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ASSOCIATION OF FOOD SCIENTISTS AND TECHNOLOGISTS

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AFFILIATED TO THE INSTITUTE OF FOOD TECHNOLOGISTS, USA

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2. To provide a forum for the exchange, discussion and dissemination of current developments in the field of Food Science and Technology.
3. To promote the profession of Food Science andTechnology.

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The following omissions have been noticed in the paper entitled 'Studies on Preservation of Moist Paddy after Harvest' by C. S. Shivanna and B. Kudarathulla.

Fig. 1.	X	Axis	represents	Storage time (days)
	Y	,,	,,	Temp (°C)
Fig. 2.	X	,,	,,	Storage time (days)
	Y	,,	,,	Temp (°C)
Fig. 3.	X	,,	,,	Storage time (days)
	Y	,,	,,	Sugars (mg)
Fig. 4.	X	,,	,,	Storage time (days)
	Y	,,	,,	Amino acids (mg)
Fig. 5.	X	,,	,,	Salt concentration (per cent)
	Y	,,	,,	Moisture content (per cent)
Fig. 6.	X	,,	,,	Storage time (days)
	Y	,,	,,	Temp (°C)
Fig. 7.	X	,,	,,	Salt concentration (per cent)
	Y	,,	,,	Breakage (per cent)

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Research Notes

The Reviewing Committee for Gardner's Award has recommended the research paper entitled 'Studies on Packaging and Storage of *Atta* (Wheat Flour) under Tropical Conditions' published in the Journal of Food Science and Technology, Vol. 8, No. 3 of 1971 by Sri S. S. Arya, M. S. Mohan and H. Nath for the award, being the best research paper published in our Journal for 1971.

A.F.S.T. announces the holding of an All India Symposium on 'Development and Prospects of Spice Industry in India' during February 1974 at C.F.T.R.I., Mysore. This will be held along with the Annual General Body Meeting of the Association. Further particulars will be announced later.

Fish Protein Concentrates: Recent Advances

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One of the most widespread nutritional deficiencies in the world today is that of high quality protein. Since many people in the world subsist on cereal grains, the improvement of cereal protein is, therefore, becoming of considerable importance[1,2].

It is difficult to anticipate the steps which will be taken during the next few years to cope with the problem of providing adequate food for the ever increasing world population. To overcome protein malnutrition, it would be better if large unutilized fishery resources in various parts of the world are profitably utilized as fish protein concentrates (FPC) for human consumption.

During the recent years, many developments have taken place for processing and utilization of FPC. Several possibilities now exist for production of FPC on a large-scale. The National Marine Fisheries Service, Aberdeen, Washington; Cardinal Proteins Ltd, Nova, Scotia, Canada; Alpine Marine Protein Industries Inc., New Bedford, Massachusetts; and Astra Nutrition, Boa, Sweden, in collaboration with the National Biscuit Company of the United States are now manufacturing FPC on large-scale[3].

The purpose of this paper is to review in brief some of the developments of FPC during the past decade. The earlier detailed review[4] covered the subject upto 1962.

Preservation of fish prior to processing

One technical problem is the economical preservation of the catch until transported to the processing site.

Fish pretreated with ammonia is safe and comparatively easy to obtain, has a good preservative action and can, at the same time, be removed easily during washing prior to its processing into FPC[5]. Ammonia has the advantage over strong bases such as caustic alkali as it does not affect the protein quality.

About 2 per cent of SO_2 in the form of $NaHSO_3$ will preserve unrefrigerated whole fish for 3-4 weeks. The cost of SO_2 for treating a ton of whole fish is $2. Processing of such chemically preserved whole fish has yielded excellent fish meal for animal use and FPC for human consumption. Biological assay showed that the protein is not damaged[6].

Isopropyl alcohol (IPA) has been shown to inhibit lipolytic enzymes and bacterial growth in fish for a period upto 3 months. Whole ground, red hake were stored in 91 per cent IPA for a period upto 11 days at room temperature. The nutritional quality of FPC was not impaired on storage. Analysis of the storage alcohol showed increase in soluble nitrogen which appears to be indicative of enzymatic proteolysis and solubilization of nitrogenous material[7].

Fish scraps stored in cans with 10 and 12 per cent IPA under refrigerated condition stored well for three months. The preserved fish can be efficiently used for FPC extraction with IPA. Use of IPA in stored herring inhibited the lipolytic enzyme activity and free fatty acids in the oil did not go above 3.2 per cent even when the fish was completely liquefied[8].

The cake pressed out of oil-sardine after cooking was preserved in ethanol of 70 per cent by volume. Though fish can be stored for a few weeks in ethanol depending upon its fat content, the adverse change in colour of fish was perceptible even after a few days of storage in the case of fish with higher fat content[9].

Processing techniques

The history of man's efforts to produce FPC is traced from Roman to modern times. The processing technology and equipment requirement including various methods of producing FPC such as biological, chemical and physical are outlined[10-12].

Major attention was first given to developing a solvent process using IPA to extract red hake (*Urophycis chuss*). Later efforts were directed towards the use of other species of fish for the production of FPC[13].

The cross-current batch extraction procedure has been replaced by counter current batch extraction system in the basic IPA extraction process. This counter current process is more applicable to commer-

cial processing[14]. Improvements have been effected in recovery of IPA with adequate purification so that the solvent is rid of amines from fish. With such recovered solvent, the amine content of FPC is very low and thus it does not contribute significantly to the flavour and odour[3]. FPC with markedly improved functional properties[3] especially oil emulsifying capacity can be produced by extracting fish with IPA at room temperature instead of at 70°C. An IPA and water ratio of 70:30 is effective in extracting lipids. The final extraction employs anhydrous IPA.

A description is given of pilot-plant operation at Quintero, Chile that can produce 300 tons of FPC (defatted, deodourised) per year. Animal and human feeding trials showed that the quality of FPC was good[15].

FPC of light colour, free from fishy flavour and reversion of flavour on storage can be prepared by successive extractions of wet whole or eviscerated fish with ethanol[16]. The FPC so obtained is slightly better than the one extracted with IPA or acetone. The process proceeds in two distinct stages: dehydration followed by lipid extraction. Six to seven extractions at the boiling point of ethanol each lasting 15-20 min yield FPC with lipid content below 1 per cent. Ethanol could be recovered, and recycled by distillation, rectification followed by azeotropic distillation. This work is confirmed by the Brazilian process, in which sardine (*Sardinella aurita*) has been used to produce FPC which has physical characteristics comparable to or better than the product extracted with IPA in terms of colour, odour and taste. Ethanol, a by-product of cane sugar industry, is available in large quantity at relatively low cost and has been used as the extracting solvent[17].

If production of FPC is carried out in two stages—first producing a concentrate of type C (non-deodourized and non-defatted) followed by a second stage of solvent extraction of type C, the quality of the finished product with respect to colour and nutritional value suffers[18].

The FPC obtained from hake by using isobutanol (2-methyl-1-propanol) as solvent was light in colour with practically no odour and contained only 0.3 per cent fat[19].

Fish is treated with a surface active agent[20] under appropriate conditions to yield acceptable quality FPC with low fat content. Sodium dodecyl sulphate was used to reduce the lipid content; the lipid content was lower than the starting material but the detergent residue was as high as 40 per cent. The use of detergents in preparation of FPC may not be feasible[21].

New process: This process utilizes a condition of high pH (12.5) and temperature (95°C) to effect a high yield of FPC solids in a relatively short time of about 20 min. Although some disaggregation of depolymerized protein occurs during the process, approximately 50 per cent of the protein is still acid precipitable. Extensive recemisation of amino acids occurs. Off-flavour was eliminated by passing the non-acid precipitable fraction of the product through charcoal. The product could then be used to produce milk like beverage which could take various flavours. Since extensive recemisation of amino acids occurs, the nutritional value of the product will be considerably lowered. Perhaps, a weaker base may yield better results[22].

Enzymatic treatment: The relative activities of more than 20 commercially available proteolytic enzymes were measured for the digestion of a washed and freeze-dried fish protein substrate. Pronase was most active but pepsin, pancreatin and papain provide much more digestive activity per unit of cost[23]. Continuous solubilization process of FPC by proteolytic enzymes showed that pepsin and pronase were particularly effective. The effects of pH, temperature, substrate and enzyme concentrations on the rate and extent of FPC proteolysis and solubilization by pronase were tested[24].

The use of enzymes for FPC with modified properties are now in development stage. They offer good scope for utilization of FPC with modified properties. Such products may be prepared by partial hydrolysis of fish proteins in the form of milk analogs. The process developed by Rutman and Heimlich[25] is an example of this type product.

Nutritional aspects of FPC

Severely protein depleted young rats fed deodourized and defatted FPC extracted with ethanol showed improved nutritional status at the end of 45-day period. The FPC was found to be acceptable, free from toxic products and possessed very high nutritional value[26].

Significant differences were not observed in the amino acids and PER between FPC from whole fish, dressed fish, raw dried fish and cooked pressed dry fish meal. FPC prepared from raw dry fish having more ethanol extractives showed, however, fishy odour when incorporated into such products at 10 per cent level[27].

FPC from silver bellies (*Leiognathus bindus*) extracted with ethanol has a high nutritive value and can be used as an effective supplement in dietaries[28]. Recent work reported showed a Protein Efficiency Ratio

(PER) of 3.5 for FPC from oil-sardine and nutritional quality of FPC from batch to batch was uniform when fish is given direct extraction with ethanol[16].

A series of experiments were conducted to determine the sequence of limitation of the essential amino acids in FPC produced by IPA extraction of whole red hake. The amino acids were grouped according to their limitation from greatest to least as follows: (*i*) methionine; (*ii*) histidine, tryptophan and threonine; (*iii*) valine, isoleucine and phenylalanine; and (*iv*) leucine, lysine and arginine[27].

FPC improved the biological quality of cereal based foods. Growth tests showed that 2.5 g FPC/kg/day as sole source of protein supported normal growth in infants, and that mixtures containing FPC and sunflower seed meal (SFM) produced normal growth in children when they supplied 70 per cent of the total dietary protein[15].

FPC has a high nutritive value which is equal to or higher than that of casein when fed to normal weanling rats. When added to vegetable proteins, FPC markedly improves their nutritive value. Evidence also indicates that it will be effective in the treatment of acute protein malnutrition in children[29-31].

Children given protein food made from low-fat peanut flour, Bengalgram flour (*Cicer arietinum*) and FPC in the ratio of 2:1:1 liked the preparation. After six months, a highly significant increase in height, weight, red blood cell counts and hemoglobin level of the subjects receiving the protein food supplement was observed as compared with the control group[32].

FPC made by Viobin Corporation, Monticello, Illinois, was used in the prevention and treatment of kwashiorkor[33]. Although the results of rise in serum albumin and total serum protein were encouraging, the weight and growth response was not comparable with that fed on skim milk powder. Since data on the total diet of the subjects are not given, influence of differences in food intake cannot be ruled out. The preparation in which FPC was incorporated was not in attractive form. Acceptability of FPC can be profoundly influenced by the form in which it is offered.[34]

Incorporation with other foods, and its supplementary effect: FPC produced from 7 species of fish by IPA extraction are shown to have nutritive value equal to or better than casein. FPC can be used in a variety of foods—bread, pasta, crackers, cookies, soups, tortillas and beverages to improve the nutritional quality[35].

Net protein utilization (NPU) of food combinations of FPC, bread, wheat, skim milk and SFM mixed in different proportions when fed to rats in diets at 10 per cent level of protein calories gave values (66-76) which compares well with that (67) found for Incaparina, an all-vegetable mixture and are higher than the values (55-56) for Peruvitas which are mixtures made up basically from cottonseed and quinoa reinforced with dried skim milk[36].

Studies were made to determine the relative supplemental value of FPC and lysine when added to wheat flour. With 10 per cent FPC, the weight gain was 36 per cent higher than with 0.4 per cent lysine. FPC supplied all essential amino acids, whereas lysine provided only the first limiting amino acid in wheat flour[37].

Nutritious pasta could be made from mixtures of corn, soy, rice and tapioca flours along with 10 or 20 per cent hake FPC and 15 to 20 per cent semolina. Generally, the protein quality of pasta containing 10 per cent FPC was as high as that for casein[38]. Addition of 3 or 6 per cent FPC to white bread increased the PER of bread proteins from 0.61 to 1.35 and 1.85 respectively[39]. Reports indicate that 5 or 10 per cent FPC supplementing any cereal makes the protein quality of the total product equal to milk, meat or eggs. For example, 0.9 lb of rice + 0.1 lb of good quality of FPC is equal in protein quality and quantity to nine eggs[40].

A study was made of the physical and sensory characteristics of bread fortified with various amounts of FPC or lysine. When 20 per cent or less FPC was added to wheat flour, more H_2O was absorbed by the dough than without FPC. Fortification of lysine at various levels did not change the characteristics of dough appreciably. Addition of FPC decreased the volume of loaves and crumb became darker, coarser and more compact. Texture and flavour of bread with 5 to 10 per cent FPC was acceptable[41]. FPC was reported to have no effect on physical properties of brown bread upto an inclusion rate of 4 per cent[30].

Residual lipids, flavouring constituents and IPA

IPA extracted samples[42,43] from red hake had 0.1-0.2 per cent residual lipids and Ethylene dichloride EDC extracted sample had approximately 0.5 per cent residual lipids. The lipids contained 50-60 per cent neutral lipids, 20-25 per cent phospholipids, 5-10 per cent acidic lipids and the rest was uncharacterised. The saturated fatty acids of the lipids were mainly palmitic and stearic, and the unsaturated fatty acids were mainly oleic and palmitoleic. The results were compared with the residual lipids in FPC from menhaden fish. There

was a lower content of highly unsaturated acids in the hake FPC which suggests[42,43] losses due to oxidative changes.

The phospholipids are extracted without obvious degradation and together with free fatty acids are found mostly in the IPA—rich phase from the first extraction. Residual lipids in FPC resemble the starting lipids of fish[44].

Thin layer and gas chromatographic separation and mass spectrometric analysis of volatile constituents of FPC revealed presence of a mixture of amines. The quantity of amine mixture present was dependent on processing conditions used in the FPC preparation and was related to the flavour intensity. The authors suggest that if FPC of high quality is to be produced, reclamation of IPA must involve more purification by distillation[45].

To maintain the quality control with respect to desolventization and to restrict the limit of residual IPA content as imposed by Food and Drug Administration (FDA), suitable methods have been recommended for estimation of the residual solvent[46,47].

A level of 6.4 mg/kg body weight of IPA consumed over a 6-week period appeared to have no toxic effect. A maximum IPA residue of 5000 ppm in FPC is suggested.

Fluorine content

Considerable interest has been shown in the fluoride content of FPC ever since the results reported by Hadjimarkos[48]. The addition of fluoride to drinking water to prevent dental caries in children, and suggested use of fluoride for prevention and treatment of osteoporosis has led to increased interest in the fluoride content of various foods and its metabolism in man.

The excretion and balance data with men indicate that fluoride is readily available from FPC, the net absorption of fluoride was 88 per cent as compared to similar concentrations of fluoride added to the casein diets in the form of sodium fluoride.[49] The difference in the availability of fluoride from FPC in the weanling rat and in adult man may be due to specific difference and/or to an age effect[50].

The concentration of fluoride in fish varied from 20-760 ppm and was dependent upon the raw materials. The major source of the fluoride was the bone[51,52]. Methods for estimation of fluoride content in foods are available[53,54].

The restriction placed on the maximum amount of fluoride in FPC is 100 ppm because of the possible intake of fluorine from daily consumption of various foods and water. This means that in some fishes, the bones where most of the fluoride is located have to be partially removed. Fluorine appears to be too restrictive at a level of 100 ppm. The permissible fluorine level should be determined in accordance with the fluoride intake from other sources of food and water. A maximum fluoride intake level of 250 ppm from FPC is suggested.

Selenium content

Since the discovery that selenium prevents liver necrosis in rats, a wide variety of animal diseases have been found to respond to selenium. Schwartz[55] discussed the possible involvement of selenium in kwashiorkor. Hadjimarkos[56] reported findings on the effect of selenium on dental caries. FPC had 1.8/mcg of selenium per gram. Daily intake of FPC by infants and children has been reported as 10-20 and upto 50 g giving 18-36 and upto 90 mcg selenium. Similar amounts of selenium have improved weight gain of children; the effect of FPC may be the combined effects of protein and selenium. There could be a deleterious effect of a higher intake of selenium from FPC.

Considerable interest has been shown in the selenium content of various foods[57]. Recent methodology facilitates estimation of selenium in foods[58].

Toxicity studies

FPC extracted with EDC has lower nutritional value[59,60]. However, it has been reported that 125 million pounds of foods have been processed with EDC for over 30 years, and these have been used in foods, feeds and pharmaceuticals. Not a single contraindication has ever been brought to the attention excepting in rare instances where persons were allergic to these foods. It is believed that 2400 mg of chlorocholine chloride per kg of diet have been fed to animals without any findings of morbidity. Moreover, there is no difference in food consumption or growth in the animals fed chlorocholine chloride as compared to the controls[61]. The present report indicates that the toxicity of EDC extracted FPC is dependent on the temperature and length of extraction time[62]. As a precautionary measure, Viobin Corporation Process uses IPA for the final extraction of the fish already extracted with EDC.

Storage and packaging

Moisture adsorption isotherms of Menhaden—FPC and hake—FPC were determined at 25, 35 and 42°C at 11-86 per cent RH. Equilibrium moisture content ranged from about 5 per cent at 11 per cent

RH to about 16 per cent at 86 per cent RH. Particle size of the hake-FPC did not appear to affect the moisture adsorption. Steam stripped samples adsorbed slightly more moisture at low relative humidities and slightly less moisture at high relative humidities than the non-steamed samples. Menhaden-FPC adsorbed slightly less moisture than hake-FPC samples[63].

Conclusion

Many types of unutilized fish in various parts of the world could profitably be utilized after processing as FPC for human consumption. FPC that meets FDA specifications could be produced from a variety of fish. However, care should be taken to separate certain types of fish that are toxic[64].

Fish caught near industrialized areas may get contaminated with heavy metals such as cadmium, mercury, lead, etc. For FPC production, since mostly pelagic fish with greater mobility, and far away from inshore waters are to be used, it should cause little concern. However, some species of fish may have a high naturally occurring levels of heavy metals. Fixation of upper limits for these toxic metallic impurities is called for.

FPC derived from sanitarily produced fish meals at much lower cost and with all the fish flavours retained might well be competitive and have a role to play[65]. One of the primary problems in the storage of such FPC is the oxidation of lipids. Lipids in many types of fish meals have fatty acids with five or six degrees unsaturation. This fact, together with the conditions of temperature and oxygen availability, render the fish meals to oxidative reactions. The interaction between proteins and oxidized lipids cause considerable decrease in nutritional value of fish meals and also associated changes such as undesirable colour[18,66,67] and flavour.

Studies on processing costs have shown that solvent extracted FPC produced by processing 25, 50 or 75 tons of fish daily will cost 31.5, 22.9 and 20.5 cents/lb respectively assuming that landed fish costs $20 per metric ton[63].

Comparing the price of FPC with other protein foods, it is perhaps the cheapest source of protein[69]. Recently[70] the cost of processing fish into FPC type A has been worked out at 16 cents per lb exclusive of raw material cost, taking into consideration daily processing of 200 tons of raw materials with 260 days of working in a year. If the cost of fish is taken at 1.5 cents/lb, the total production cost of FPC works around 26 cents/lb. It is considered that FPC may not be competitive with oilseed proteins. However, it should be remembered, that FPC contains more protein than oilseeds and the biological value of FPC proteins is at the same time much superior. FPC protein is particularly rich in lysine and methionine—the two essential amino acids limiting in vegetable proteins. It has now been well established that supplementation of diets based on staple cereals and other farinaceous materials with FPC even at low levels greatly improves the growth promoting value of the former.

The possibilities for incorporation of FPC into a variety of foods without affecting their desirable characteristics are limitless[71-73]. FPC, however, has limited functional properties. New processes based on use of enzymes or solubilization, are now being developed which have improved functional properties and they offer wider scope for use.

No novel food, regardless of its intrinsic merits, is likely to be accepted immediately. With skill and patience, acceptance can be won for a novelty food. Since FPC is odourless, tasteless and without apparent attraction to the consumer, it is not surprising that it will take sometime for people to accept it as an addition to their diet[74,75]. To quote a 1968 United Nations Report which admirably summarizes the views: 'The greatest obstacle to the commercial use of new protein foods is not so much in their production in a safe, nutritious, palatable and sufficiently inexpensive form, but the requirements for effective marketing and promotion'.

It is disturbing to realize that the entire world progress regarding the marketing of FPC has been comparatively small. This is in no small measure due to lack of adequate market development and education. Making FPC competitive in price with other vegetable proteins is a secondary problem.

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Sorption of Water Vapour by Wheat Flours

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Studies in the sorption of water vapour on three high yielding varieties of wheat flour have been made at 35°C, by using quartz fibre spring technique. The adsorption was related to the protein content of the flours. The values of the specific surface area of adsorbents have been calculated by the BET and HJ methods. The thermodynamic values show that there is no chemical interaction between the adsorbent and the adsorbate.

