, AFST Southern Region, Department of Food, Government of India, Shashtri Bhavan, 3rd Floor, 35 Haddows Road, Madras-600006.

Hyderabad Chapter

The Hyderabad Chapter of AFST in collaboration with Food Crafts Institute, Hyderabad, organised a four day "Food Exhibition 1975" from 17th to 20th October 1975. This was inaugurated by the Hon. Minister for Agricultural and Transport Government of Andhra Pradesh Shri J. Chokka Rao. Prize distribution was done by Smt. W. Lakshmidevi, Minister for women-welfare, Govt of Andhra Pradesh. Smt. Yamuna Ranga Rao, Principal, Food Crafts Institute, delivered the welcome address while Shri P. V. Suryaprakasha Rao concluded the meeting with vote of thanks.

Hyderabad Chapter planned for a Seminar on "Scope for Food Industries in Andhra Pradesh" sometime during December 1975. People interested may please contact, Mr. B. Raghuramiah, Development Officer, Foods Warner Hindustan Ltd., Uppal, Hyderabad-500039.

Eastern Regional Branch, Calcutta

A national seminar on "Mango and Its Utilization" is planned for 6th and 7th March 1976 by the Eastern Regional Branch of AFST, Calcutta. People interested may contact Sri Sunil Kanti Das Gupta, Hon. Gen. Secretary, AFST (Eastern Region), Department of Food Technology and Biochemical Engineering, Jadavpur University, Calcutta-700032.

List of New Members

Sri A. Subbarayan, Tamilnadu Nutrition Project 7A, Gopalapuram Ist Street, Madras-600 086.

Sri P. S. Nataraja Sharma, Technical Adviser, Sri Ganesh Ram's Analytical Labs 51, Thambu Chetty Street, Madras-600 001

Sri R. N. Ramani, Sri Ganesh Ram & Co., 46, Thambu Chetty Street, Madras-600 001.

Sri Krishna, Laxmanrao Sarode Institute of Catering Technology and Applied Nutrition, Adyar, Madras-600 020.

Sri R. V. Subba Rao, 292, T. H. Road, Madras-600 081.

Smt. Mariamma Tharakan, No. 8, S.B.I. Staff Colony, Madras-600 029.

Sri J. Maria Joseph, Institute of Catering Technology Madras-600 020.

Sri K. Manoharan, CARE—Tamilnadu College Road, Madras-600 006.

Sri A. V. Kuppuswamy, Principal, Institute of Catering Technology, Adyar, Madras-600 020.

Sri A. L. Joseph, Rubka Enterprises, 100, Poonamalle High Road, Madras-600 010.

Sri S. Jayapaul, Office of the Deputy Tech. Adviser Southern Region, Shastri Bhavan, Madras-600 006.

Sri Viravanallur Subbiah Indira, "Jayanthi" 13, IInd Cross, St. Ramakrishna Nagar, Madras-600 028.

Sri N. Ibrahim, Technical Officer Department of Food Government of India, Shashtri Bhavan, Madras-600 006.

Sri A. K. Gupta, Engineering Manager, Modern Bakeries (I) Ltd., Adyar, Madras-600 020.

Sri D. S. Ganapathy, 3F, Kalinga Colony, K. K. Nagar, Madras-600 078.

Sri A. S. Desai, Senior Inspecting Officer (Fruit & Veg. Preservation) Food & Nutrition Board, Dept of Food, Ministry of Agriculture & Irrigation, Shashtri Bhavan 35, Haddows Road, Madras-600 006.

Sri C.B. Cariappa, Ministry of Agriculture, Dept of Food, Food & Nutrition Board, Office of the Deputy Technical Adviser, 3rd Floor, Shashtri Bhavan, Madras-600 006.

Mrs. Anandhi Ramachandran, Institute of Catering Technology, Adyar, Madras-600 020.

Sri C. Ali, Asst. Instructor, Instt. of Catering Technology & Applied Nutrition, Adyar, Madras-600 020.

Sri M. D. Mukerjee, Technical Manager, Madras Commercial Corporation, Tamilnadu Flour Mills, New Avadi Road, Ambattur, Madras-600 058.

Sri G. Radhakrishnan, Assistant Director, Central Machinery Corpn. Regional Office 42 A, Third Street, Abhiramapuram, Madras-600 018.

Sri K. M. Appaiah, House No. 4, Block II, Jayalaxmipuram, Mysore-570 012.

Sri J. N. Rao, Macneill & Magor Limited, Unity Buildings, Bangalore-560 002.

Sri Ravi P. Awasthi, C/o. UNDP., P.O.B. No. 9182 Dar-e-Salaam, Tanzania.