Water is a unique liquid in its ability to form flour doughs. The commercial uses of flour accordingly depend almost entirely on this important property of water. In the quality characterisation of wheat flours, results of such physico-chemical tests as adsorption, loaf volume and surface area are often used to give an indication of the baking potentialities. In general, higher the protein content of the flour, the better will be its baking value but this is not always true, because of variations in the physical and mechanical characteristics of proteins from different wheat types and varieties. There can be no doubt that physical dough testing devices measure the characteristics of flours that are related to the quality of the resulting bread. It is essential to know the quantity of water required to form a dough of proper consistency.

Wichser *et al.*,[1] studied the effect of particle size on certain characteristics of flour. An increase in ash, protein, water absorption and loaf volume was found with a decrease in the size of the flour particles. NeKryach[2] found that water absorptive power and heat of wetting of the flours did not differ greatly but increased from oat through barley to rye and wheat. Gur-Arieh *et al.*,[3] concluded that the adsorption by wheat flours is not a surface phenomenon but takes place on specific sites within the pores inside the particles. Udani *et al.*,[4] compared the moisture adsorption rates of seven kinds of flours and observed that adsorption did not change with particle size and was not related to the cake volume.

Although considerable progress has been made, an understanding of the hydration mechanism is yet to be investigated. The present studies were, therefore, undertaken to obtain some more information about the interaction of water with flour and the specific mechanism of cross-linking. An attempt has been made to account for the sorptive capacity of flours on the basis of their physical and chemical structure.

Materials and Methods

Three high yielding varieties of wheat flour namely 'C-273,' 'S-308' and 'PV-18' were procured from the Punjab Agricultural University, Ludhiana. The three samples had the protein content of 14.69, 12.33 and 10.57, and ash content of 0.40, 0.38 and 0.38 per cent respectively. The adsorbents were passed through 85 mesh British Standard Sieve.

The quartz fibre spring technique described elsewhere[5] was employed in the present work. The sorption apparatus was kept at 35°C inside an air thermostat. An Edwards high vacuum pump which produces a pressure of 10^{-3} mm was used.

Results and Discussion

The studies of the adsorption process revealed that the rate of adsorption of water on flours was quite rapid and complete equilibrium was established in less than 6 hr. Considerable caking was observed when water was sorbed at high relative pressures. At higher relative pressures the degree of adsorption was sufficient to completely and permanently alter the physical nature of flour and hence the studies were carried out upto 0.80 relative vapour pressure. The sorption isotherms at 35°C, are shown in Fig. 1, which are similar to those reported by Babbitt[6]. The amounts of water absorbed at 0.80 relative vapour pressure by 'C-273', 'S-308' and 'PV-18' wheat flours are respectively 15.00, 16.75 and 19.50 per cent. The protein content of these flours is 10.57, 12.33 and 14.69 per cent respectively. A comparison shows that

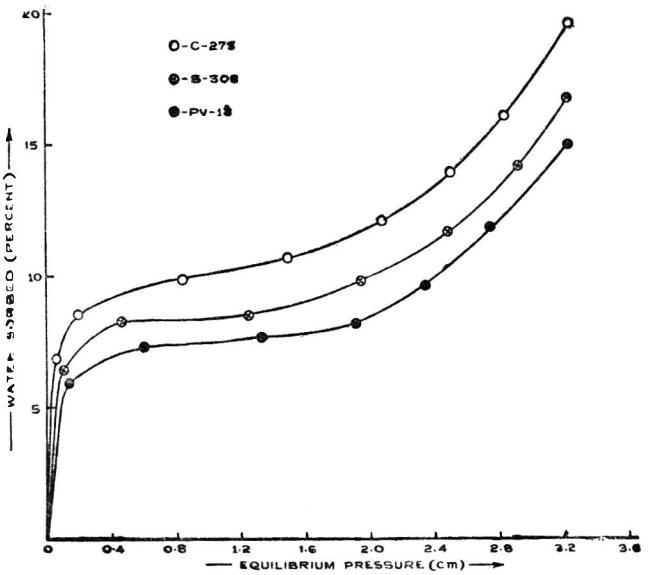

FIG. 1. Adsorption isotherms

adsorption is directly proportional to the protein content. These differences reflect real differences in the ability of these materials to bind water. The main force that causes the vapour to be adsorbed on flours seems to be the attraction between the dipoles. To reach these adsorption sites the sorbate molecules have to diffuse through the flour medium. The protein, especially gluten can sorb much more water than starch. Similar observations were made by Udani *et al.*[4]

Application of the BET equation: The adsorption isotherms (Fig. 1) are of sigmoid shape, which according to BET theory are classified as type II isotherms. This indicates that the adsorption of water by wheat flours is multi-molecular in nature. The BET equation[8]

$$\frac{p}{x(p_o-p)} = \frac{1}{x_m.c} + \frac{c-1}{x_m.c} \cdot \frac{p}{p_o}$$

where p/p_o is the relative vapour pressure, x is the quantity of water (in g) adsorbed per 100 g of sorbent, x_m is the monolayer capacity c and c is a constant related to the heat of adsorption, has been applied to the isotherms. The plots of $\frac{p}{x(p_o-p)}$ versus $\frac{p}{p_o}$ results in straight lines as shown in Fig. 2. From the slope and intercept, the values of the monolayer capacity (x_m) have been calculated for 'C-273', 'S-308' and 'PV-18' varieties to be 0.085, 0.092 and 0.068 g/g of adsorbent, respectively. From these values and the cross sectional area of water molecule taken[9] as $10.6A^{o2}$ the specific surface area[8] of the flours 'C-273', 'S-308' and 'PV-18' have been calculated to be 299.7, 321.5 and 241.0 m^2/g respectively.

Bushuk and Winker[10] observed disagreement between the values of the specific surface area obtained for the flour-water vapour and flour-argon systems in which there is an extensive swelling of the adsorbing material. Flour is also a swelling system with water and hence it is interesting to check the values of the specific surface area by the application of Harkins and Jura[11] method.

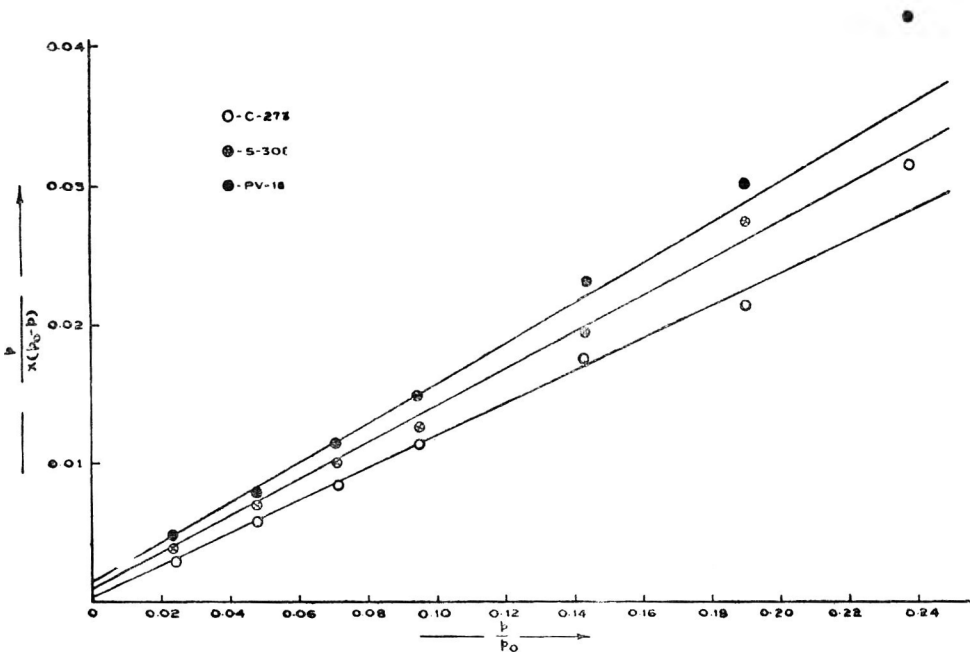

FIG. 2. Application of the BET equation

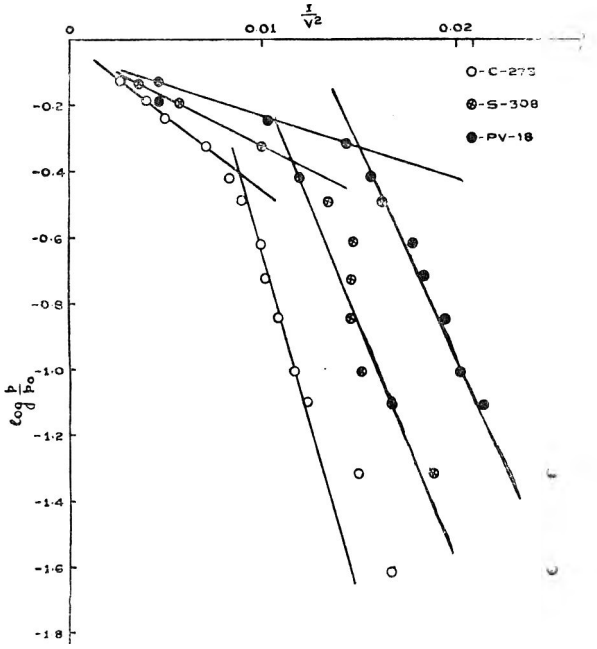

FIG. 3. Harkins-Jura plots

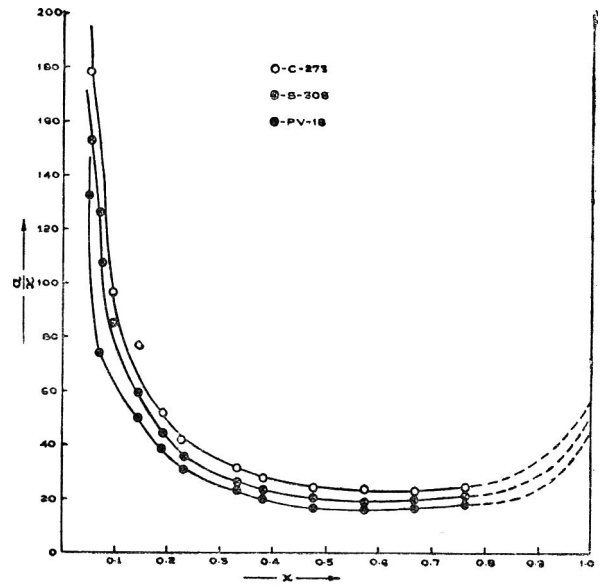

FIG. 4. Plot of $\frac{a}{y}$ *vs.* y

Harkins-Jura method: The Harkins-Jura (HJ) equation is

$$\log \frac{p}{p_o} = B - \frac{A}{V^2}$$

where V is the amount of adsorbate taken at any pressure p and A and B are constants. The plots of log p/p_o against $1/V^2$ are shown in Fig. 3. The relationship seems to be valid over an appreciable range of vapour pressure. The specific surface area(S) of the adsorbent is related to the slope (-A) of the HJ plot through the equation:

$$S = KA^{\frac{1}{2}}$$

where K is a constant. By taking the value of K for water vapour[12] as 3.83 the specific surface areas of the three varieties in the higher and lower vapour pressure range are calculated to be 540.3; 256.6 for 'C-274', 432.8, 250.3 for 'S-308' and 355.5, 168.5 for 'PV-18' wheat flour respectively.

Comparison of the HJ results with those of BET method reveals that the values obtained by the HJ method in lower pressure range are comparatively nearer to the BET values, than those in the higher pressure range.

These differences can probably be explained on the basis of the different postulates involved, for example in the BET method, one requires the knowledge of the molecular size of the adsorbate whereas in the HJ method, this value is not required and the value of the constant K is used instead. There is some indication that at least a part of the discrepancy might be due to the swelling that occurs during the sorption of vapours, although it does not seem likely that the increase in surface area owing to swelling would be extensive enough to account for all these differences.

Thermodynamic functions: In order to gather some information about the interaction of water with flour, the values of net heat of adsorption in the BET equation has been calculated, from the equation[8]:

$$c = e^{(E_1 - E_2)/RT}$$

where E_1 is the heat of adsorption in the first layer and E_2 is the latent heat of condensation. The values for the three types of wheat flours, namely, 'C-273', 'S-308' and 'PV-18' have been calculated to be 1.46, 1.25 and 1.34 kcal/mole respectively. These values are not sufficiently high to indicate any chemical interaction.

Further an isothermal process involving the transfer of water from vapour state to the surface of the adsorbent, the free energy change, which is usually taken as a measure of the affinity of the adsorbent for the vapour and is given by the equation.

$$\triangle G = \frac{RT}{M} \int_o^1 a. \frac{dy}{y}$$

where a is the amount adsorbed at a relative vapour pressure y and other letters have their usual significance.

In order to integrate the above equation a/y *vs* y has been plotted in Fig. 4. The area under the curve was multiplied by RT/M value. The values of the free energy change for the flours 'C-273', 'S-308' and 'PV-18' have been calculated to -919.9, -793.4 and -688.4 cal respectively. These values, again are not sufficiently high to indicate any chemical interaction.

It must be mentioned at this stage that the swelling of the material used in these studies would be endothermic also. Accordingly to get a true picture of the variation of the heat of adsorption with amount adsorbed, the values obtained have to be corrected for the heat of swelling. Such a correction requires a number of physical constants for the adsorbent, which unfortunately are not yet available for flours.

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Comparative Antioxygenic Properties of Citrous Bioflavonoids and Synthetic Antioxidants in Foods

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The antioxygenic properties of commercially available citrous bioflavonoids (CBF) have been compared with synthetic food grade antioxidants like propylgallate (PG), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) when incorporated in safflower oil, ghee, whole milk powder and fried potato chips . The antioxygenic activity of CBF has been found to be comparable to BHT and better than BHA in ghee. In whole milk powder it is as effective as BHA but its carry through properties are inferior to BHA during frying. Antioxygenic activity of CBF is inferior to PG which has been found to be most effective in all the products tested.

A minimum shelf life of six months, in moisture proof flexible packaging, is considered essential for various dehydrated and ready-to-eat convenience foods intended for use in pack rations of armed forces. Rancidity resulting from oxidation of lipids is a primary cause of spoilge of edible oils and dehydrated foods. Vacuum and inert gas packing have not proved satisfactory because of poor gas retention in flexible packs during transportation and storage. Food grade antioxidants like propylgallate, butylated hydroxyanisole and butylated hydroxytoluene have been widely used for prevention of off flavour development in oxygen sensitive foods. But none of these antioxidants are produced commercially in our country. Citrous bioflavonoids (CBF) is being indigenously produced in the country and therefore its antioxygenic properties were compared with synthetic phenolic antioxidants in oxygen sensitive foods.

Bioflavonoids are a major group of plant phenolics occurring in various food crops and some of them have been reported to have oxidation inhibiting properties.

Antioxygenic properties of hot water extracts of a number of vegetables have been reported to be due to flavonoids[1]. Higgins[2] reported that citrous peel and pulp could stabilize animal fats against rancidity. Charley[3] reported the remarkable flavour stability of citrous fruit drinks prepared from the communited

base of whole fruits. Naturally occurring orange flavonoid, pomiferin, has been reported to have remarkable antioxygenic activity[4]. The antioxygenic activity of naturally occurring flavonoids is due to their ability to act as free radical acceptors and to complex with metal ions[5-7].

The present paper describes the comparative antioxygenic potency of commercially available citrous bioflavonoids (CBF) with propylgallate (PG), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in safflower oil, ghee, whole milk powder and potato chips fried in refined groundnut oil and hydrogenated oil (vanaspati) containing the above antioxidants.

Materials and Methods

Edible oils: Sample of citrous bioflavonoid used in this study was obtained from American Pharmaceutical Company, Bombay. Two hundred milligrams each of CBF, BHA, PG and BHT were dissolved separately in 100 ml portions of propyleneglycol. One ml aliquots of these solutions were transferred in beakers containing 100g samples of commercially refined safflower oil or ghee and stirred thoroughly with glass rod. The treated samples were kept in an incubator maintained at 37±0.5°C. Control samples were treated with one ml of propylene-glycol and stored along with the treated samples. Initially and periodically one beaker of each type was removed and the contents analysed for peroxide value[8], malonaldehyde content[9] and organoleptic assessment for rancidity. These tests were selected because they have been found most suitable for measuring the rate of autoxidation in fats and oils[9,10].

Whole milk powder: Ten litre lots of cow's milk were concentrated to half of their initial volumes with constant stirring and filtered through muslin cloth. Filtered samples were treated respectively with PG, BHA and CBF at 0.02 per cent concentration of antioxidant on total fat weight basis. The treated samples were dried in a spray drier equipped with disc type automiser at an inlet temperature of 160°-170°C and outlet temperature of 70°-75°C. Fifty gram samples were packed in paper (65 g BC)/Al. foil (0.02 mm)/polyethylene (150 gauge) laminate pouches and stored in an incubator maintained at 45±0.5°C. Initially and periodically one sample of each type was removed and analysed for peroxide value[8], malonaldehyde content[11] and organoleptic acceptability of the reconstituted product.

Fried potato chips: Two kg refined groundnut oil and hydrogenated oil (vanaspati) samples were treated respectively with 0.02 per cent of PG, BHA and CBF. Potato chips were fried in each sample at 180°C and 100 g samples were stored in cellopoly (300 gauge) packs at 45±0.5°C. Initially and periodically one sample of each type was analysed for peroxide value and organoleptic evaluation.

Results and Discussion

The changes in peroxide value and malonaldehyde content of ghee and safflower oil containing CBF, BHA, PG and BHT are depicted in Fig. 1 and 2 respectively. It is seen that PG is most effective in inhibiting the peroxidation and rancidity development in both safflower oil and ghee samples. CBF has limited antioxygenic potency in safflower oil but in ghee its oxidation inhibiting capacity is comparable to BHT and greater than BHA. In safflower oil, both BHT and BHA are more effective in prevention of rancidity development than CBF. It will thus be evident that the nature of the fat to be stabilised has been found to influence the antioxygenic potency of different antioxidants. Higher antioxygenic potency of PG and tertiary butylated hydroquinone than BHA have also been reported by Sherwin and Thompson[12,13], in safflower oil. This has also been observed previously[13] e.g., BHA was superior to BHT and PG in lard but practically ineffective in stabilising highly

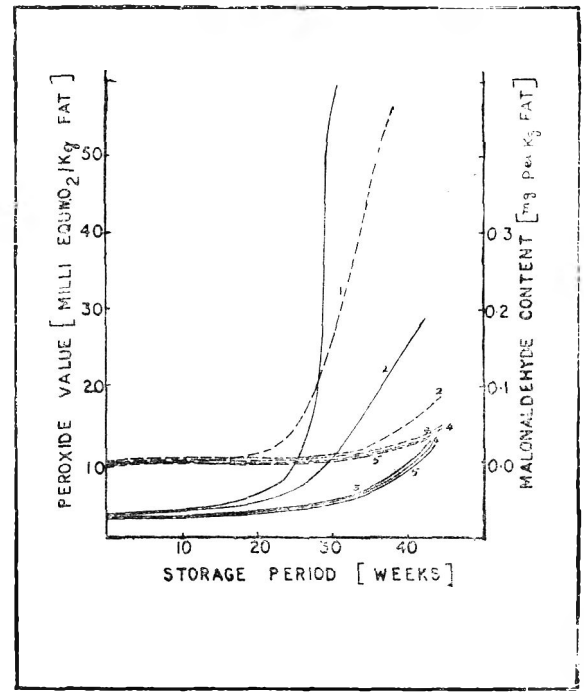

FIG. 1. Peroxide value and malonaldehyde content of ghee samples stored at 37 ±0.5°C; peroxide value (—); malonaldehyde content (- -); 1. ghee; 2. ghee+BHA; 3. ghee+CBF; 4. ghee+BHT; 5. ghee+PG.

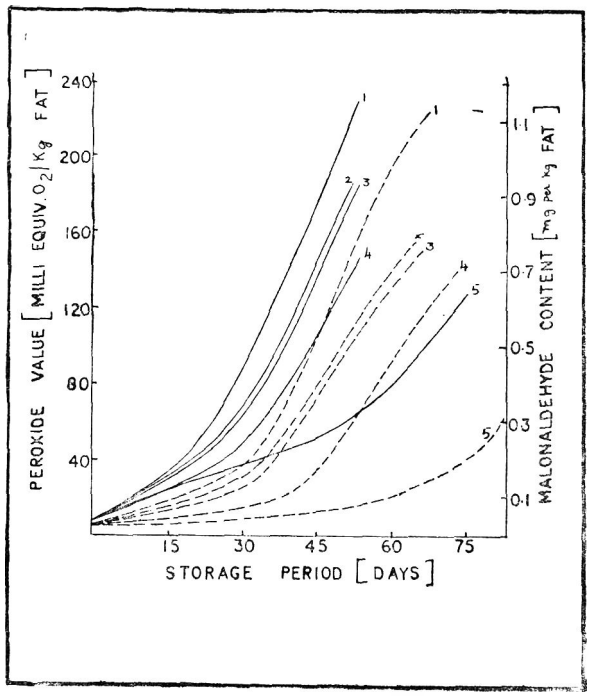

FIG. 2. Peroxide value and malonaldehyde content in safflower oil stored at 37 ± 0.5°C; peroxide value (—); malonaldehyde content(- -); 1. safflower oil; 2. safflower oil + CBF; 3. safflower oil + BHA; 4. safflower oil + BHT; 5, safflower oil + PG.

polyunsaturated safflower and soybean oils. Synergistic effect of PG with naturally occurring tocopherols may be responsible for this effect.

Naturally occurring pentahydroxy, and tetrahydroxy plant flavones and fruit juice of *Amla* (*Emblica officinalis*) have also been reported to be as effective as PG and nor dihydroguiaretic acid (NDGA) in stabilising milk fat against rancidity development[14-16].

The peroxide value, malonaldehyde content and acceptability scores of whole milk powder samples containing different antioxidants, on storage at 45°C are presented in Table 1. Both peroxide value and malonaldehyde content of samples containing PG were lowest indicating its highest antioxygenic potency. The antioxygenic potency of CBF and BHA have been found to be comparable in spray dried milk samples as is indicated by chemical and acceptability scores of the stored samples. Earlier workers[17-19] have also observed the higher antioxygenic potency of cetyl, dodecyl and propyl esters of gallic acid in spray dried whole milk samples against oxidation than BHA, NDGA, quercetin and ascorbylpalmitate. However, in samples stored under nitrogen only naturally occurring flavonols and ascorbic acid were effective in imparting flavour stability. This is due to their ability to form complexes with metallic ions which impart metallic and 'cardboard-like' flavours. The use of CBF along with gallates may therefore prove most effective in development of milk powders having good flavour stability.

The stability of the products fried in fats treated with different antioxidants depends upon the oxidation inhibiting and carry through properties of the different antioxidants and has widely been used to assess the suitability of different antioxidant treatments. The stability of potato chips fried in refined groundnut oil and hydrogenated oil containing PG, BHA and CBF, against peroxidation at 45°C are depicted in Fig. 3 and Table 2 respectively. It is observed that potato chips fried in oils containing PG were more stable against peroxidation than those fried in samples containing CBF and BHA. Although BHA had been reported[20] to have better carry through properties than PG but synergistic effect of PG with naturally

TABLE 1. CHANGES IN PEROXIDE VALUE (PV), MALONALDEHYDE CONTENT (MA) AND ACCEPTABILITY SCORES (AS) OF WHOLE MILK POWDER CONTAINING DIFFERENT ANTIOXIDANTS ON STORAGE AT 45°C

Storage period (weeks)	Control			BHA			PG			CBF		
	P.V.	M.A.	A.S.*	P.V.	M.A.	A.S.*	P.V.	M.A.	A.S.*	P.V.	M.A.	A.S.*
0	1.3	0.00	+2	...	...	...	...	...	...	...	...	...
8	2.5	0.002	+1	1.9	0.002	+2	1.7	0.002	+2	1.9	0·002	+2
16	6.2	0.086	0	3.6	0.026	+1	2·9	0.026	+1	3.7	0.029	+1
20	8.8	0.20	−1	4.3	0.096	+1	3.5	0.062	+1	4.4	0.098	+1
24	10.3	0.29	−1	6.0	0.10	0	4.8	0.098	+1	5.9	0.10	0

* +2, +1, Acceptable; −1, −2, Not acceptable.
P.V.: Milliequivalents O_2/kg fat
M.A.: mg. malonaldehyde/kg substance.

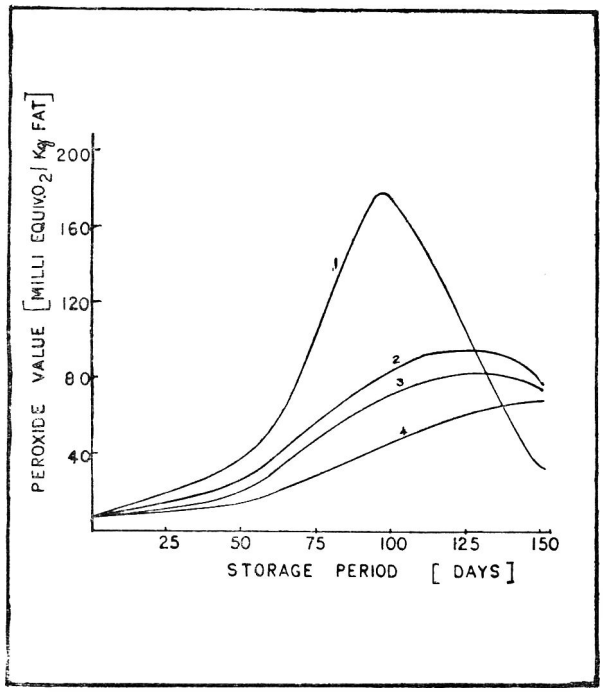

FIG. 3. Peroxide value of potato chips fried in groundnut oil containing different antioxidants on storage at 45±0.5°C in cellopoly packs. 1. control; 2. CBF; 3. BHA; 4. PG.

occurring tocopherols may probably be responsible for the higher stability of chips fried in oil samples containing PG. BHA does not act synergistically with tocopherols and is less effective in vegetable oils[20]. The peroxide value of chips increased on storage, registered a peak and then decreased on further storage (Fig. 3). The highest value reached was in sample without antioxidant and lowest is in case of PG. The carry through properties of CBF were inferior to BHA and PG both in refined groundnut oil and hydrogenated oil as is indicated by higher peroxide value of potato chips fried in CBF samples.

TABLE 2. CHANGES IN PEROXIDE VALUE (PV) AND ACCEPTABILITY SCORES (AS) OF POTATO CHIPS FRIED IN HYDROGENATED OIL (VANASPATI) CONTAINING BHA, PG AND CBF, ON STORAGE AT 45°C

Period of storage (weeks)	Control		BHA		PG		CBF	
	P.V.	A.S.*	P.V.	A.S.*	P.V.	A.S.*	P.V.	A.S.*
0	2.5	+2	...	...	...	...	...	...
4	4·7	+1	3.2	+1	3.9	+1	3.9	+1
8	5.7	+1	3.5	+1	3.8	+1	3.9	+1
12	6.4	+1	4.8	+1	3.7	+1	5.0	+1
16	11.5	+1	4.9	+1	4.1	+1	5.2	+1
24	17.6	−1	5.8	+1	5.3	+1	6.1	+1
32	28.1	−1	10.4	+1	8.4	+1	12.3	+1

*+2, +1, Acceptable; −2, −1, Not acceptable.

In the present study antioxygenic properties of citrous bioflavonoids have been found to be dependent on the nature of the food to be stabilised. Citrous bioflavonoids have appreciable oxidation inhibiting potency in ghee and milk powder but in vegetable oils and fried foods their antioxidant potential is not economically significant. Use of citrous bioflavonoids in combination with synthetic phenolic antioxidants like PG, BHA, etc., may prove more useful in processed foods because of their metal complexing activity.

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Studies on Vegetable Rennet from *Withania coagulans*

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Studies were made on the preparation of vegetable rennet from *Withania coagulans*, its keeping quality and use in cheese manufacture. A 5 per cent NaCl solution at 0-4°C containing 1 per cent boric acid was found to be best for extraction. Acetone, alcohol and NaCl were tried for the preparation of enzyme from the extract. The best preparation resulted from saturation with NaCl and it lost only 10 per cent of its activity upon storage in refrigerator for 3 months. Cheese of satisfactory quality from cow milk could be prepared with this rennet.

Milk coagulating enzymes from different sources are called as vegetable rennet, animal rennet and microbial rennet. The main sources of vegetable rennet are *Ficus carica* (edible fig), *Withania coagulans* and *Streblus asper*. In Northern India, *Withania coagulans* are available in plenty and is a potent source of milk clotting enzyme. Animal rennet is almost exclusively used for cheese manufacture since vegetable rennet and microbial rennet preparations have not yet been fully standardised. As the vegetarian population may not like to take cheese prepared from animal rennet, it is necessary to standardise and use vegetable rennet for cheese manufacture. Kothavalla and Khubchandani[1], Yeshoda[2], Narain and Singh[3], Dastur, *et al.*[4] have found the berries of *Withania coagulans* to be a potent and satisfactory source of enzyme for manufacturing cottage cheese. Qureshi, *et al.*[5] used *Withania coagulans* extract for preparing modified *dahi* having lower acidity, higher lactose content and greater palatability than normal *dahi*. In the present study the method for preparing vegetable rennet from *Withania coagulans*, keeping quality of preparations and its use in cheese manufacture are investigated.

Materials and Methods

The *Withania coagulans* berries (*Panir Dodi*) were purchased from the local market at the rate of Rs 1.50 per kg.

Extraction of enzyme: The extraction of enzyme from the berries was carried out under different conditions using various solvents namely water, different concentrations of NaCl upto 10 per cent and NaCl containing boric acid. The effects on the activity of extract obtained due to the factors like extraction of berries in instalments, grinding the berries, drying of berries in the sun, stirring upto 6 hr during extraction, addition of boric acid, addition of Fuller's earth or animal charcoal and centrifuging the extract at 3,000 rpm and 10,000 rpm in Sharple's centrifuge were studied.

Precipitation of enzyme from the extract: The precipitation of enzyme was carried out with non-aqueous solvents like acetone, alcohol and also by saturation with sodium chloride. One volume of extract was treated with 2 volumes of acetone at a temperature of 0°C with stirring. The upper opaque layer was decanted, the greyish black precipitate was filtered and washed thrice with small quantities of acetone and dried in vacuum desiccator.

In case of precipitation with alcohol, one volume of extract was treated with 3 volumes of distilled alcohol, at below 0°C. The addition of alcohol was made slowly with stirring at 0°- 4°C. After allowing to stand for one hour, the upper clear layer was decanted and the remaining portion was filtered in cold. The precipitate was washed with a small amount of alcohol and then dried under vacuum in cold.

For precipitation of enzyme from the extract by saturation with NaCl, 65.2 g of NaCl was required for 200 ml of extract. After allowing to stand for 24 hr at 0°C, it was centrifuged for 30 min at 2,500 rpm. The sediment containing the enzyme was collected. The insoluble portion from the precipitated rennet was removed by dissolving rennet in 5 per cent NaCl solution and centrifuging. The powder rennet was obtained on reprecipitation in the same way and drying

under vacuum. The process of centrifuging and drying was carried out in cold.

Activity of rennet preparations: The activity of rennet extract and subsequent preparation was studied by determining the clotting time of reconstituted milk using the technique of Dastur *et al.*,[4] and Berridge *et al.*[6] The reconstituted milk was prepared from skim milk powder stored in a sealed container in a refrigerator. Each time 10 g of powder were reconstituted with 100 ml of 0.01 M $CaCl_2$ solution in a Braun Multimix Blender for 1 min at speed of 8,900 rpm. The clotting time was determined in a constant temperature water bath at 40°C using 10 ml of substrate and 0.2 ml of rennet solution. Before adding the rennet solution, the milk was allowed for 5 min to maintain the required temperature. The activity of any rennet preparation under the given conditions was described[7] as X units per ml or per g, where

$$X=\frac{100\times D}{t}$$

(D is the dilution i.e., quantity (ml) containing 1 ml or 1 g and t is the clotting time in seconds).

Nitrogen content of rennet preparations: The nitrogen in rennet extracts and in different preparations was estimated by Kjeldahl method[8]. In case of original extract, 10 g of the sample was taken for digestion. In the case of solid rennet precipitated with acetone, alcohol, and NaCl the amount of rennet taken for each estimation was 0.2-0.3 g. All estimations were carried out in duplicate.

Keeping quality of vegetable rennet: The rennet preparations from sodium chloride precipitation was studied for its keeping quality in the solid as well as liquid form by storing at room temperature (28-35.5°C) and also in the refrigerator at 0-4°C. The solid rennet was also mixed with 50 per cent NaCl to examine the effect of salt on the keeping quality.

For storing in the liquid form, a 5 per cent solution was prepared in 5 per cent NaCl solution from solid rennet as such, while a 10 per cent solution was prepared in distilled water from the solid rennet containing 50 per cent added NaCl. Each solution was divided into two portions. One portion was stored at room temperature in amber coloured glass bottles while the other portion was kept in refrigerator. For storing in the solid form, the samples of solid rennet as such and solid rennet containing 50 per cent added common salt were stored in refrigerator and at room temperature in amber coloured bottles. In order to examine the activity of these solid rennet samples after each interval of storage, each time a fresh 5 per cent solution was prepared in 5 per cent NaCl from solid rennet as such and a 10 per cent solution was prepared in distilled water from solid rennet containing 50 per cent added salt. The activities were determined using 0.2 ml of rennet solution for 10 ml of reconstituted milk at 40°C prepared from skim milk powder of constant composition.

Preparation of cheese: The vegetable rennet prepared was used for making cheddar cheese from cow and buffalo milks. For the sake of comparison, cheese was also prepared using Hansen's liquid rennet and the qualities of cheese were examined.

Results and Discussion

Activities of the extracts obtained indicated that two parts of 5 per cent NaCl solution containing 1 per cent boric acid used for one part of berries was suitable for extraction. As shown in Table 1, the seed coat possessed some activities but the volume of extract obtained when the berries were extracted along with seed coat was appreciably less. The powder component was the most active. The seeds contained a very little enzyme and this activity was due to the powder sticking to the surface of seeds. Therefore, it was considered desirable to extract the berries after removing the seed coat. Extraction in two instalments using the same volume of saline solution resulted in a better extraction. But the extraction of first instalment became somewhat difficult due to less volume of the solution and hence 2 volumes of the saline solution were used in single instalment only.

The grinding of berries slightly increased the activity of extract but resulted in a greater deposit of sediment on keeping. Since a clearer extract was desirable for precipitation of enzyme, the grinding was considered unsuitable. The drying of berries in the sun for 8 hr brought about a loss in the activity of extract. Stirring for a period of 6 hr during extraction resulted in slightly decreased activity of extract. Soaking of

TABLE 1. EXTRACTION OF BERRIES AND ITS COMPONENTS

Particulars	Whole berries	Berries without seed coat	Sample from 20 g of berries		
			Seed coat	Seed	Powder
Weight of samples (g)	20	20	5.67	11.32	2.56
Vol. of 5% NaCl sol. taken for extraction (ml)	40	40	40	40	40
Vol. of extract (ml)	15	23	19	25	22
Total activity in extract (rennet units)	2.89	5.58	0.50	2.46	1.99
Activity (rennet units) from 20 g of sample	2.89	5.58	1.76	4.35	15.58

berries for 24 hr and working with hand or stirring for about 5 min before filtration through muslin cloth was found to be rather more efficient. The addition of 1 per cent boric acid as preservative resulted in a better activity of the extract. Table 2 shows the effect of adding 5 g Fuller's earth or animal charcoal to the extract from 100 g of berries. The activity of extract was found to decrease due to the addition of Fuller's earth or animal charcoal and the colour of extract indicated that the colouring materials were not absorbed on these. It was observed that after centrifuging in Sharple's centrifuge, the extract was much clearer due to the removal of fine suspended particles and the activity was also good.

The finally adopted method for extraction consisted in soaking the berries (free from stems, leaves and seed coat) with 2 volumes of 5 per cent NaCl solution containing 1 per cent boric acid. After keeping for 24 hr at 0.5°C, it was stirred or worked for about 5 min and filtered through muslin cloth. The filtrate was then centrifuged at 10,000 rpm through the Sharple's centrifuge in a cold room. The brown coloured extract was collected and stored in refrigerator.

Table 3 shows the activity of rennet preparations. The enzyme obtained by precipitation with acetone or alcohol possessed a good activity but some loss occurred due to denaturation. It was felt that precipitation of enzyme with solvent especially acetone was not practicable due to large consumption. The precipitation with ammonium sulphate at 30 per cent saturation and NaCl at saturation using pH of 2.0, 3.0, 4.6 and 5.6 at room temperature and at 0°C showed that the precipitation with NaCl saturation at 0°C using pH 4.6 is the most favourable.

From Table 4 it can be seen that the nitrogen content of rennet powder from sodium chloride precipitation is lower than acetone and alcohol precipitated rennets. As shown earlier in Table 3 the rennet precipitated with sodium chloride has a comparatively greater total activity than alcohol and acetone precipitated enzyme, but its nitrogen content is rather lower showing thereby that the enzyme obtained by sodium chloride precipitation is in a purer form.

The effect of storage on the activity of vegetable rennet in liquid and solid forms has been presented in Table 5 and 6 respectively. For a 5 per cent solution of liquid rennet stored in refrigerator, its activity decreased from 6.44 to 3.35 rennet units after a period of 3 months (about 50 per cent loss). In the case of solid rennet stored in refrigerator the activity decreased from 6.43 to 5.79 rennet units (about 10 per cent loss) after 3 months and from 6.43 to 4.83 rennet units (about 25 per cent loss) after 6 months of storage. It

TABLE 2. EFFECT OF ADDING FULLER'S EARTH OR ANIMAL CHARCOAL AND CENTRIFUGING THE EXTRACT

Nature of extract	Vol. of extract from 100g. berries (ml)	Total activity in extract (rennet units)
Original extract centrifuged at 3,000 rpm	115	31.7
After addition of animal charcoal	110	24.8
After addition of Fuller's earth	108	17.0
After centrifuging at about 10,000 rpm	111	30.2

TABLE 3. ACTIVITY OF ACETONE, ALCOHOL AND NACL PRECIPITATED ENZYMES

Particulars	Original extract	Vegetable rennet powder precipitated with		
		Acetone	Alcohol	NaCl
Wt. of rennet powder from 100 ml original extract (g)	...	3.0	2.5	2.1
No. of precipitations	...	5	16	20
Av. coagulation time (sec)*	367	454	480	311
Activity in rennet units/g of powder	...	4.4	4.2	6.4
Total activity (rennet units) in powder from 100 ml extract	27.2	13.2	10.4	13.5

*Using 5 per cent rennet solution/original extract, for reconstituted milk.

TABLE 4. NITROGEN CONTENT OF VEGETABLE RENNET PREPARATIONS

Particulars	Original extract	Veg. rennet powder precipitated with		
		Acetone	Alcohol	NaCl
Nitrogen (mg %)	448	3,136	6,500	2,800
Nitrogen content in rennet from 100 ml original extract (mg)	...	94.0	162.5	58.5
Protein (N×6.34) (mg)	2,842	596	1,020	373
Specific activity (activity in rennet units/mg protein)	0.0096	0.0220	0.0102	0.0370

TABLE 5. EFFECT OF STORAGE ON THE ACTIVITY OF VEGETABLE RENNET (LIQUID FORM)

Period of storage week	Activity (in rennet units) per gram powder stored at indicated temperatures			
	5% solution		10% solution	
	(28-35.5°C)	(0-4°C)	(28-35.5°C)	(0-4°C)
0	6.44	6.44	6.44	6.44
1	5.80	6.21	5.74	6.20
2	5.21	5.94	5.20	5.95
3	4.41	5.66	4.45	5.68
4	4.56	5.33	3.60	5.36
5	2.80	5.05	2.85	5.05
6	1.95	4.83	1.99	4.84
8	...	4.30	...	4.32
10	...	3.82	...	3.80
12	...	3.25	...	3.24
14	...	2.76	...	2.80
16	...	2.20	...	2.22

TABLE 6. EFFECT OF STORAGE ON THE ACTIVITY OF VEGETABLE RENNET (SOLID)

Period of storage week	Activity in rennet units/g powder			
	Rennet powder		Rennet powder + 50% NaCl	
	(28-35.5°C)	(0-4°C)	(28-35.5°C)	(0-4°C)
0	6.43	6.43	6.43	6.43
1	6.34	6.40	6.33	6.41
2	6.23	6.38	6.24	6.39
3	6.10	6.36	6.12	6.36
4	5.94	6.38	5.90	6.34
6	5.76	6.10	5.73	6.11
8	5.45	6.02	5.41	6.02
10	5.16	5.90	5.15	5.88
12	4.88	5.79	4.87	5.78
14	4.64	5.63	4.62	5.65
16	4.41	5.45	4.40	5.46
20	3.92	5.14	3.90	5.14
24	3.45	4.83	3.46	4.84

is clear that the loss in activity on storage of solid rennnet was much less as compared to liquid rennet. Further, the loss in activity of rennet in the liquid as well as solid form was significantly large at room temperature. In the case of storage in refrigerator, the loss in activity was appreciably less. Hence the storage of solid rennet in refrigerator was favourable for retaining the activity. The addition of excess salt did not help to improve the keeping quality of rennet.

As regards the studies on the preparation of cheddar cheese from cow milk, the flavour and taste of green cheese from vegetable rennet compared well with that of calf rennet. There was a slight bitterness in vegetable rennet cheese, the control cheese with calf rennet had no bitterness. The cheese from buffalo milk with vegetable rennet as well as calf rennet was comparatively inferior in quality and possessed bitter flavour. After 3 months of ripening of vegetable rennet cheese, it was observed that quality of cheese prepared from cow milk was better than that from buffalo milk. The increase in ripening period was found to decrease the bitter flavour in cheese but the defect could not be remedied completely. The quality of cheese from cow milk using vegetable rennet compared fairly well with cheese from calf rennet. For their quality, the samples of cheese were examined by a panel of judges.