Sri E. M. Fernandes, C/o. N. Fernandes, Kalpane, Kulshekar P.O. Mangalore-575 005.

Sri D. N. Samanta, C/o. The APV Engg. Co., Ltd., 2, Jessore Road, Calcutta-700 028.

Sri J. K. Jagtiani, 505, Asia House, Kasturba Gandhi Marg, New Delhi.

Sri Dayanand, D II/14, Kidwai Nagar (East), New Delhi.

Mrs. Sucy Koshy, Division of Agricultural Extension, Indian Agricultural Research Instt., New Delhi-110 012.

Dr. J. D. Contractor, The Coca-cola Export Corpn., 14-A, Nizamuddin West, New Delhi-110 013.

Mr. M. Bhatia, A-419, Defence Colony, New Delhi.

Sri R.L.Dang, Food Technologist, Fruit Processing Plant JAROL, Sundernagar Dist Mandi (Himachal Pradesh).

Sri V.S.Mathur, Deputy Director (Agri. & Food), Indian Standards Institution, Manak Bhavan, 9, B. S. Zafar Marg, New Delhi-110 001.

Sri H. C. Das, D-3/18. Model Town, New Delhi.

Sri Sharma Autar Krishan, Pomona Canning Co., Kastoorchand Mills Estate, Dadar, Bombay-400 028.

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Sri Balwant K. Sehgal, Kasavli, Cannery Kasavli Himachal Pradesh.

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EASTERN REGIONAL BRANCH

Association of Food Scientists and Technologists (India)

DEPARTMENT OF FOOD TECHNOLOGY & BIOCHEMICAL ENGINEERING
Jadavpur University, Calcutta—700 032

NATIONAL SEMINAR ON "MANGO AND ITS UTILISATION"
6th and 7th March 1976

The Eastern Regional Branch of the Association of Food Scientists and Technologists (India) is planning to hold a two-day Seminar on "Mango and its Utilisation" in March 1976.

The two-day Seminar will consist of Technical Sessions allocated to:

1. Raw materials–varieties, quality and horticultural aspects
2. Technology of green mango products and quality control aspects
3. Technology of ripe mango products and quality control aspects
4. Marketing of mango and mango products including export.

We look forward to your encouragement and support in organising a successful seminar. Papers for presentation and discussion at the Seminar are most welcome.

The Registration fee for delegates is Rs. 20/- for members of Association of Food Scientists and Technologists (India) and Rs. 50/- for non-members.

It is proposed to publish a brochure for the Seminar containing the abstracts of the papers and information on the activities of Mango Processors and Exporters. We shall be happy if you wish to reserve space for your advertisement in the brochure. It is intended to publish the proceedings of the Seminar in book-form as a comprehensive review of the subject.

An exhibition of Mango product and related machinery and equipment will be held on the occasion of the Seminar.

For further details, please correspond with the Convenors, Jadavpur University, Calcutta—700032.

Chairman
Seminar Committee
Dr A. N. Bose

Convenors
Sri A. K. Banik and Sri P. Chattopadhyay
AFST (Eastern Regional Branch)
Jadavpur University, Calcutta-700 032

ALL INDIA SYMPOSIUM ON

"FATS AND OILS IN RELATION TO FOOD PRODUCTS AND THEIR PREPARATIONS"

It is proposed to organise a two-day All India Symposium on "Fats and Oils in Relation to Food Products and their Preparations", sometimes in April 1976 at Central Food Technological Research Institute, Mysore under the Joint Auspices of Association of Food Scientists and Technologists (India), Central Food Technological Research Institute, Mysore and Oil Technologists Association of India (Southern Regional Branch), Hyderabad-500009.

The Symposium will be organised with the following objectives.

i) To provide a common platform for the processors, planners, government executives and scientists connected with R & D problems

ii) To focus attention on the problems of Lipid Technology connected with the usage of fats and oils in Indian food items

iii) To discuss and identify areas for future research and developmental activities relating to

a) Raw-material survey, resources and newer sources of fats and oils,

b) Processing, Hydrogenation, Emulsification, Interesterification, Refining and Modification of Fats and Oils

c) Fat-based food products--Indian confectionary, deep fat fried products, bakery products, margarine, chocolate, pickles, salad cream, myonaise, butter, cheese and ghee,

d) Nutrition and toxicity,

e) By product utilisation for edible purposes,

f) Autoxidation, antioxidants and storage characteristics of fats and oils,

g) Chemistry, analytical techniques, adulteration and quality control.