Narain and Singh[3] prepared an active enzyme preparation from *Withania coagulans* berries by extraction with 20 per cent alcohol and precipitating with further addition of commercial alcohol. Dastur *et al.*,[4] extracted the berries with water and precipitated the enzyme with two volumes of acetone. The precipitated enzyme possessed good activity. Yeshoda[2] extracted *Withania coagulans* berries with water and precipitated the enzyme with 65 per cent saturated ammonium sulphate.

Some workers have tried to use vegetable rennet from *Withania coagulans* for cheese making. Kothavala and Khubchandani[1] showed that the rennet extract was promising for the manufacture of cheddar cheese and soft (Surti) cheese. They suggested some modification of the ordinary method of cheese manufacture and employed a higher temperature with a longer cooking time than the normal. Narain and Singh[3] prepared cheddar cheese under the usual conditions of temperature and the quality of cheese was as good as that prepared with animal rennet. Dastur *et al.*,[4] prepared soft cheese of quality indistinguishable from that made from animal rennet. But the cheddar cheese developed open texture on keeping and had a bitter taste. The time required for ripening was longer and probably more than 6 months. As the ripening time increased the bitter taste diminished but could not be remedied completely.

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Moisture Sorption Behaviour and Packaging of *Papads*

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Moisture sorption studies of black gram (*Phaseolus mungo*) *papads*, as influenced by the commonly used additives have shown that (i) sodium chloride alone contributes to increased moisture sorption, while, alkaline additives or Ca and Mg, present as impurities in common salt, have no significant effect, (ii) *papad khar*, an alkaline salt commonly used in *papad* making could be conveniently replaced by food grade carbonates, (iii) to prevent fungal spoilage in *papads*, the package should not allow the moisture to exceed the critical limit of 21 per cent on dry weight basis at the end of desired storage period, and (iv) a level of common salt higher than 8 per cent should be avoided, as it affects organoleptic acceptability, crystallises on surface at lower relative humidities (RH) and increases the moisture pick-up significantly at higher RH, thereby rendering *papads* susceptible to fungal spoilage.

Packaging studies with respect to ingress of moisture in polyethylene bags have revealed that 340 g unit pack of 200 gauge low density polyethylene (LDPE) or 170 g unit pack of 400 gauge LDPE has a shelf life of more than 4 months at 92 per cent RH and 38°C. This corresponds to a shelf life of nearly 12 months under normal conditions of storage. 350 gauge high density polyethylene was 2-2½ times as protective as the 400 gauge LDPE against ingress of moisture.

Traditionally confined to household, *papad* making in recent years has developed into a small scale industry. Consequently, the industry is facing the hitherto unknown problems pertaining to their packaging and adequate storage, more so, in view of the increasing export potential[1]. Freshly rolled *papads* contain 30-35 per cent moisture and as such are highly susceptible to fungal attack. It is, therefore, desirable to bring down the moisture level by drying and maintain the same within safe limits during the storage period.

Interestingly enough, *papad* has two levels of critical moisture contents: the upper limit for its susceptibility to fungal spoilage and the lower one to prevent adverse changes in pliability, resulting in warping and breakage. As such, a study of moisture sorption characteristics of such a product under extremes of climatic conditions is important for designing a right package for transhipment to export as well as inland markets. The effects of (*i*) the commonly used additives on the sorption behaviour, and (*ii*) packaging of *papads* in polyethylene bags on the shelf life of black gram (*Phaseolus mungo*) *papad* are reported in this paper.

Materials and Methods

Preparation of papads: Using the procedure described earlier,[2] *papads* were rolled into circular discs from doughs based on the following recipes:

(*i*) Black gram flour alone.

(*ii*) Black gram flour+1 per cent sodium carbonate.

(*iii*) Black gram flour+1 per cent sodium carbonate +7 per cent common salt (pH. 7.3) or sodium chloride (AR).

(*iv*) Black gram flour+8 per cent common salt+ one of the following alkaline salts: 0.66 per cent sodium carbonate, 2.16 per cent trisodium phosphate, 0.24 per cent sodium hydroxide or 3.6 per cent sodium bicarbonate.

The above concentrations of the alkaline solutions were predetermined so as to give a product of comparable pH.

Commercial samples purchased locally were also included in the study for comparison.

Moisture sorption behaviour: Papads used in this study were equilibriated to 64±2 per cent RH at 27°C. The samples were broken into small pieces and weighed quantities of the same in petri-dishes were placed in desiccators maintained at relative humidities ranging from 32 to 86 per cent. Saturated solutions of appropriate salts[3] were used to maintain constant humidity. The samples were periodically weighed to study gain or loss in moisture of *papads*. Weighings were continued till the samples attained a constant weight or till the onset of visible fungal

growth, whichever was earlier. The moisture sorption characteristics for sodium chloride (AR) alone were also studied for comparison.

Packaging of papads*:* *Papads* used were based on a recipe of blackgram flour: 100 parts, common salt: 7 parts and sodium carbonate: 1 part. When equilibriated to 64±2 per cent RH at 27°C, they had a moisture content of 14.8 per cent, as determined by drying the samples at 105°C for 5 hr. The packaging materials tried were low density polyethylene (LDPE) of 200 and 400 gauge and high density polyethylene (HDPE) of 350 gauge. Water vapour transmission rates (WVTR) of these films were determined by standard method (ASTM E96-66 procedure E)[4]. Two unit packs were tried for *papads*: 170 g (approximately 35 nos.) in 16 cm × 16 cm bags and 340 g in 16 cm × 17 cm bags.

Shelf life studies: All packets (except the commercial ones) taken in quadruplicates were heat sealed and were subjected to accelerated storage tests in a humidity chamber maintained at 92 per cent RH and 38°C. For comparison, 250 g unit packs of a commercial sample purchased locally were also included in this study. All packets were weighed periodically to assess the moisture pick-up and examined for visible fungal growth. When one of the packets indicated a moisture pick-up of about 3.0 per cent all the packets were withdrawn from the humidity chamber and moisture was estimated in *papads*. The uniformity in moisture pick-up in the same packet was checked by selecting *papads* from periphery and center.

Results and Discussion

Moisture sorption of papads: Processed food products in general are susceptible to fungal spoilage at 70 per cent RH[5] and above, where the moisture sorption indicates a steep rise. The following discussion of isotherms and results presented in Fig. 1 and 2 and Table 1, therefore refers mainly to moisture sorption of *papads* at 70 per cent RH.

Effect of common salt: It is seen from Fig. 1 that *papad* made from blackgram flour alone has an equilibrium moisture content (EMC) of 14.6 per cent at 70 per cent RH. Inclusion of 1 per cent sodium carbonate in the recipe had little effect on the EMC of *papad.* In contrast, 7 per cent common salt markedly increased the EMC to 20.3 per cent, indicating thereby, that common salt is the sole factor, contributing to a significant increase in the EMC. The data (Table 1) on EMC of sodium chloride (AR) alone, also confirm this observation. Iyengar and Sen[6]

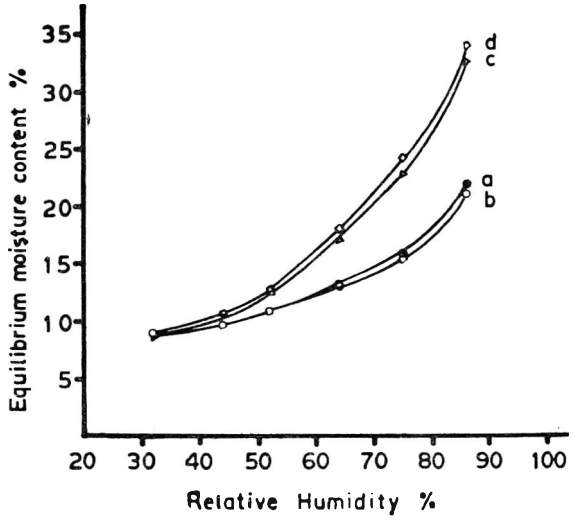

FIG. 1. Sorption isotherms of *Papads* at 27°C
(a) Blackgram flour (BF) only — control
(b) BF + Na_2CO_3 (1%)
(c) BF + Na_2CO_3 (1%) + Common salt (7%)
(d) BF + Na_2CO_3 (1%) + NaCl (AR) (7%)

have reported a marked lowering of the water holding capacity of salted fish, when the salt used contained impurities like calcium and magnesium. However, in *papads*, replacing common salt by sodium chloride (AR) did not bring about any significant change in their sorption behaviour.

The data (Table 1 and Fig. 2) on the effect of different levels of common salt also confirm the above mentioned trend of results. Inclusion of even 4 per cent common salt increased significantly the EMC to 20.5 per cent at 70 per cent RH as compared to 14.6 per cent of *papads* containing no salt. Increasing the salt level in *papads* further upto 16 per cent did not bring about significant changes in their EMC (20-22 per cent).

From the above results, it may be concluded that moisture level in *papads* should not exceed the critical limit of 21 per cent on dry weight basis (17.5 per cent on fresh weight basis) for preventing the onset of fungal spoilage.

The increases in EMC were strikingly conspicuous at 86 per cent RH being 22, 31, 39, 51 and 62 per cent for *papads* containing 0, 4, 8, 12 and 16 per cent salt respectively. These data on the moisture pick up of *papads* are of considerable practical importance in designing a package for providing adequate protection to *papads* against ingress of moisture during overseas transhipment at high humidities. It may also be concluded from the nature of isotherms (Fig. 2), that

TABLE 1. EQUILIBRIUM MOISTURE CONTENT (EMC) OF *papads* AT 27°C*

Ingredients	EMC (%) at indicated relative humidities (%)							Initial moisture content (%)	
	32	44	52	64	72.5	75	86	Dry wt. basis	Fresh wt. basis
Common salt Vs NaCl (AR)†									
Blackgram flour (BF) only	8.9	9.8	10.8	13.1	...	15.8	21.8	10.8	9.7
BF + Na_2CO_3 1%	8.9	9.8	10.9	13.0	...	15.4	21.1	11.4	10.3
,, ,, + Common salt 7%	8.9	10.5	12.5	17.1	...	22.7	32.5	16.6	14.2
,, ,, + NaCl (AR) 7%	9.0	10.7	12.6	18.0	...	23.7	33.8	16.6	14.2
Common salt									
BF + Na_2CO_3 1% + Common salt 0%	8.8	10.5	11.5	13.5	14.0	18.0	22.3	18.0	15.3
,, ,, ,, 4%	9.2	11.0	12.6	18.0	21.3	22.9	31.4	23.1	18.8
,, ,, ,, 8%	9.2	11.0	13.7	15.8	22.2	25.7	38.7	17.4	14.7
,, ,, ,, 12%	8.9	10.7	12.4	14.4	22.1	35.1	50.8	17.6	14.1
,, ,, ,, 16%	8.8	10.3	12.0	14.4	23.2	42.4	61.8	16.2	13.9
Alkaline salts									
BF + Common salt 8% + $(NH_4)_2CO_3$ 0.12%	7.8	9.6	11.5	18.6	20.3	27.8	41.2	26.7	21.1
,, ,, + Na_2CO_3 0.66%	7.3	9.3	11.3	17.5	19.0	27.3	39.9	25.4	20.2
,, ,, + Na_3PO_4 12 H_2O 2.16%	7.9	9.8	9.7	17.4	20.3	27.3	42.0	27.6	21.6
,, ,, + NaOH 0.24%	8.2	10.1	11.9	17.9	19.9	26.6	39.8	25.7	20.5
,, ,, + $NaHCO_3$ 3.6 %	8.7	10.8	13.0	19.5	20.4	28.8	44.1	22.7	18.5
Commercial samples (5 Nos.)									
Range	8.0-8.6	9.7-10.5	...	14.3-17.5	21.0-22.6	23.7-25.8	...	12.3-17.2	10.9-14.7
Average	8.3	10.1	13.8	16.3	22.0	24.8	...	15.8	13.0

* The recipe consisted of blackgram flour, 100 parts; common salt, 7-8 parts; sodium carbonate, 1 part. When required, common salt, sodium carbonate or any other alkaline salt was varied keeping the other ingredients constant. The EMC values are as per cent on dry weight basis.

† The EMC values at 27°C for common salt were 0.1, 0.9, 4.5 and 72.5 per cent and for NaCl (AR) 0.03, 0.05, 0.08 and more than 100 per cent at 64, 72.5, 75 and 86 per cent RH respectively.

a level of more than 8 per cent common salt in *papads* is not desirable, in view of the steep increases in their EMC at more than 75 per cent RH. In addition, at lower humidities, salt bloom (crystallisation of salt on the surface) was observed during storage of *papads* containing more than 8 per cent salt. Taking into consideration the taste factors and increased hygroscopicity, a level of 7 per cent salt was found to be acceptable in a standard recipe.

Effect of alkaline salts: The data on EMC of *papads* containing different alkaline salts like sodium carbonate, sodium bicarbonate, trisodium phosphate, sodium hydroxide were comparable and also resembled those of *papads* containing 8 per cent common salt and 1 per cent sodium carbonate. It was evident therefore, that use of different alkaline salts did not bring about a significant change in the EMC of *papads*, as long as the common salt level in the recipe was maintained the same.

Commercial papads*:* The EMC of commercial samples ranged between 21.0 and 22.6 at 72.5 per cent RH. It is interesting to note that the moisture sorption data for the commercial samples containing *papad khar* resemble well with those of *papads* containing 7 per cent common salt and 1 per cent sodium carbonate. It may be inferred that alkaline salts like *papad khar* having widely varying composition[7] could be easily replaced by food grade carbonates with advantage for maintaining uniform alkalinity in *papad*.

Packaging and shelf life of papads*:* The sorption studies have shown that *papads* equilibriate to 17.5 per cent moisture on fresh weight basis, i.e., 21 per cent on dry weight basis at 70 per cent RH. Thus, in the present study, *papads* with an initial moisture content of 14.8 per cent (at the time of packaging) will have a permissible uptake of about 3.0 per cent moisture during storage, without any onset of fungal attack. The data on the moisture pick-up of *papads* during storage at 38°C and 92 per cent RH are presented in Table 2. After 78 days storage, the moisture pick-up in 170 g unit packs of 200 gauge LDPE had exceeded a permissible limit of about 3.0 per cent. Though, no visible fungal growth was observed, these *papads* had developed unpleasant musty odour, thereby

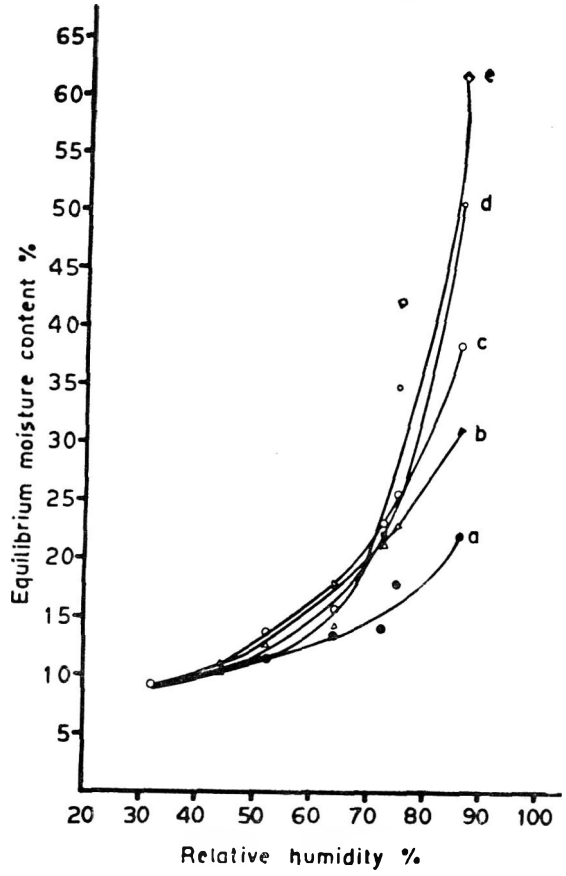

FIG. 2. Sorption isotherms of *Papads* (at 27°C) containing different levels of common salt.
(a) Control — no salt :
(b), (c), (d) and (e) represent 4, 8, 12 and 16 per cent salt respectively.

rendering them organoleptically unacceptable. Under similar conditions, 340 g unit packs having only 10 per cent more package area than 170 g unit pack, had a moisture pick-up of 2.6 per cent even after 128 days. From these observations, it may be inferred that the shelf life of *papads* could be nearly doubled by packing twice the quantity of *papads* in the same gauge LDPE at less than 10 per cent extra cost of the film. A similar trend was observed in case of 170 and 340 g of product packed in 400 gauge LDPE.

Remarkably low moisture pick-ups of 1.4 and 1.0 per cent respectively were recorded at the end of 128 days storage by *papads* in 170 and 340 g unit packs in 350 gauge HDPE. The WVTR values of different films presented in Table 2 justify such low moisture pick-ups. In contrast to LDPE, no appreciable change in the ingress of moisture was observed in 170 and 340 g unit packs of HDPE respectively. It is however evident from Table 2, that on the same gauge basis, HDPE with nearly one third WVTR as that of LDPE provides excellent protection against ingress of moisture. As such, HDPE is preferable as packaging material for product like *papads* where ingress of moisture is the main criterion for spoilage.

It was found that the commercial *papads* were packed in bags (15.5 cm × 17 cm) of 250 gauge LDPE. Interestingly enough, this unit pack had a high moisture pick-up of 5.4 per cent in 93 days. On gauge to gauge basis, however, one would expect a moisture pick-up similar to that in 340 g unit packs of 200 gauge LDPE i.e., 1.9 per cent in 93 days. This high moisture pick-up in commercial packs may be attributed to changes in WVTR resulting from improper sealing or pin holes on the surface of the bags caused by the sharp edges of *papads*.

The shelf life studies reported have been carried out under accelerated storage conditions (38°C and 92 per cent RH), which are too drastic, when compared to ambient conditions. As such, the actual shelf life according to Paine's correlation[8] may be nearly 3 times longer i.e., about a year, when 400 gauge LDPE is used.

TABLE 2. MOISTURE PICK-UP IN *papads** STORED IN POLYETHYLENE BAGS AT 92 per cent RH AND 38°C

Polyethylene gauge	WVTR‡ g/m²/24 hr	Quantity packed g.	Moisture pick-up (%) during storage at indicated periods (days)							
			15	29	43	64	78	93	99	128
LDPE—200	6.4	170	0.73	1.31	2.14	2.84	3.42	...	...	...
		340	0.34	0.61	0.93	1.36	1.69	1.92	...	2.57
LDPE—400	4.5	170	0.44	0.86	1.24	1.81	...	...	2.63	3.23
		340	0.26	0.49	0.71	1.04	...	...	1.52	1.93
HDPE—350 HD	1.9	170	0.19	0.35	0.53	0.76	...	...	1.13	1.35
		340	0.12	0.24	0.35	0.52	...	...	0.78	1.00
LDPE 250†	...	250	0.81	1.61	2.46	3.69	4.46	5.36	...	...

*Initial moisture content of the *papads*: 14.8 and 17.4 % on fresh wt. and dry wt. basis respectively.
†Commercial samples with initial moisture 14.4 and 16.9 % on fresh wt. and dry wt. basis respectively.
‡Water vapour transmission rate under 90 per cent RH gradient at 38°C.

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Further Studies on Microbiological Quality of Cashewnut (*Anacardium occidentale*)

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The incidence of microorganisms on cashewnut at different stages of processing in 7 different cashew processing units in Tamilnadu sector has been examined. The extent and type of contamination depended on the level of sanitation that prevailed in the units studied. Yeasts and moulds, aerobic mesophilic spores, aerobic-thermophiles, thermophilic flat-sours and staphylococci (non-coagulase type) were present to a limited extent. Salmonella and *Clostridium perfringens* were not present.

The cashewnut is our biggest foreign exchange earner and it is imperative to maintain high standards of quality to meet the foreign trade requirement. Although cashewnut processing has long been established in the country, not much has been done to evaluate the microbiological quality of the product. Russel[1] has stressed the importance of fungal infestations of cashewnut. A microbiological survey of some cashew processing plants in Quilon sector was carried out by CFTRI which suggested remedial measures against possible *Escherichia coli* contamination. Krishnaswamy *et al.*,[2] have studied the incidence of microflora of cashewnut in certain processing units in Kerala.

The present study envisages the microbiological survey of cashew processing units in Tamilnadu and presents the types of some of the coliforms, aerobic mesophilic spores and yeasts and moulds encountered on the cashewnut.

The processing steps followed in the units under study were as follows:

Raw nut → Raw shelling → Sun drying of kernels
↓
hand peeling
↓
Packing ← Grading ← Drying ← Kernels.

In the previous study[2] nuts were shelled after heat processing (drum/oil roasting). Pressing was in a primitive way and consisted of drying the nuts in the sun on flat roofs to facilitate shelling. Further processing involved drying of shelled-kernels on floors, hand-peeling, conditioning, grading and packing of peeled kernels.

Materials and Methods

Samples of cashewnuts were collected at random at various stages of processing from 7 different units representing various levels of sanitation.

Microflora in samples of cashewnut as collected above was examined as in an earlier study[2].

Coliforms were enumerated and identified by IMVIC tests[3].

Aerobic mesophilic spores[4] (Table 3) and yeast cultures were identified in accordance with the descriptive charts[5,6].

Mould isolates were purified by single-spore isolation or hyphal tip transfer technique and cultivated on (*a*) czapek (*b*) gorod kowac, and (*c*) corn-meal agars. The patterns of growth, colour and nature of spores were observed at intervals. Correlating the observed data with the descriptive charts, the cultures were identified.

Results and Discussion

Incidence of microorganisms: From the data in Table 1, it is seen that the extent and type of contamination depended on the level of sanitation that prevailed in different units. Raw nut was much more contaminated than the processed material. Yeast and moulds, aerobic mesophilic spores, aerobic thermophiles, thermophilic flat-sours and staphylococci (non-coagulase type) were present to a limited extent at different stages of processing. *Salmonella* and *Clostridium perfringens* were absent.

Coliforms: The occasional incidence of coliforms (Table 2) is probably due to the raw shelling of nuts

TABLE 1. INCIDENCE OF MICROFLORA IN PROCESSING OF CASHEWNUT

No. of unit	Stage of processing	Microflora/g.						
		Standard plate count	Yeast and moulds	Aerobic thermophiles	Aerobic mesophilic spores	Thermophilic flat-sours	Mesophilic anaerobes (MPN)	*Staphylococci* (non-coagulase type)
1.	Nut (raw)	37×10^5	2,000	1,500	90	28	2	1,600
	W210	8.3×10^3	340	20	20	0	...	0
	W240	1×10^3	200	30	0	0	0	30
	SW	6×10^3	100	200	120	1	0	50
2.	Nut (raw)	21×10^3	160	150	80	0	4	120
	Kernel (raw)	33×10^3	1200	520	600	15	7.0	410
	,, (peeled)	54×10^3	400	840	550	7	0	590
3.	Kernel (unpeeled)	48×10^3	190	110	400	7	0	280
	,, (peeled)	10×10^3	160	250	200	4	0	70
4.	Kernels (raw)	Spreader	2,000	4,200	4100	70	27	500
	,, (dried)	5.8×10^2	100	255	285	1	7.8	25
	SPS	30×10^3	50	150	100	15	0	70
5.	SPS	6.4×10^4	50	210	100	15	0	70
	W-320 (raw)	38×10^3	1800	320	105	0	0	600
	W-280 (dried)	11×10^3	450	230	100	1	4	310
	Nut (raw)	26×10^3	3000	100	20			360
	Peeled kernels							
6.	W210	6×10^3	170	0	50			340
	W240	5×10^3	270	0	300			110
	BB	4×10^3	240	0	80			170
	B	2×10^3	10	10	...	↑	↑	40
	S	1×10^3	...	...	1300	Absent	Absent	50
7.	Nut (raw)	22×10^3	300	140	0			210
	Kernel (peeled)	10×10^3	20	0	0			0
	,, (Borma dried)	10×10^3	0	0	0	↓	↓	0
	,, (conditioned)	6×10^3	0	110	10			80
	Grades:							
	LWP	3×10^3	0	30	0			50
	SWP	8×10^3	100	0	20			440

Salmonella and *Clostridium perfringens* were not present.

W = Whole; S.W. = Scorched wholes; SPS = Scorched pieces; B.B. = Baby bits; B = Butts; S = Splits; LWP = Large white pieces; SWP = Small white pieces.

TABLE 2. DENSITY OF COLIFORMS IN PROCESSING OF CASHEWNUT

No. of processing unit	Stage of processing	Coliform density/g (MPN)
1.	Raw nut (stored)	13.0
	W—320	6.8
	W—240	27.0
	SPS	23.0
2.	Raw nut (stored)	42.0
	Shelled kernel (raw)	17.0
	Peeled kernel (raw)	4.0
3.	Peeled kernel (raw)	17.0
	Peeled kernel (sundried)	4.0
4.	Peeled kernel (raw)	4.5
	" " (Borma dried)	4.0
	B	24.0
5.	SW	6.3
	W (raw)	70.0
	W (dried)	6.1
	S	13.0
6.	Raw nut	9.0
	LWP	4.0
	SWP	4.0
7.	Raw nut	23.0

W=whole; SPS=scorched pieces; B=butts; S.W.=scorched wholes; S=splits; LWP=large white pieces; SWP = small white pieces.

and unhygienic processing. Out of 41 isolates examined, 2 were *E. coli* type I (typical). Rest of the cultures belonged to *E. coli*, type II (10), intermediate type I (11), *A. aerogenes* type I (15) and *A. aerogenes* type II (3). The presence of coliforms including *E. coli* in cashewnut may be traced to soil contamination as in the case of almonds[7]. *E. coli* contributed to the coliform contamination of black walnut[8].

TABLE 3. IDENTIFICATION OF SOME OF THE AEROBIC SPORE-FORMING SPECIES IN PROCESSING OF CASHEWNUT

Stage of processing	No. of isolates examined	*B. subtilis*	*B. pumilus*	*B. polymyxa*	*B. pantothanticus*	*B. lichiniformis*
Raw nuts (stored)	5	2	2	1	...	...
Kernels (unpeeled)	3	1	1	...	1	...
Kernels (peeled and dried)	3	3	...	...	...	...
Kernels (assorted)	2	1	...	...	...	1
White pieces	3	1	...	...	1	1
Butts (scorched)	4	...	2	1	...	1

Aerobic mesophilic spores: From Table 3, it is seen that aerobic mesophilic spores consisted of *B. subtilis* (8), *B. pumilus* (5), *B. polymyxa* (2), *B. pantothanticus* (2) and *B. lichiniformis* (3). Aerobic mesophilic spores are soil inhabitants and when present in large numbers may cause spoilage of foods.

Yeasts: Yeasts identified were *C. pelliculosa*, *C. brumptii* and *C. melini*, respectively.

Moulds: Mould isolates belonged to *Penicillium* sp. (2), *Aspergillus niger* (4), *Aspergillus* sp. (2), and *Mucor* (2).

Cashewnut has been infested with *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizophus*[10]. Mould infestation of foods result in the liberation of metabolites termed 'mycotoxins' causing physiological disorders. Aflatoxin production in cashewnut by *Aspergillus flavus* and other types of moulds is of concern to the exporters since some of the importing countries have instituted limits for aflatoxin content.

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Studies on Packaging and Storage of Wheat Flour *(Atta)* under Tropical Conditions. II.

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Atta was packed in three types of polyethylene laminated bags and stored at two ASC depots located in north eastern region. Changes in moisture, sugars, diastatic activity, glutenin, gliadin, salt soluble proteins, free and bound lipids and their phosphorus and galactose content are reported. _Atta_ remained acceptable to units during entire period of storage (7 months) and the lipids which govern the baking properties of damaged wheat flours change slightly on storage indicating its wholesomeness in functional characteristics. The role of moisture and oxygen on biochemical changes that take place during storage is discussed.

Biochemical deterioration resulting from rapid moisture ingress is one of major causes of spoilage of *atta* in conventional jute bag packaging under high humid climatic conditions. In our previous communication[1] the suitability of various packaging materials available in the country including polyethylene laminated bags for storage of *atta* under high humid climatic conditions (37°C/90 per cent RH) simulated in the laboratory, was reported. It was found that *atta* can be successfully preserved up to 9 and 5 months in polyethylene/canvas and polyethylene/hessian bags respectively without appreciable damage in its biochemical and *chapati* baking characteristics. Due to hot humid climate in north eastern region, preservation of army rations in conventional jute bags is a problem under Defence storage conditions especially during monsoon season. Therefore laminated bags were tried for storage of *atta* at two Army depots located in this region. The results of storage along with the biochemical and *chapati* baking properties of stored *atta* are reported in this communication.

Materials and Methods

Packaging: *Atta* was milled from commercially available wheat at Assam Flour Mills, Gauhati and packed in 30 kg lots in bags made from the following materials:

(*a*) Polyethylene (400 gauge)/canvas; (laminate).
(*b*) Polyethylene (200 gauge)/hessian; (laminate).
(*c*) Polyethylene (200 gauge)/high density polyethylene (woven) 16 mesh; (laminate).

Storage: Four bags of each type were transported by train from Gauhati to respective trial centres and stored in ASC depots from July, 1971 to January, 1972 under ambient conditions. The climatological data for these places are indicated in Table 1.

Analysis: After four and seven months storage, two bags of each type were removed and *atta* samples were analysed for moisture, reducing and total sugar and diastatic activity by AACC methods[2]. Glutenin, gliadin and 5 per cent potassium sulphate soluble proteins were analysed by methods described by Kent-Jones and Amos[3]. Free and bound lipids and free fatty acids were determined by methods used by Daftary and Pomeranz[4]. Lipid phosphorus and galactose were estimated by methods as reported by Fiske and Subbaraw[5] and Svennerholm[6] respectively.

Organoleptic evaluation: Samples of *atta* from each bag were also issued to four army units at each place for acceptability trials of *chapaties* prepared from stored samples. Units were asked to grade *atta* samples based on colour, texture, taste and leavening characteristics of *chapaties*.

Results and Discussion

Moisture ingress and acceptability: The changes in moisture content of *atta* stored in different packaging materials are presented in Table 2. It is seen that moisture ingress was minimum in polyethylene/canvas and maximum in polyethylene/hessian bags. However, in all cases it remained below 12 per cent during the entire period of storage at both the places.

TABLE 1. CLIMATOLOGICAL DATA OF TRIAL CENTRES I AND II

Storage month	Trial Centre I				Trial Centre II			
	Temperature (°C)		Relative humidity(%)		Temperature (°C)		Relative humidity(%)	
	Mean daily		Mean of observation at		Mean daily		Mean of observation at	
	Max	Min	08.00 hr	17.00 hr	Max	Min	08.00 hr	17.00 hr
July	32	25	88	82	32	25	89	78
Aug.	32	24	89	82	32	25	90	78
Sept.	32	24	87	81	31	25	89	79
Oct.	31	22	85	80	30	22	85	77
Nov.	29	17	83	75	27	16	84	76
Dec.	26	13	86	72	24	12	91	75
Jan.	25	11	86	63	23	11	91	68

TABLE 2. CHANGES IN MOISTURE CONTENT OF *ATTA* STORED IN DIFFERENT PACKAGING MATERIALS

Packaging material	Increase in moisture content (%) at indicated months*			
	Trial Centre I		Trial Centre II	
	4 Months	7 Months	4 Months	7 Months
Polyethylene/ canvas	0.2 (0.05)	0.15 (0.02)	0.3 (0.07)	0.2 (0.03)
Polyethylene/ hessian	1.5 (0.39)	1.3 (0.19)	1.6 (0.40)	1.4 (0.20)
Polyethylene/high density polyethylene (woven)	1.2 (0.3)	0.8 (0.16)	1.3 (.032)	1.0 (0.14)

* Values in brackets indicate the moisture absorption/month calculated for 4 and 7 months period respectively; Initial moisture content, 10.4%.

At 12 per cent moisture level *atta* becomes in equilibrium with 70 per cent interspace relative humidity which has been found to be the critical limit for mold attack at 32°C[7]. This much interspace relative humidity at 32°C has been found to be safe for 6 months storage of wheat flour without noticeable damage in biochemical and baking characteristics. Rate of moisture increase per month was higher during first four months than during average of total seven months thus indicating the loss of moisture from *atta* during later three months. Decrease in ambient temperature during later months raises the interspace humidity inside the bags. This along with the decrease in ambient relative humidity leads to moisture migration from *atta* to atmosphere.

The *atta* stored in all the three types of packaging materials remained in good condition at both the places upto seven months storage. There was no evidence of cake formation and musty odour during entire period of storage. The *chapaties* prepared from stored *atta* were acceptable in taste, texture and colour to all the units. Comparatively the samples stored in polyethylene/canvas bags were rated better than the one stored in polyethylene/hessian and polyethylene/high density polyethylene (woven) bags. This is due to comparatively lower moisture level in the samples stored in polyethylene/canvas than the other packaging materials because of its lower water vapour permeability[1].

Changes in sugar and diastatic activity: The changes in reducing and non-reducing sugars and diastatic activity of *atta* stored in three types of bags are shown in Table 3. The reducing sugar increased by 40, 77 and 82 per cent at one trial centre and by 43, 85 and 80 per cent at another during seven months storage in polyethylene/canvas; polyethylene/hessian and polyethylene/high density polyethylene woven bags respectively. Non-reducing sugar decreased by 17-23 per cent and by 15-23 per cent during seven months storage in two trial centres. The diastatic activity of *atta* stored at both the places increased but percentage increases were significantly different at two places. Although the microbial data on stored samples could not be collected, it is possible that differential microbial load in stored samples may be responsible for their differential diastatic activity. The frequency of changes in temperature and humidity is also liable to alter the active surface area of the starch granules by continuous hydration and dehydration and may also be responsible for differential diastatic activity in the samples stored at these two places.

Changes in proteins and lipids: The changes in glutenin, gliadin (70 per cent alcohol soluble proteins) and 5 per cent potassium sulphate soluble proteins are presented in Table 4. As observed previously[1], the glutenin and gliadin content of stored *atta* did not change to a significant extent. There was a slight but significant decrease in salt soluble proteins of *atta* stored at both the places. The decrease was more in *atta* samples stored in polyethylene/hessian and polyethylene/high density polyethylene woven bags where the moisture content was higher. The decrease in solubility of wheat globulins and albumins may be due to their interaction with sugars and other carbonyl compounds formed by lipid peroxidation. Change in solubility of proteins by interaction with sugars and carbonyls has previously been reported[8,9]. Interaction of lipid hydroperoxides formed during storage

with proteins as suggested by Roubal and Tappel.,[10] also lowers the solubility. Pomeranz *et al.*,[11] have also reported the decreased amounts of globulins and albumins in storage damaged flours. By starch gel-electrophoresis of proteins from starch tailings they have also demonstrated the association of gliadins with starch in mold damaged flours[12]. However, there was no noticeable change in these protein fractions in *atta* stored under above conditions.

The wheat flour lipids (phospho- and galacto-lipids) play an important role in determining the baking properties of flours. Pomeranz *et al.*,[11] reported the restoration of bread baking potential of mold damaged flours by incorporating the free polar lipids from good quality flours. This is due to the ability of galacto lipids to form electrovalent and hydrophobic linkages between glutenin and gliadin on mixing with water[13].

The changes in percentage of free and bound lipids, free fatty acids, lipid phosphorus and galactose both in free and bound fractions are presented in Table 5. There was slight decrease in petroleum ether extractable lipids (free) but butanol extractable lipids (bound) remained almost constant on storage under these conditions. Earlier workers have also reported the slight decreasing tendency of petroleum ether extractables with increasing storage[11]. This has been reported to be due to the degradation of glycerides forming lower chain fatty acids and carbonyl compounds which become air-born or interact with other constituents forming petroleum ether insoluble compounds. The same has been supported by lower iodine value of petroleum ether extractables during storage indicating the oxidative breakdown of unsaturated glycerides. The free fatty acid levels in *atta* increased on storage both in free and bound lipids. But the percentage increase was more in bound lipids than in free lipids. The decrease was comparatively higher in samples having higher moisture contents. Pomeranz and coworkers[11] have reported decreased amounts of triglycerides phospho- and galacto-lipids in mold damaged wheat flours. Since under the above storage conditions, phospho- and galacto-lipids decreased to a slight extent, increased free fatty acids might have been liberated mostly from the hydrolysis of triglycerides. Slight increase in free fatty acids may also be

TABLE 3. CHANGES IN REDUCING AND NON-REDUCING SUGARS AND DIASTATIC ACTIVITY OF *ATTA* ON STORAGE IN LAMINATED BAGS

Packaging material	Trial Centre I						Trial Centre II					
	Polyethylene/ canvas		Polyethylene/ hessian		Polyethylene/ high density polyethylene (woven)		Polyethylene/ canvas		Polyethylene/ hessian		Polyethylene/ high density polyethylene (woven)	
Storage period (months)	4	7	4	7	4	7	4	7	4	7	4	7
Reducing sugar (mg maltose/ 10 g flour)	47	55	57	69	54	71	47	56	50	72	52	68
Non-reducing sugar ,,	249	233	239	195	228	219	238	219	215	175	226	211
Diastatic activity (mg maltose/10g flour/per hr)	151	170	151	176	156	161	172	209	175	213	170	209

Initial values: reducing sugar, 39; non-reducing sugar, 269; diastatic activity, 138

TABLE 4. CHANGES IN 5 PER CENT POTASSIUM SULPHATE SOLUBLE PROTEIN, GLIADIN AND GLUTENIN ON STORAGE IN LAMINATED BAGS

Packaging material	Trial Centre I						Trial Centre II					
	Polyethylene/ canvas		Polyethylene/ hessian		Polyethylene/ high density polyethylene (woven)		Polyethylene/ canvas		Polyethylene/ hessian		Polyethylene/ high density polyethylene (woven)	
Storage period (months)	4	7	4	7	4	7	4	7	4	7	4	7
Potassium sulphate soluble proteins (%)	2.10	1.97	2.03	1.84	2.08	1.86	2.04	1.96	2.06	1.83	2.03	1.88
Gliadin (70 % alcohol soluble proteins, %)	4.92	4.85	4.88	4.80	4.85	4.83	4.88	4.78	4.82	4.78	4.91	4.81
Glutenin (%)	3.96	3.91	3.87	3.80	3.78	3.87	3.90	3.36	3.82	3.83	3.85	3.83

Initial values: 5 per cent potassium sulphate soluble proteins, 2.16; gliadin, 4.86; glutenin, 3.90

TABLE 5. CHANGES IN PHOSPHORUS, GALACTOSE AND FREE FATTY ACIDS IN FREE AND BOUND LIPIDS OF *ATTA* ON STORAGE IN LAMINATED BAGS

Packaging material	Trial Centre I				Trial Centre II			
	Polyethylene/canvas		Polyethylene/hessian		Polyethylene/canvas		Polyethylene/hessian	
Storage period months	4	7	4	7	4	7	4	7
Free lipids (%)	1.43	1.34	1.24	1.26	1.33	1.31	1.28	1.24
Lipid phosphorus (%)	0.52	0.50	0.46	0.44	0.49	0.44	0.44	0.45
Lipid galactose (%)	1.10	1.03	0.96	0.98	0.97	0.90	0.93	0.91
Free fatty acids (% oleic acid)	46.5	59.9	62.2	67.3	47.2	78.0	50.9	73.3
Bound lipids (%)	0.36	0.38	0.42	0.44	0.45	0.43	0.42	0.40
Lipid phosphorus (%)	1.03	0.98	1.01	1.05	1.10	1.06	0.97	1.05
Lipid galactose (%)	3.81	3.95	3.65	3.78	4.02	4.00	3.89	3.78
Free fatty acids (% oleic acid)	31.8	37.1	34.6	44.5	34.6	42.8	39.2	...

Initial values: Free lipids 1.50; phosphorus, 0.54; galactose, 0.96 and free fatty acids, 22.9. % Bound lipids 0.40 phosphorus, 1.04; galactose, 3.55 and free fatty acids, 12.1 %.

explained due to oxidative degradation of unsaturated glycerides by scission of double bonds.

Conclusion : Atta can be successfully preserved in polyethylene/canvas, polyethylene/hessian and polyethylene/high density polyethylene woven bags up to 7 months under high-humid climatic conditions existing in Assam during monsoon months. The stored *atta* was acceptable to units during entire period of storage. The proteins and polar lipids which govern the baking potential of stored *atta* changed slightly indicating its wholesomeness in functional properties. Use of laminated bags for storage of *atta* will considerably eliminate losses due to biochemical spoilage.

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Amylose Content and Amylolytic Studies on High Yielding Varieties of Bajra (*Pennisetum typhoides*)*

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An investigation, aimed at determining the nature and relative utilization of carbohydrates of seven high yielding varieties of bajra generally grown in northern part of this country was carried out. Qualitative analysis of water-soluble carbohydrates did not indicate any difference in the general pattern. In all, spots of fructose, glucose, sucrose, maltose and two higher oligosaccharides were observed. T-55 variety showed highest value for amylose (24.58 per cent) while the amylose content of HB_1 was the lowest (18.32 per cent) in the range. The rates of in vitro digestibility using bacterial alpha-amylase were compared. The patterns of alpha-amylolysis of raw and cooked grains and isolated starches were identical.

Bajra (*Pennisetum typhoides*) is an important millet crop of the dry and semi-dry regions of northern and peninsular India. Both by acreage and grain production it ranks fourth among the cereal crops. As it constitutes a major component in the dietaries of a vast segment of the Indian population, the digestibility of the carbohydrate fraction in this millet acquires importance. As no information is yet available on the carbohydrates of bajra, the present study was undertaken to investigate the make up of the various classes of carbohydrates in some of the high yielding varieties of bajra. The susceptibility of the starch to digestion by amylase (*in vitro*) was also studied.