We plan to bringout a Souvenir on the eve of the symposium with lead papers from eminent specialists in the field. Papers on different aspects as given above are most welcome. Abstracts not exceeding 200 words should reach us before 15th February 1976 and detailed papers may be sent by 15th March 1976. An exhibition of various charts, products, machinery, equipment and raw-materials will be another feature of this Symposium. All the readers of the journal from India as well as from abroad, interested in any of the aspects of the Symposium are cordially invited to participate as exhibitors, advertisors for the souvenir, delegates or visitors.

For further details please feel free to write to Mr. M. V. Sastry, Honorary Executive Secretary, AFST, CFTRI, Mysore—570 013, India.

Honorary Executive Secretary
AFST

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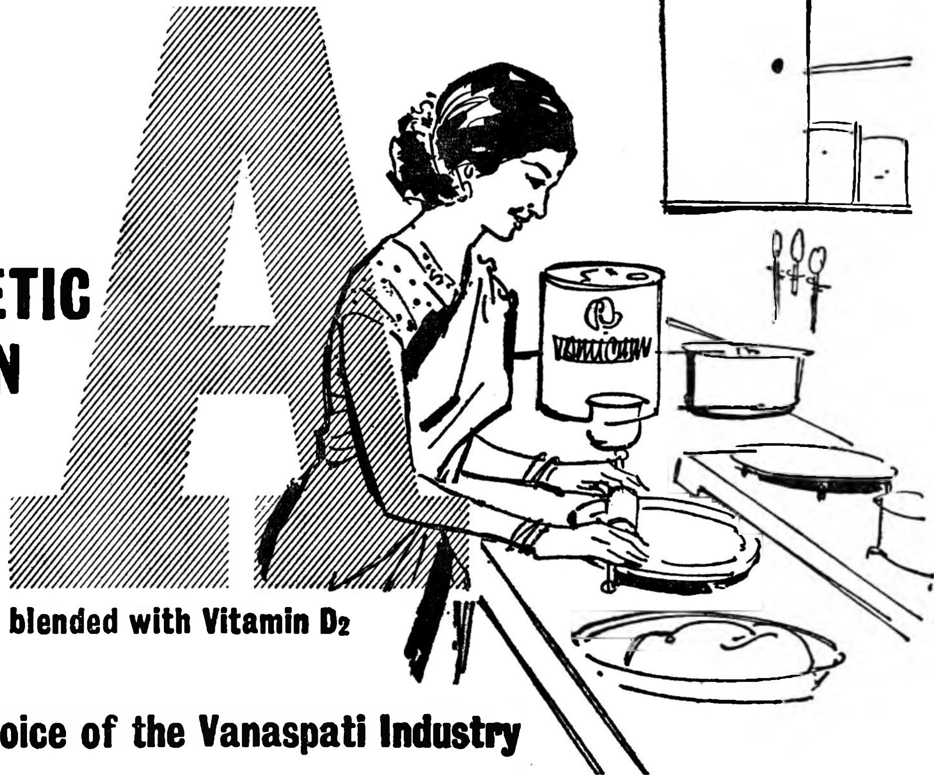

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1. Manuscripts of papers should be typewritten in double space on one side of the paper only. They should be submitted in **triplicate.** The manuscripts should be complete and in final form, since no alterations or additions are allowed at the proof stage. The paper submitted should not have been published or communicated anywhere.

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

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

4. **Abstract:** The abstract should indicate the scope of the work and the principal findings of the paper. It should not normally exceed 200 words. It should be in such a form that abstracting periodicals can readily use it.

5. **Tables:** Graphs as well as tables, both representing the same set of data, should be avoided. Tables and figures should be numbered consecutively in Arabic numerals and should have brief titles. Nil results should be indicated and distinguished clearly from absence of data.

6. **Illustrations:** Line drawings should be made with *Indian ink* on white drawing paper preferably art paper. The lettering should be in pencil. For satisfactory reproduction, graphs and line drawings should be at least twice the printed size. Photographs must be on glossy paper and contrasty; *two copies* should be sent.

7. Abbreviations of the titles of all scientific periodicals should strictly conform to those cited in the World List of Scientific Periodicals, Butterworths Scientific Publication, London, 1962.

8. **References:** Names of all the authors should be cited completely in each reference. Abbreviations such as *et al.*, should be avoided.

   In the text, the references should be included at the end of the article in serial order.

   Citation of references in the list should be in the following manner:

   (*a*) *Research Paper:* Menon, G. and Das, R. P., J. sci. industr. Res., 1958, **18,** 561.

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

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

   (*d*) *Proceedings, Conferences and Symposia:* As in (*c*).

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

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

REG. No. 24918/64

Printed and published by Shri M. V. Sastry, Secretary AFST (India) CFTRI, Mysore-570013, at Sharada Press, Mangalore-575001