Materials and Methods

Samples of high yielding varieties of bajra, viz., 'HB_1', 'HB_4', 'T-55', 'HB_3', 'S-530', 'A 1/3' and 'R-559' obtained from the Bajra Breeder, Haryana Agricultural University, were ground to pass through 60 mesh. For qualitative analysis of water-soluble carbohydrates; 1 g sample was extracted twice with distilled water over boiling water bath and the filtrates were then concentrated to 5 ml. Carbohydrates in the concentrate were studied by descending paper chromatography following the techniques of Bealing and Bacon[1], Partridge[2] and Bacon and Edelman[3]. Reducing, non-reducing and total sugars and diastatic activity were determined by standard AOAC methods[4].

The total carbohydrates were determined by substracting the sum of moisture, ash, protein, ether extractives and crude fiber from 100. Starch was estimated by the method of Hassid and Elizabeth[5], while amylose was determined according to the procedure of Williams *et al*[6]. Starch was isolated by following the method of Wolf[7], as adopted for wheat.

Relative digestibility of the starch was studied by *in vitro* α-amylolysis. Known amounts (100 mg) of the samples suspended in 25 ml of 0.02 M glycerophosphate buffer (pH 6.9) were incubated with 25 mg of α-amylase (Bacterial, BDH) at 37°C. The rate of hydrolysis was followed by estimating the reducing sugars formed at 15 min intervals for 90 min. Amylolysis was done on raw and boiled flour suspensions as also on the isolated starch.

Results and Discussion

Paper chromatography of the extracts using n-butanol: acetic acid: water (4:1:5) as the solvent system revealed the presence of fructose, glucose, sucrose, maltose and two higher oligosaccharides in extracts of 'HB_1', 'T-55', 'HB_3' and 'S-530'. Fructose was absent in extracts of 'HB'_4', 'A 1/3' and 'R-559'. Varietal differences in the content of total, reducing and non-reducing sugars were found (Table 1). Total and non-reducing sugars were highest in 'A 1/3' while lowest values were found for 'R-559'. Reducing sugars were higher in 'S-530', 'HB_3' and 'A 1/3' varieties than in the others.

Amylose content was highest (24.6 per cent) in 'T-55'. Others contained 18 to 22 per cent amylose.

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

TABLE 1. SUGARS (REDUCING, NON-REDUCING AND TOTAL), DIASTATIC ACTIVITY, STARCH, AMYLOSE AND TOTAL CARBOHYDRATES CONTENT OF DIFFERENT BAJRA VARIETIES

Variety	Protein %	Total carbohydrates %	Starch %	Amylose %	Total sugars mg maltose/10g	Reducing sugars mg maltose/10g	Non-reducing sugars mg sucrose/10g	Diastatic activity mg maltose/10g
HB_1	14.3	65.4	56.3	18.3	254	44	185	338
HB_3	14.5	65.9	58.4	18.5	299	39	223	403
T-55	11.3	69.2	59.6	24.6	285	42	212	551
HB_2	13.8	66.1	61.9	19.4	270	55	185	422
S-530	8.4	71.2	63.7	18.7	288	57	200	405
A 1/3	10.3	69.4	59.2	21.4	331	54	233	435
R-559	9.4	70.3	62.5	22.6	244	42	181	474

Diastatic activity was highest in 'T-55', moderate for 'R-559', and 'A 1/3' and lowest for 'HB_1'. There was good correlation between diastatic activity and amylose content.

In vitro digestibility studies were carried out on suspensions of raw (uncooked) and cooked bajra flour and on suspensions of isolated starch from the varieties. These data are presented in Fig. 1. Amylolysis was maximum with 'T-55' and lowest for 'HB_1'. The rate of hydrolysis was significantly improved by cooking, but the differences among the varieties persisted even after cooking. *In vitro* digestion of suspensions of starches isolated from the varietes presented the same pattern as for the raw or cooked flours. The above results confirm that there are pronounced differences in the *in vitro* digestibility of the starch in different varieties of bajra. It is interesting to note that the release of sugars by *in vitro* amylolysis bear a relation to the amylose content as also to diastatic activity. Varieties with higher amylose content were therefore susceptible to faster attack by amylase. This observation is similar to the result obtained with rice varieties[8] but is at variance with the results obtained with pulses[9] and maize[10] where varieties with high amylose content had poor *in vitro* digestibility with regard to their starch.

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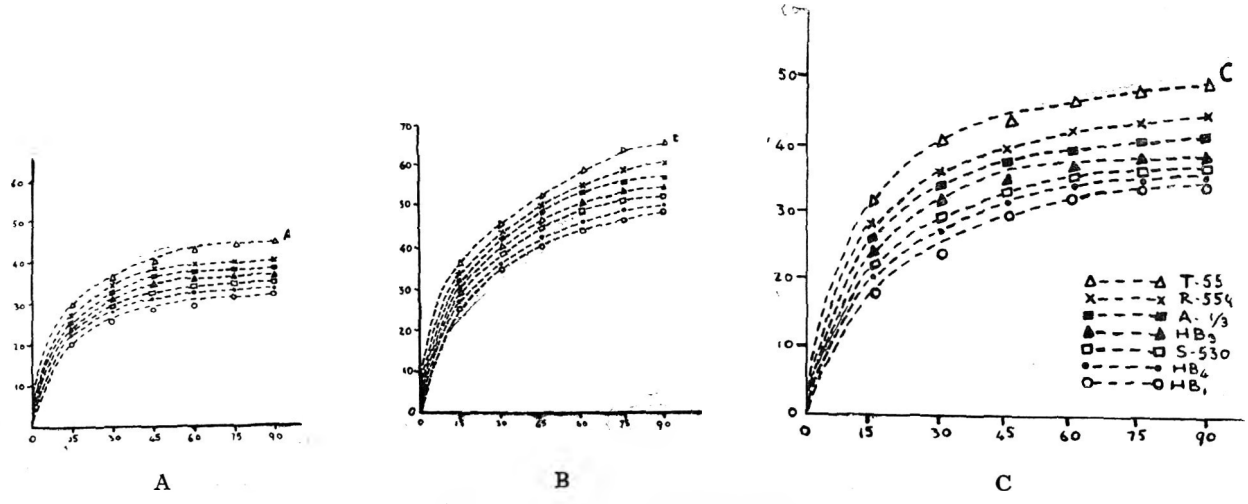

FIG. 1. Amylolytic activity of bajra
A. Raw. B. Cooked. C. Isolated starches.

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Non-permitted Colours in Food and their Toxicity

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Adulteration of day to day eatables is a serious problem. A study undertaken at this Centre revealed that, on an average, 70 per cent coloured samples examined contained non-permitted colours and only 30 per cent had permitted colours. Six commonly used colours out of 18 non-permitted dyes detected in the foodstuffs were placed in the following order on the basis of their frequency of use: metanil yellow, 29 per cent; Orange II, 11 per cent; auramine, 9 per cent; rhodamine B, 8 per cent; Blue VRS, 6 per cent and malachite green, 4 per cent. Three permitted colours used were tartrazine, 12 per cent; sunset yellow, 11 per cent and carmoisine, 5 per cent. Rest of the 5 permitted colours were sparingly incorporated in foodstuffs. From the known toxicity of various non-permitted colours, it is obvious that stringent measures should be applied to check the malpractice of colour adulteration of our common eatables.

The use of colours for making foodstuffs more attractive has been known for centuries. The consumer acceptability of any article of food is greatly influenced as much by its appearance as by its flavour and taste. Artificial colouring is also necessary in case of processed foods, canned and pulped fruits, and vegetables which often lose their natural colour during processing or storage.

At present eight synthetic dyes namely amaranth, carmoisine, erythrosine, fast red E and ponceau 4R (red), sunset yellow FCF (orange), tartrazine (yellow), and indigo carmine (blue) are permitted in India for colouration of foodstuffs. However, because of cheapness a number of non-permitted colours like auramine (yellow), blue VRS (blue), Congo red, Sudan II and III (red), malachite green (green), metanil yellow and orange II (yellow to orange) and rhodamine B (pink) are used very commonly, and this constitutes a hazard to health. From time to time the authorities have tried to enforce various regulations to check the criminal practice of food adulteration but without any appreciable success.

In the present communication the extent of use of various permitted and non-permitted colours in foodstuffs in the state of Uttar Pradesh has been studied. Food samples collected by Food Inspectors, according to the prescribed scheme (P.F.A. Rules)[1], from entire Uttar Pradesh were analysed in the laboratory of the Public Analyst to Govt. of U.P., Lucknow. Data obtained over a period of 11 years (1960-70) have been compiled, analysed and systematically categorized. The aim and objective of the study is to identify and inform the consumers of the most commonly used non-permitted colours, their known toxicity and the articles in which they are frequently used.

Materials and Methods

Extraction, separation and identification of colours: The colour(s) from various foodstuffs was extracted according to the scheme outlined in AOAC[2]. The

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analyses were carried out by adopting the multiple solvent group separation technique of Koch[3] and the final identification of individual colours was achieved by spot tests on dyed wool thread.

Classification of eatables: Since description of individual colour in each sample is not possible, the samples were divided into four broad groups as follows:

1. *Milk products:* All types of sweet preparations based on milk and milk products including *Khoya*, *chenna*, ice cream, etc.
2. *Non-milk products:* Sweets and *namkins* prepared from *maida*, *suji*, *besan* and other powdered pulses.
3. *Pulses:* Raw pulses such as *Cajanus indicus* (arhar), gram, peas, etc.
4. *Miscellaneous:* Sugar products and general confectionery, soft drinks, alcoholic and other beverages, tea, spices and condiments.

Results

A total of 12,575 coloured foodstuff samples were examined; 3,755 samples contained permitted and the remaining 8,820 non-permitted colours. The extent of adulteration and the percentage of individual colours used in the four tentative groups is as follows:

(1) *Milk products:* Out of 1,154 coloured samples of this group, 498 samples contained permitted and 656 non-permitted colours. Thus non-permitted colours were detected in 57 per cent of samples. During this period minimum adulteration of 44 per cent was recorded in the year 1967 and maximum of 84 per cent in 1962. Among the permitted colours, tartrazine was used in 25 per cent, sunset yellow in 7 per cent, carmoisine and amaranth in 5 per cent samples each. Two other permitted colours, indigo carmine and ponceau 4R, were detected in the remaining 1 per cent samples. Among non-permitted colours, metanil yellow and orange II were each found in 13.6 per cent, blue VRS in 12.6 per cent, auramine in 10 per cent, rhodamine B in 5 per cent and malachite green in 2 per cent samples (Fig. 1). Occasionally used colours were acid magenta and red 6B.

(2) *Non-milk products:* Among 6,182 coloured samples, only 1,021 had permitted colours whereas 5,161 contained non-permitted colours; average extent of adulteration was 83 per cent in this class of food products. Minimum adulteration was 76 per cent in the year 1969 and maximum 95 per cent in 1962. Of the permitted colours, tartrazine was used in 9 per cent, sunset yellow in 6.5 per cent, ponceau 4R and amaranth in 0.5 per cent each and carmoisine in 0.4 per cent samples. In non-permitted colours, metanil yellow was detected in 51 per cent, orange II in 20 per cent, auramine in 9 per cent, rhodamine B in 1.5 per cent and blue VRS in 0.8 per cent samples (Fig. 1). Rarely used non-permitted colours were butter yellow, crocein scarlet and Congo red.

(3) *Pulses:* All the 940 coloured samples of this group contained non-permitted colours. Metanil yellow was used in 97 per cent cases, and the remaining 3 per cent samples contained auramine, blue VRS, orange II and rhodamine B (Fig. 1). Maximum adulterated pulse was *Cajanus indicus* (arhar) which was coloured bright yellow with metanil yellow. In a few cases white dried peas were restored to natural refreshing green colour with the help of a yellow dye and blue VRS.

(4) *Miscellaneous:* Of 4,299 coloured samples, 2,236 had permitted and 2,063 contained non-permitted colours, average extent of adulteration being 48 per cent. Minimum and maximum adulteration with non-permitted colours were 40 and 60 per cent in the years 1963 and 1960 respectively. Permitted colours used were sunset yellow in 19 per cent, tartrazine in 17 per cent, carmoisine in 10 per cent, ponceau 4R in 3 per cent, amaranth in 2 per cent and erythrosine and indigo carmine in about 1 per cent samples. Non-permitted colours used were rhodamine B in 13 per cent, auramine in 10 per cent, blue VRS in 9 per cent, malachite green in 6 per cent, metanil yellow in 5 per cent and orange II in 4 per cent (Fig. 1). A number of other non-permitted colours such as red 6B, Sudan II, Sudan III, Congo red, brilliant crocein scarlet, methyl violet, butter yellow, acid magenta, fluorescein, naphthol yellow, nigrosine, onion yellow and inorganic pigments like lead chromate, iron oxide and copper sulphate have also been occasionally detected in this group of eatables.

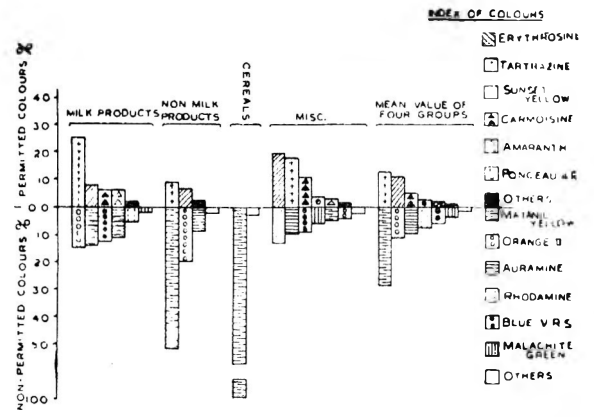

FIG. 1. FREQUENCY OF USE INDIVIDUAL PERMITTED AND NON-PERMITTED COLOURS (EXPRESSED AS % OF TOTAL PERMITTED PLUS NON-PERMITTED COLOURS) IN FOUR GROUPS OF EATABLES

TABLE 1. COLOURS IN INDIVIDUAL EATABLES OF MISCELLANEOUS GROUP

Eatables	Total number	Permitted colours	Non-permitted colours	Adulteration %
Sugar confectionery	1903	835	1068	56
Soft drinks	755	729	26	3
Ice candy	462	334	128	28
General confectionery	190	74	116	61
Tobacco (surti)	160	6	154	96
Country wine	124	116	8	6*
Chilli powder	81	...	81	100
Tomato ketchup/sauce	73	66	7	9
Turmeric	64	...	64	100
Heeng	60	2	58	97
Pan ka Masala	32	19	13	41
Saffron	15	2	13	87
Sago papar	14	1	13	93
Tea	5	5	...	...*

* These figures should be regarded as 100% adulteration because addition of colour (even permitted) in these preparation is totally prohibited.

TABLE 2. FREQUENCY OF INDIVIDUAL COLOURS

Colour	Total number	Percentage within the group (permitted or non-permitted)	Percentage of total colours (permitted and non-permitted)
Permitted			
Tartrazine	2043	38.5	12.2
Sunset yellow	1812	34.3	10.8
Carmoisine	785	14.8	4.7
Ponceau 4 R	300	5.7	1.8
Amaranth	272	5.1	1.6
Erythrosine	71	1.3	0.4
Indigo carmine	13	...	...
Fast red E	2	...	...
Non-permitted			
Metanil yellow	4833	42.3	28.9
Orange II	1903	16.6	11.4
Auramine	1581	13.8	9.5
Rhodamine B	1253	10.9	7.5
Blue VRS	1037	9.1	6.2
Malachite green	586	5.1	3.5
Others*	263	2.5	1.5

* Colours such as red 6B, Sudan II, Sudan III, Congo red, brilliant, crocein scarlet, methyl violet, butter yellow, acid magenta, fluorescein, naphthol yellow, nigrosine, onion yellow and inorganic pigments like lead chromate, copper sulphate, iron oxide, etc.

Details of the number of samples with permitted and non-permitted colours in various miscellaneous eatables are tabulated in Table 1. Metanil yellow was the major colourant for powdered turmeric and *heeng* (85 per cent each) and chewing tobacco (surti) samples (62 per cent). Colours like Sudan II and III were used in samples of powdered red chillies to the extent of 30 and 50 per cent respectively. Congo red was also detected occasionally. Permitted colours were found in majority of samples of aerated water, tomato sauce/ketchup, tea and country wines. However, it is to be noted that even permitted colours are not allowed in country wine and tea.

The total number of any individual permitted or non-permitted colour in various eatables (all the four groups combined) over a period of 11 years, in order of their frequency of use, is shown in Table 2.

Discussion

Of the 8 permitted colours, amaranth, sunset yellow and tartrazine are reported to be least harmful and kept in category *A* on the basis of toxicological classification by Joint FAO/WHO Expert Committee on Food Additives[4]. Category *A* colours are considered to be acceptable for use in food and their minimum permissible daily intake for man has been worked out. It is, therefore, suggested that any other colour, for which no specific daily intake data in man is available, should not be allowed for incorporation in foodstuffs. It will be useful if toxicological studies are repeated in all the remaining five permitted colours under Indian conditions where a normal diet is deficient in protein.

At present Government Analysts Departments undertake only the qualitative detection of colours in various eatables. However, quantitative estimation is equally important to determine the amount of colour used. It may be pointed out that even permitted colours, if taken in excessive quantity, may prove harmful. Provision should, therefore, be made for the quantitative estimation of these colours in various foodstuffs. It is advised that sugar confectionery, ice candy, etc., mainly consumed by children should have minimum amounts of even the permitted colours.

Non-permitted colours have thrived in the market on account of their cheapness. If the price of permitted colours is controlled or subsidized by the Government, and their easy availability is ensured, there is every reason to believe that foodstuff manufacturers will use only permitted colours. At the

same time, the use and production of harmless natural pigments should also be encouraged.

Despite strong legislative control measures, harmful colours are still added in most commonly used condiments and spices like powdered turmeric, red chillies and *heeng*, etc. Under these circumstances it would be safe to avoid the purchase of these ground or powdered preparations from the market. Pulses (especially arhar) should be washed with ordinary water prior to cooking since the colours used are water soluble and can be easily removed.

As the unethical practice of colouration of food materials with non-permitted dyes might expose the consumer to various health hazards, it will be pertinent to give, in short, a toxicological profile of the non-permitted colours that have been detected in food preparations.

Animal toxicity experiments revealed that commonly used non-permitted dyes such as orange II[5], auramine[6], rhodamine B[7], blue VRS[8], malachite green[9] and Sudan III[10] have produced pathological lesions in vital organs like kidney, spleen and/or liver. Metanil yellow the most popular among the non-permitted dyes, has recently been shown to cause testicular degeneration[11]. Administration of Congo red resulted in the development of hydrocephalus, hydronephrosis and ocular defects[12]. Auramine[13], rhodamine B[14] and blue VRS[4] are reported to be carcinogenic. Malachite green has been shown to cause increase in the incidence of tumours of lung, breast, overy and liver and teratogenic abnormalities of bone, eyes, skin and lung[15]. Besides organic dyes, totally prohibited inorganic pigments like lead chromate, copper sulphate and iron oxide have been used in a few cases. Lead chromate, which was mixed in powdered turmeric and also coated on the rhizomes of turmeric, is known to cause anaemia, paralysis and abortion[16].

Thus, the available experimental studies indicate that most of the commonly used non-permitted colours are toxic. More stringent measures should, therefore, be taken to check this malpractice of colour adulteration by gearing up the enforcement machinery and laboratory facilities, by the State Governments, Union Territories and local bodies. Also, in order to protect community health, the PFA act should be ammended to provide for deterrent punishment for this heinous crime.

Acknowledgement

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Changes in the Nitrogenous Constituents of Staple Foods and Feeds During Storage under Controlled Temperature and Relative Humidity. III. Changes in the Ratio of Free Glutamic Acid to Free Aspartic Acid

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Non-fat dry milk, cottonseed, peanut, and soybean meals, chickpeas, rice, and soybeans were stored for 24 months in controlled environments. Temperatures (20°, 30°, 40°C) were selected to simulate practical conditions for storage: relative humidities (40 and 60 per cent) were within a range that would not favour the growth of microorganisms. Samples were stored in cloth bags or in closed metal containers under vacuum. The ratio between the free amino acids, glutamic and aspartic (GAR) was determined by paper electrophoresis. The general decrease in the GAR is directly related to time of storge for grains and seeds, as well as for non-viable commodities such as meals and milk powder. For the storage conditions tested, the influence of temperature on the decrease of the GAR was greater than the influence of humidity. It is unlikely that the mechanism involved was one of the glutamic acids, decarboxylase, already reported. Possible mechanisms for explanation of the results are discussed.

Storage of agricultural commodities is an essential common practice. Long-term storage under practical conditions of temperature and humidity may result in the impairment of the quality of the foodstuffs stored[1-3]. Storage of grains and seeds may decrease their nutritive value[4], impair their technological qualities[5], and damage their value as seeds[6] if storage conditions are not properly controlled.

A procedure for the prediction of wheat, rice, and barley germination, based on storage time, temperature, and humidity was proposed by Roberts[7]. The changes in the pattern of the free amino acids in wheat during storage were proposed by Linke[8] as an index of the degree and type of wheat spoilage during storage. The main changes in the pattern of the free amino acids observed by Linko were the general increase in free amino acids content and an increase in the gamma-amino butyric acid at the expense of the free glutamic acid. Increase in the free amino acids content was found by Zeleny and Coleman[9] and by Fisher[10] in stored flours as well as in grains; and Jones and Gersdorff[11] showed that the increase in the free amino acids content is followed by a decrease in the trichloroacetic acid precipitated protein.

Under certain conditions, the changes in the total free amino acids may have the opposite trend. The amount of free amino acids decreases during high temperature (83°C) storage[12]. A more complicated picture may exist during developmental stages. Although Chibnall[13], regards the breakdown of storage protein, followed by increase in free aspargine and glutamine as a major process during germination, synthesis of amino acids, mainly aspartic, alanine, glycine, and lysine, was discovered by Folkers and Yemm[14], with free glutamic acid, proline, and amides serving as nitrogen source. Synthesis, during germination, of proline, arginine, and aspartic acid, when glutamic acid serves as the nitrogen source, was observed also[15]. Linko[9] suggested that more detailed information, rather than the total amount of free amino acids, would be useful in evaluation of storage damage to wheat. He proposed the ratio of free glutamic acid to free aspartic acid, along with information regarding the gamma amino butyric acid concentration, as an index for viability of wheat.

In the present study, several staple foods and feeds were stored for 24 months in controlled environments, representing possible practical climatic conditions, and

* Since deceased.

the ratio of the free glutamic acid to the free aspartic acid was determined.

Materials and Methods

The materials tested were non-fat dry milk, chickpeas, wheat, rice, soybeans, and meals of soybean, cottonseed, and peanut.

Storage equipment was composed of storage cabinets with controlled temperature and relative humidity. Storage temperatures were 20°, 30°, and 40°C; relative humidities were 40 and 60 per cent. Samples were stored in cloth bags and in closed metal containers under 25″ vacuum.

Five grams of a sample, ground to pass a 60 mesh sieve, were extracted with 50 ml of 70 per cent (w/v) ethanol, by shaking in a horizontal shaker for 1 hr. The extract was centrifuged for 15 min (Servall SS-4 centrifuge 5,500 rpm). After separation, the supernatant liquid was passed through an Amberlite IR-120 (H^+) resin column[8]. The electrophoretic separation was performed by an LKB paper electrophoresis apparatus 3276BN, during 90 min at 400 v, using 0.025 M potassium-hydrogen-pthalate (pH 4.0) as buffer. The electrophorograms were dried horizontally in an oven at 60°C, dipped in a 0.25 per cent solution of ninhydrin in acetone, heated for 10 min at 80°C, and dipped in methyl salycilate. Light absorption by the different amino acid bands were measured and plotted as the electrophorogram and scanned manually with the LKB manual densitometer. The ratio of free glutamic acid to free aspartic acid (GAR) was calculated by measuring the area of the corresponding amino acid peaks.

Results and Discussion

Results of the determined ratio of GAR are given in Table 1A and 1B. Within each set of storage conditions,

TABLE 1A. CHANGES IN THE RATIO OF FREE GLUTAMIC ACID TO FREE ASPARTIC ACID

Foodstuff	Non-fat dry milk				Cottonseed meal				Peanut meal				Chickpeas			
Initial	7.4				3.0				4.7				4.9			
Storage period (months)	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24
Storage conditions																
40% R.H.																
20°C	5.8	5.6	5.2	5.0	2.6	2.7	2.6	2.3	4.2	4.2	4.0	3.6	3.1	3.2	2.8	2.4
30°C	4.2	4.0	3.9	3.5	2.8	2.7	2.8	2.5	4.1	4.2	3.7	3.4	3.1	3.3	2.2	2.4
40°C	2.1	1.7	1.3	1.5	1.9	1.8	1.6	1.5	4.1	4.0	3.8	3.2	2.1	1.9	1.3	1.2
60% R.H.																
20°C	5.0	4.2	3.5	3.0	2.4	2.4	2.2	2.0	4.1	4.2	4.1	4.0	3.1	3.0	2.6	2.5
30°C	3.5	2.1	1.7	1.4	2.0	2.1	2.2	1.8	3.9	3.8	3.9	3.8	3.1	2.9	2.3	2.4
40°C	1.2	1.3	1.1	1.1	1.7	1.4	1.2	1.2	3.9	3.4	3.0	2.9	1.8	1.5	1.1	1.3
Cans, vacuum																
20°C	7.3	6.9	6.4	6.3	2.3	2.2	2.2	2.3	4.1	4.2	4.0	3.5	3.3	3.1	2.5	2.6
30°C	6.1	6.2	5.7	5.4	2.0	2.1	2.0	1.8	3.9	3.8	3.5	3.3	2.8	2.7	2.3	2.2
40°C	6.0	5.8	5.7	5.1	1.8	1.5	1.3	1.2	2.7	2.4	2.1	2.1	1.5	1.3	1.2	1.0

TABLE 1B. CHANGES IN THE RATIO OF FREE GLUTAMIC ACID TO FREE ASPARTIC ACID

Foodstuff	Wheat				Rice				Soybean meal				Soybeans			
At zero time	1.2				5.0				1.4				1.8			
Storage period (months)	6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24
Storage conditions																
40%R.H.																
20°C	1.2	1.1	1.1	1.1	4.6	4.5	4.3	4.1	1.4	1.3	1.3	1.4	1.7	1.7	1.5	1.4
30°C	1.1	1.2	0.9	1.1	4.6	4.5	4.2	3.8	1.4	1.4	1.3	1.2	1.6	1.4	1.4	1.2
40°C	0.9	0.8	0.7	0.4	4.2	4.1	3.7	3.4	1.4	1.5	1.3	1.2	1.7	1.5	1.5	1.4
60%R.H.																
20°C	1.1	1.1	1.0	1.0	4.9	4.7	4.4	4.1	1.5	1.4	1.5	1.3	1.8	1.7	1.4	1.6
30°C	1.0	1.1	1.1	1.0	4.9	4.6	4.0	3.2	1.4	1.4	1.3	1.4	1.7	1.7	1.4	1.4
40°C	0.9	0.7	0.7	0.3	4.1	3.9	3.3	3.1	1.4	1.5	1.4	1.1	1.5	1.6	1.7	1.2
Cans, vacuum																
20°C	1.1	1.1	0.9	0.9	5.0	4.5	4.1	4.1	1.4	1.5	1.4	1.3	1.7	1.6	1.5	1.4
30°C	0.9	1.1	1.0	1.0	4.8	4.5	3.8	3.4	1.3	1.4	1.4	1.3	1.8	1.7	1.5	1.3
40°C	0.9	0.5	0.5	0.2	4.1	3.8	3.1	2.7	1.4	1.5	1.4	1.4	1.3	1.1	1.0	0.9

TABLE 2. LEVELS OF CONFIDENCE FOR INFLUENCE OF STORAGE CONDITIONS ON CHANGES IN THE RATIO OF THE FREE GLUTAMIC ACID TO THE FREE ASPARTIC ACID

Stored material	Samples in cloth bags (A)			Samples in cloth bags (B)		Samples under vacuum (C)	
	Storage temp. °C	Time	Relative humidity	Time	Temp	Time	Temp
Non-fat dry milk	20	0.05	0.01				
	30	0.05	0.01			0.01	0.01
	40	0.05	0.01				
Cottonseed meal	20	0.05	...				
	30	0.05	0.05	0.05	...	0.05	0.01
	40	0.01	...				
Peanut meal	20	0.05	...				
	30	0.05	...	0.05	...	0.01	0.01
	40	0.01	0.05				
Chickpeas	20	0.01	...				
	30	0.01	...	0.01	0.01	0.01	0.01
	40	0.01	...				
Wheat	20	...	...				
	30	...	...			0.01	0.01
	40	0.01	...				
Rice	20	0.05	...				
	30	0.01	...	0.01	0.01	0.01	0.05
	40	0.01	0.05				
Soybean meal	20	...	...				
	30	...	...	...	...	...	...
	40	...	...				
Soybeans	20	...	...				
	30	...	...			0.05	0.01
	40	0.01	0.05				

(A) Check for significant differences as a result of storage time and relative humidity in each storage temperature level.

(B) Check for significant difference as a result of storage temperature level in cases where changes were not found to be attributed to relative humidity levels.

(C) Levels of significance for the influence of time and temperature during storage of samples in closed containers.

moisture content of samples stored in cloth bags remained nearly constant throughout the storage period[1]. Moisture content of these samples, however, differed among the different storage conditions, varying directly with relative humidity and inversely with temperature[1]. The existence of a gradient of moisture content, as a result of higher storage temperature, was taken into consideration in the design of the statistical analysis[1]. Table 2 gives the confidence levels for the influence of storage time, temperature, and relative humidity on the changes in the GAR measured throughout the storage period.

A significant decrease in the GAR was noticed in the case of non-fat dry milk (NFDM) stored for six months in cloth bags at 40 and 60 per cent RH. Under similar conditions there was a significant decrease in case of the other foods also except for soybean meal. In the case of wheat and soybean the decrease was not significant. Under these conditions of storage the decrease of the GAR with increasing temperature (30° and 40°C) was significant in the case of NFDM and cottonseed meal. The GAR at 40°C was significantly lower than at 20°C in almost all the cases. With storage above 6 months there was a significant and progressive decrease in the case of NFDM and rice. Even in other cases there has been a similar tendency.

In the case of cans kept in vaccum, there has been a significant decrease in the ratio over the first six months, the decrease being more as the temperature of storage increased. In the case of rice and soybean meal the decrease is significant at 30° and 40°C and not at 20°C. In the case of soybeans the decrease is significant only at 40°C. In the case of NFDM, cottonseed meal, peanut meal and chick pea the progressive decrease during storage at all temperatures was significant.

In order to understand the changes that take place in the free amino acids of the stored commodities, it is necessary to mention several mechanisms which are known to influence this system.

The reaction of amino acids with carbonyl compounds is well summarized in many reviews[16]. The reaction was studied in numerous model systems as well as in many native and processed food products. Storage studies of grains and milled products showed that this reaction may take place under unfavourable storage conditions. Germ discolouration followed by reduced germination as well as the decrease in the free ϵ-NH_2 groups of lysine were attributed to the reaction between carbonyl compounds and free amino groups in free and in protein-incorporated amino acids[1,17-19]. The general effect of these deteriorative changes on the pattern of the free amino acids would result in a decrease in the free amino acids content[20,21]. In addition to the non-enzymatic browning reaction, under certain storage conditions, or at certain developmental stages in the seed, the total amino acids content may increase as a result of an enzymatic breakdown of the proteins[22].

Glutamic acid decarboxylase activity in the extracted germs of wheat, rice, and corn tested on glutamic acid solutions[23-25] or measurement of the glutamic acid content, compared with aspartic acid content, with information concerning the advancement of the decarboxylation process and possible proteolysis, as represented by measurement of the gamma-aminobutyric acid, were correlated with storage damage as measured by germination[8,22]. Although the results

reported in the present paper show the decrease in the ratio of free glutamic acid to free aspartic acid, it seems unlikely for several reasons that the observed changes were a result of an enzymatic activity. A certain degree of heat treatment was incorporated in preparation of the cottonseed and peanut meals, and in preparation of the milk powder. Although heat treatment is known to reduce enzymatic activity by enzyme denaturation, the decrease in the GAR in the mentioned commodities was not smaller than in the non-treated ones. The moisture content which activates the glutamic acid decarboxylase was found to be about 15 to 18 per cent[26]. The moisture content of the stored foodstuffs ranged from 3 per cent, in case of the milk powder, up to 12 per cent in rice, which is much lower than that required to activate the decarboxylase. In addition, only traces of gamma-amino-butyric acid were found in the study. An earlier report[27] confirmed that with the advancement of storage time there is a decrease in the activity of the glutamic acid decarboxylase in the stored grain samples.

Table 3 shows the result of heat treatment on the ratio of the free glutamic acid to the free aspartic acid. The decrease in the GAR in this experiment cannot be explained on the basis of an enzymatic reaction. The possibility of a general decrease in the free amino acids content as a result of the aldehyde-amino condensation reaction was mentioned before. The bigger decrease in the free glutamic acid, as compared with the free aspartic acid, which is expressed by the decrease in the GAR must be explained by higher reactivity in the aldehyde-amine reactions of the glutamic acid as compared with the aspartic acid. This fact was established in 1921 by Grunhut and Weber.[28]

The decrease in the GAR is generally greater than the decrease in available lysine.[1] This decrease in the available lysine represents the number of ε-amino groups of lysine that are blocked by carbonyl compounds such as reducing sugars and fat oxidation breakdown products. Thus, another reaction that might lead to a decrease in the free glutamic acid is the formation of a Lactam—the pyrolidone carboxylic acid (II)—as a result of the cyclization of the glutamic acid, (Fig. 1).[29] Both glutamic acid and glutamine can

TABLE 3. INFLUENCE OF HEAT TREATMENT* ON THE RATIO OF THE FREE GLUTAMIC ACID TO THE FREE ASPARTIC ACID IN THE VARIOUS FOODSTUFFS

	Non-fat dry milk	Cottonseed meal	Peanut meal	Chickpeas	Wheat	Rice	Soybean meal†
Before heat treatment	7.4	3.0	4.7	4.9	1.2	5.0	1.9
60 min. heat treatment	1.4	2.0	4.2	3.8	0.7	4.3	1.5
120 min. heat treatment	1.3	1.2	3.1	2.0	0.4	2.7	1.2

* 120°C, oven heating
† Untoasted, native soybean meal.

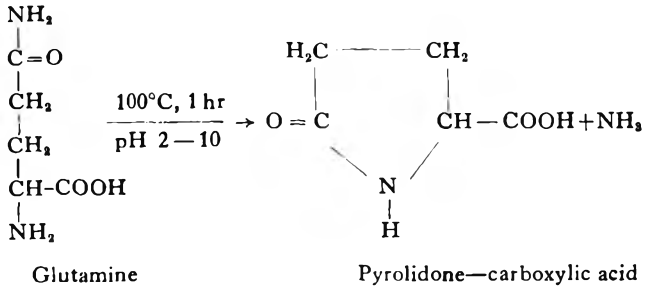

FIG. 1. Cyclization of glutamine by heat treatment

go through this cyclization process[29] and although it advances rapidly only at temperatures above 100°C,[26] it does take place at lower storage temperatures, provided the storage time is long enough.[30] The formation of the lactam causes a decrease in the GAR since its mobility differs from that of glutamic acid, and thus the lactam travels separately (in the neutral amino acids group) on the electrophorogram, and since a much higher energy level is required for a similar process in the case of the free aspartic acid.[31]

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EFFECT OF PROCESSING ON ASCORBIGEN IN CABBAGE (*BRASSICA OLERACEA*)

Ascorbigen disappears when cabbage is subjected to dehydration, canning or fermentation.

Cabbage is one of the important sources of ascorbigen,[1] the combined form of ascorbic acid. Whereas changes in free ascorbic acid during processing of cabbage have been studied,[2-4] there is no information with regard to the effect of processing on ascorbigen. In this communication, we report the total disappearance of ascorbigen when cabbage was dehydrated, canned or subjected to fermentation for making sauerkraut.

Commercial variety of cabbage available in the local market was used in all the experiments. The outer leaves were removed, cabbages were cut into quarters, the cores removed and then shredded in a Stephan universal machine. The shredded cabbage was used for dehydration, canning or for making sauerkraut as indicated below. These shreds were placed on stainless steel trays and blanched in steam for 3 min at atmospheric pressure, sulfited in 0.2 per cent potassium metabisulfite for 10 min (1000 ppm SO_2) and then dehydrated in a cabinet drier at 60°C for 4-5 hr to a final moisture content of 14.9 per cent. The dried cabbage was kept in sealed polythene bags until used for analysis. For canning, the shreds were blanched in steam for one min at atmospheric pressure, filled into A2½ cans (401×411), covered with 2 per cent brine, exhausted in steam to a can centre temperature of 79.4°C sealed and processed for 30 min at 115°C and cooled. The contents of each can were homogenised and then used for analysis. For preparing sauerkraut, the shreds were thoroughly washed in water and drained completely. Salt was then added at 2.25 per cent level and mixed well. The shreds were tightly filled into glass jars, covered by polyethylene sheets and pressure applied by putting a rubber stopper followed by a screw cap. Fermentation was allowed to proceed at room temperature (25°C). The contents of each jar were homogenised and then used for determining ascorbigen.

Moisture was determined by drying the sample in an oven at 105°C. Acidity was determined according to the AOAC procedure[5]. Ascorbigen was estimated according to the method of Bose *et al.*[1], and also with the modification that the 2, 4-dinitrophenylhydrazine (DNPH) method was used for estimating ascorbic acid.[6] The results are shown in Table 1.

TABLE 1. EFFECT OF DEHYDRATION, CANNING AND SAUERKRAUT FERMENTATION ON ASCORBIGEN IN CABBAGE

Cabbage sample	Free reducing substances	Total reducing substances	Ascorbigen (mg/100g dry matter)
Raw	350.1	386.5	36.4
Dehydrated	16.0	16.0	0
Raw	351.2	369.2	18.0
Steam Blanched 1 min	324.6	335.5	10.9*
Canned	123.6	104.8	— ve
Raw	258.6	267.9	9.3
Sauerkraut 1 week	557.5	535.0	— ve
,, 2 week	490.0	399.1	— ve

* Determined by dye titration

With the exception of sauerkraut, data obtained by the dye titration procedure agreed closely with that from the DNPH procedure. On boiling sauerkraut with 5 per cent metaphosphoric acid, a pink colour was formed rendering it difficult to judge the end point by dye titration. In view of this and the greater specificity of the DNPH procedure, only data obtained by this method is presented. Changes in pH and acidity during fermentation indicated that it was proceeding normally and was similar to published data.[7] It is seen that ascorbigen disappeared when cabbage was subjected to dehydration, canning or fermentation. Ascorbigen is known to be hydrolysed during cooking; a similar condition would be prevalent during blanching or canning and would account for the decrease on blanching and for the absence of ascorbigen in the canned product. Although the temperature during dehydration was only 60°C, the prolonged time of heating in addition to sulphitation may explain the absence of ascorbigen in dehydrated cabbage. Ascorbigen decreased even during fermentation. For determining ascorbigen, a pH of less than 1 (5 per cent metaphosphoric acid, final concentration) and hydrolysis in boiling water are necessary. It is therefore surprising that ascorbigen seems to be hydrolysed

even at a pH of 3.5-4.5 during fermentation. This raises the question regarding the possibility of hydrolysis of ascorbigen by factors other than the pH of the medium during fermentation. This possibility is to be ascertained by further investigation.

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A STUDY OF IDENTIFICATION OF PAPAYA SEEDS IN PEPPER

A paper chromatographic method was found suitable for the differentiation of the seeds.

Due to similarity in physical appearance papaya seeds (*Carcia papaya*) are used to adulterate pepper (*Piper nigrum*)[1]. Based on our studies on phenolic compounds it was possible to devise a method to detect papaya seeds in pepper on the paper chromatogram, depending on the difference in Rf and colour of various spots with aluminium trichloride under ultra-violet light.

About 5 g each of finely powdered and defatted pepper, papaya and a mixture of these two were extracted with 100 ml of hot 70 per cent ethanol, cooled and filtered. The filtered extracts after concentrating to dryness and removing residual fat were re-extracted with 10 ml of 10 per cent iso-propanol, concentrated to about 4 ml and filtered.

About 50 microlitres of each filtrate was separated on a Whatman No. 1 filter paper by descending chromatographic technique overnight, using *n*-butanol-acetic acid-water (12:3:5) as developing solvent. The chromatogram after drying was sprayed with 5 per cent aluminium trichloride solution in ethanol[2], air dried, and observed under UV light.

The chromatographic pattern and UV characteristics of phenolic compounds with Rf values of pepper and papaya seeds are given in Table 1.

TABLE 1

CHROMATOGRAPHIC AND UV CHARACTERISTICS OF PAPAYA AND PEPPER SEEDS

Sample	Rf of characteristic spots	Colour with $AlCl_3$ under UV light
Pepper	0.75	Greenish yellow
	0.62	Yellow
	0.52	Intense yellow
Papaya seeds	0.15	Bluish white fluorescence

The method can conveniently be used to detect papaya seeds in pepper.

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INFLUENCE OF SODIUM CHLORIDE AND SODIUM CARBONATE ON THE DISTRIBUTION OF FLOUR COMPONENTS IN BLACKGRAM (*PHASEOLUS MUNGO*) *PAPAD*

Influence of the commonly used additives, sodium chloride and sodium carbonate on the distribution of flour components in blackgram (*Phaseolus mungo*) *papad* has been studied. Histological picture of the *papad* indicates that sodium chloride helps in uniform distribution of the flour components in the *papad*. Frying characteristics of *papad* show that sodium chloride brings about significant lateral expansion, while sodium carbonate has no effect. The beneficial effect of sodium chloride could not be correlated with the solubility of blackgram proteins.

In an earlier communication the role of common salt and *papad khar*, the two additives commonly used in the preparation of blackgram (*Phaseolus mungo*) *papad* was reported.[1] Of the two additives, common salt was found necessary for the lateral expansion of *papad* during frying while sodium carbonate, the main component of *papad khar* was beneficial to overcome brown patches in fried *papads*. The manner in which sodium chloride (as common salt) and sodium carbonate function to impart the desirable attributes of expansion and colour to the *papad* is not known.

In the present communication the histological changes effected by these two additives with respect to

the distribution of flour components in *papad* are presented.

The *papads* were prepared as described earlier[1] using a recipe consisting of 100 g blackgram flour and 40 ml water; 10 g of sodium chloride and/or 1 g sodium carbonate being included in the recipe when required.

The *papads* were cut into small bits, dehydrated in graded alcohol, cleared in xylol and embeded in paraffin. Lateral sections were taken at 10 μ thikness and were stained for proteins, starch and mucilage by the Ferricyanide[2], Periodate-Schiff reaction[3] and Alcian Blue[4] methods respectively. The lateral expansion of *papads* as a result of frying was measured as described by Shurpalekar, *et al.*[5] The solubility of proteins was estimated as follows: 5 g blackgram flour was dispersed in 100 ml of the extracting solution and after shaking for 30 min the dispersion was centrifuged at 2700 rpm for 15 min in an MSE centrifuge. Protein in an aliquot of the supernatent was then estimated by micro-Kjeldhal method.

Distribution of flour components: The distribution of flour components viz., proteins, mucilage and starch in sections of *papads* made from flour alone, flour with 1 per cent sodium carbonate, flour with 10 per cent sodium chloride as compared to that in a commercial sample is shown in Fig. 1, 2 and 3 respectively. The microscopic structure of *papad* made from flour with 10 per cent sodium chloride and 1 per cent sodium carbonate was similar to that of the *papad* made from flour and 10 per cent sodium chloride and hence is not presented.

The proteins in sections of *papad* made from flour alone appear as clumps (stained black) as seen in Fig. 1 (*a*) indicating uneven distribution. Inclusion of 1 per cent sodium carbonate in the recipe did not bring about significant change in protein distribution as seen in Fig. 1 (*b*). However, on including sodium chloride the distribution became uniform as shown in Fig. 1 (*c*). This compares well with the commercial *papad* which contains both sodium carbonate and sodium chloride.

The distribution of mucilage and starch followed a trend similar to that of proteins (*i*) being uneven in *papad* made from flour alone, (*ii*) showing little improvement with the addition of sodium carbonate, and (*iii*) becoming uniform with the inclusion of sodium chloride in the recipe.

Lateral expansion in papad: Data in Table 1 show that the lateral expansion on frying is only 20 per cent in *papad* made from flour alone or the *papad* containing sodium carbonate. However, with the inclusion of sodium chloride in the recipe, the expansion increased considerably to 44 per cent on frying. Addition of sodium carbonate along with sodium chloride did not bring about any further increase in expansion.

TABLE 1. EFFECT OF SODIUM CHLORIDE AND SODIUM CARBONATE ON THE LATERAL EXPANSION IN *PAPAD* AFTER FRYING

Dough ingredients	Diameter of *Papad* (cm)		Diametrical expansion %
	Before frying (a)	After frying (b)	$\frac{b-a}{a} \times 100$
Flour alone	11.4	13.6	20
Flour+1% sodium carbonate	11.5	13.8	20
Flour+10% sodium chloride	11.9	17.1	44
Flour+10% sodium chloride+1% sodium carbonate	11.4	16.7	46
A commercial sample	11.4	16.9	48

TABLE 2. SOLUBILITY OF BLACKGRAM PROTEINS IN SODIUM CHLORIDE AND SODIUM CARBONATE SOLUTIONS

Medium of extraction	Solubility* of proteins %
Water	39.6
Sodium carbonate 1%	91.5
Sodium chloride 5%	91.7
Sodium carbonate 1%+sodium chloride 5%	85.3

* Total proteins (N×6.25) in blackgram flour is 25%

These results corroborate well with the histological observation indicating that uniform distribution of components in *papad* is essential for obtaining the flour desirable lateral expansion (which is one of the indices of quality[2]).

The manner in which sodium chloride imparts the desirable quality of lateral expansion is not clear. The data in Table 2 show that there is no significant difference in solubility of the proteins of blackgram in the solutions of sodium chloride, sodium carbonate and a mixture of the two. The expansion characteristics of the *papad* cannot therefore be attributed to the solubility behaviour of the proteins of the blackgram.

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A. PAUL JAYARAJ

23 June 1972

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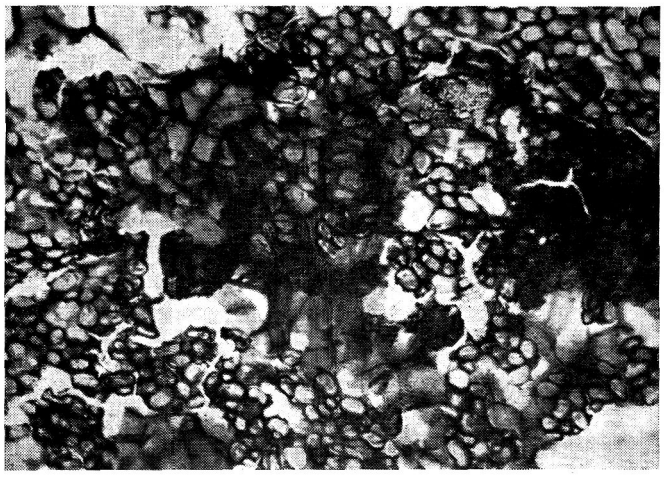

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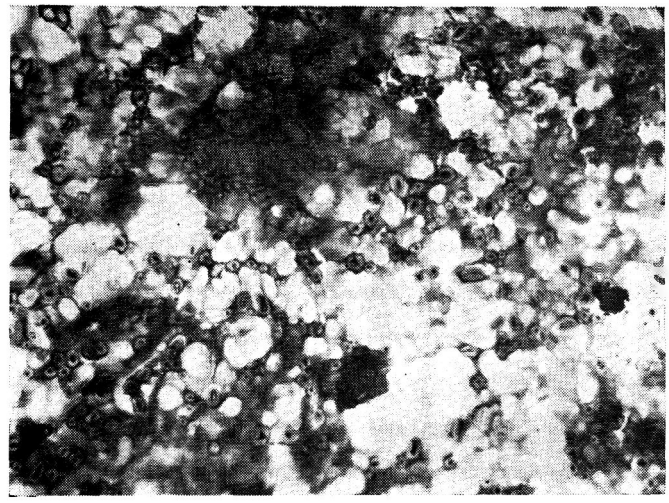

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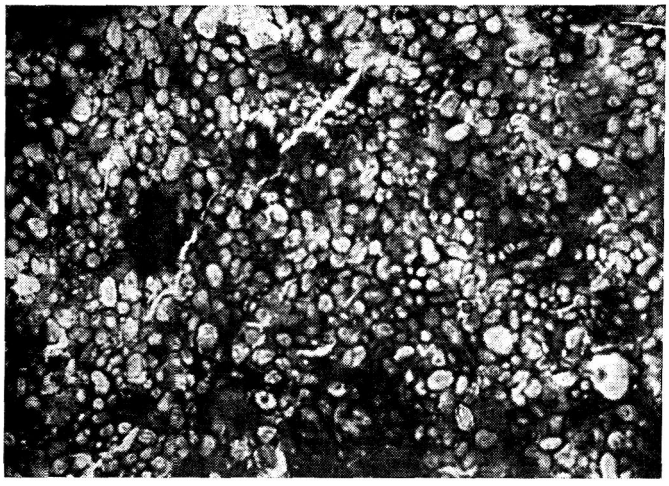

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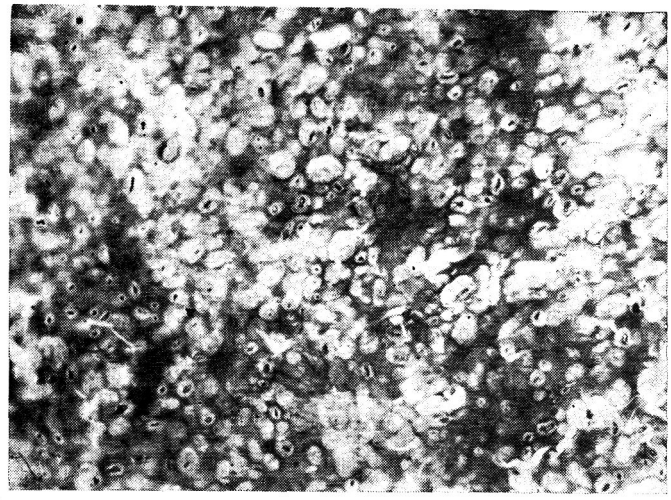

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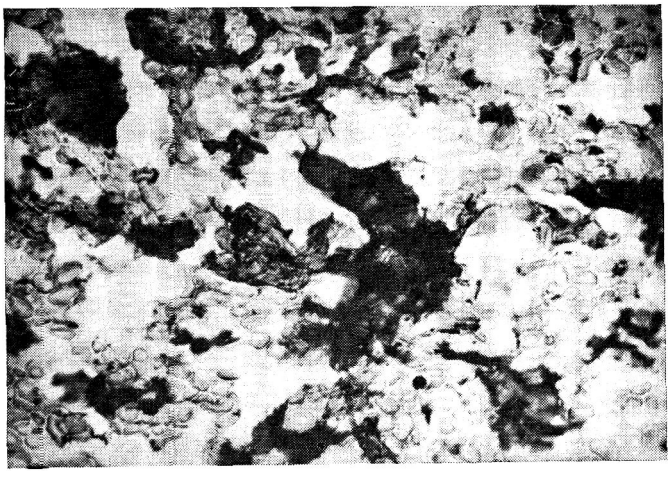
FIG. 1. Photomicrographs of sections of *papad* stained for proteins × 200. Ingredients of *papad:* (a) flour, (b) flour + 1% sodium carbonate, (c) flour + 10% sodium chloride, (d) A commercial sample.



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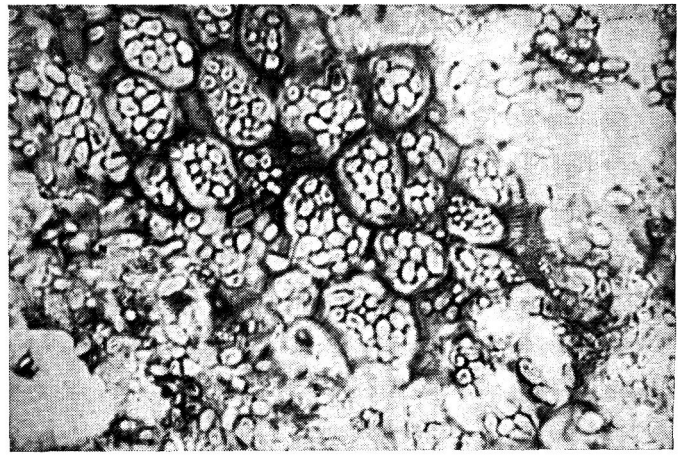

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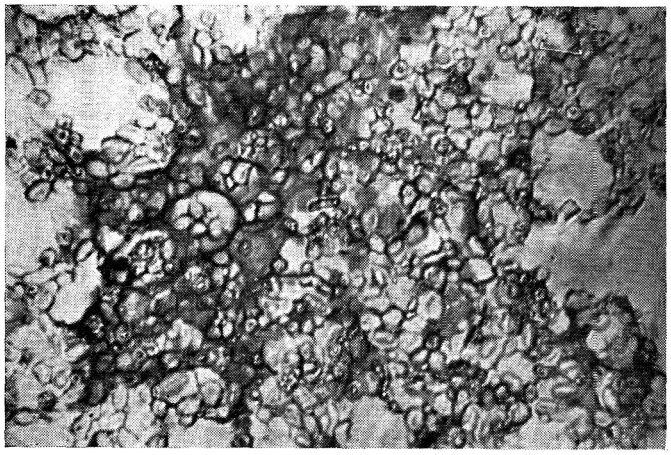

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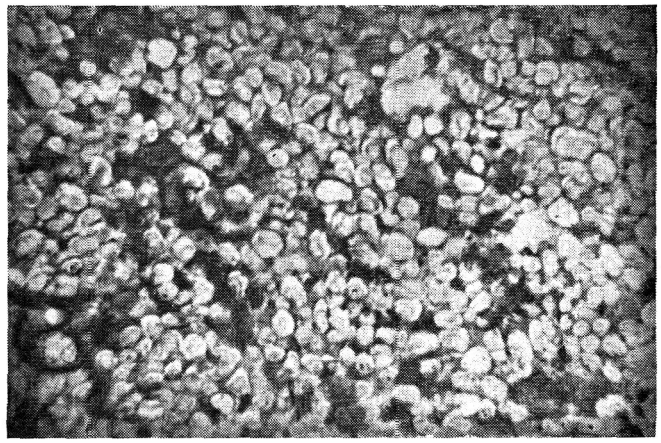

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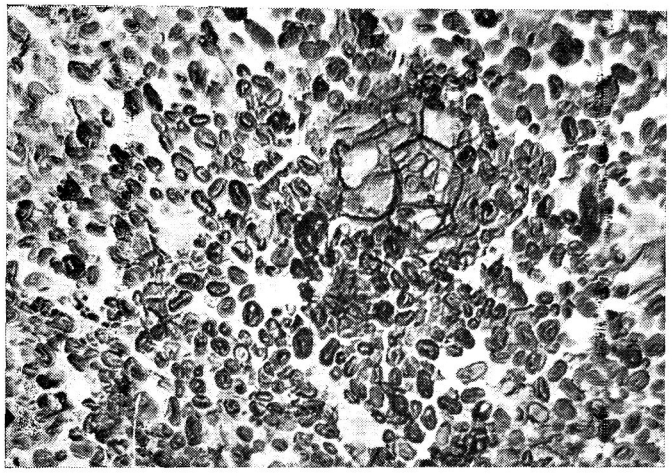
FIG. 2. Photomicrographs of section of *papad* stained for mucilage×200. Ingredients of *papad:* (a) flour, (b) flour+1% sodium carbonate, (c) flour+10% sodium chloride, (d) A commercial sample.



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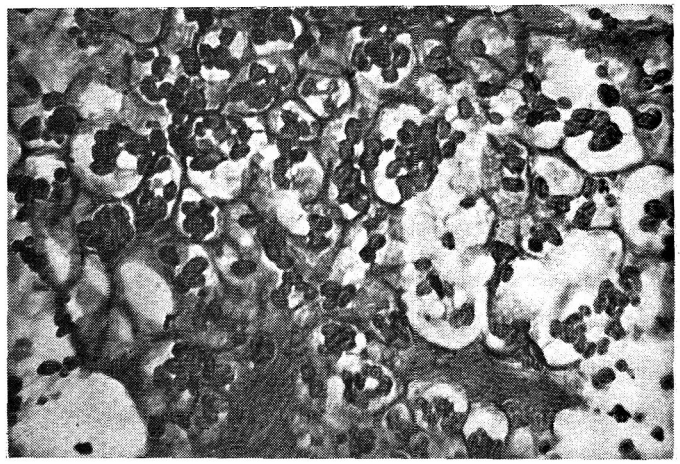

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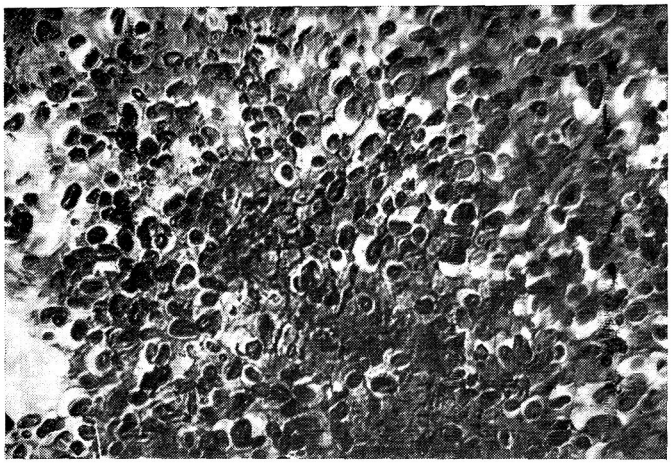

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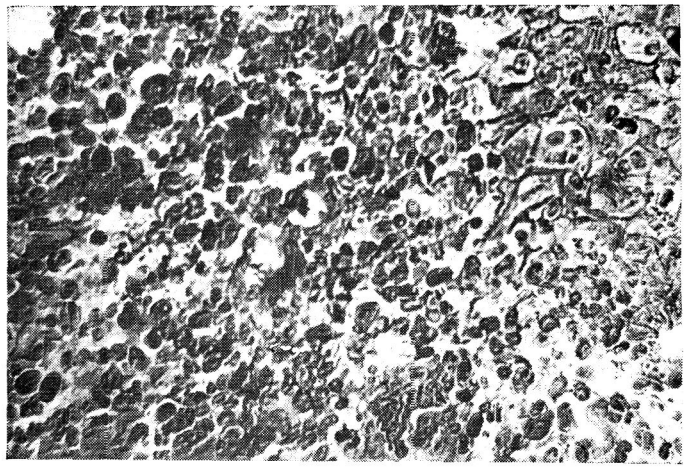

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FIG. 3. Photomicrographs of sections of *papad* stained for starch×200. Ingredients of *papad:* (a) flour, (b) flour+1% sodium carbonate, (c) flour+10% sodium chloride, (d) A commercial sample.

A SIMPLE AND RAPID METHOD FOR ESTIMATION OF TOTAL CAROTENOID PIGMENTS IN MANGO

A direct colorimetric method developed.

Mango is a rich source of carotenoid pigments which have been studied by a number of workers[1-3]. Ramasarma *et al.*[4], reported that the epiphasic pigment isolated from 'Badami' mango consists almost entirely of β-carotene, which is of particular importance from the point of view of nutrition. The estimation of carotene in mango takes a long time and it is not possible to analyse a large number of samples in a day. A rapid method for routine estimation of total carotenoid pigments is essential for mango particularly for comparative studies e.g., variety, effect of manurial trial or other growing conditions, selection for breeding, stability in processing, storage, etc. Booth[5] reported a simplified procedure for estimation of total carotenoids in carrots. An attempt has been made to estimate the total carotenoid pigments in mango by applying Booth's technique with a little modification.

The fat soluble pigments were extracted from the fresh mango pulp (1 g) with 3:2 petroleum ether (60°-80°C)-acetone mixture by grinding with sand in a 50 ml silica dish with a glass mortar. The extracts were decanted into a 50 ml volumetric flask. The extraction was continued till all fat soluble pigments were taken out. Four to five extractions were found to be enough. The volume was adjusted to 50 ml and reading was taken in a Spectronic-20 at 450 nm; the result was expressed in terms of β-carotene. Potassium dichromate was used as standard in absence of standard β-carotene[6].

The total carotenoid pigments of the five varieties viz., 'Dashehari', 'Langra', 'Chausa', 'Bombay Green' and 'Safeda' were determined by this method and also by the method prescribed by the Association of Vitamin Chemists[7] (Table 1). It was observed that 'Bombay Green' contained maximum carotenoids followed by 'Dashehari,' 'Langra', 'Chausa' and 'Safeda'. Similar trend was reported by Sadana and Ahmad[3] with 'Dashehari', 'Langra' (Calcutta) and 'Chausa'. It appears from the Table that the method adopted here had given in general a slightly higher value of carotenoid pigments compared to the method of the Association of Vitamin Chemists, maxium being in 'Safeda', i.e., 4.2 per cent. This may possibly be so because, in the present method, the extract contained not only acetone but also a small quantity of water, whereas according to the Association of Vitamin Chemists the acetone is washed away and the pigment is taken mainly in petroleum ether layer. The method adopted from Booth[5] for estimating total carotenoid pigments in mango is simple and rapid; about 5 to 6 samples can be analysed per hour depending on the efficiency of the worker. The results obtained from this method are also consistent.

TABLE 1. TOTAL CAROTENOID PIGMENTS IN SOME OF THE VARIETIES OF MANGO

Varieties	Total carotenoid pigments expressed as β-carotene		Increase from method 1 to 2. %
	Method-1 μg/100g	Method-2 μg/100g	
Dashehari	6691	6885	2.9
Langra	4035	4187	3.7
Chausa	3087	3216	4.1
Bombay Green	8136	8352	2.6
Safeda	2331	2439	4.2

Method 1: Approved by the Association of Vitamin Chemists.[7]
Method 2: Simplified method based on Booth[5].

Author is thankful to Dr R. N. Singh, Head, Division of Horticulture and Fruit Technology for his keen interest in the study.

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16 November 1972

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BACTERIOLOGICAL QUALITY OF MARKET MILK IN POONA

The bacteriological quality of milk available in Poona City from different sources was evaluated. 220 samples of raw and pasteurised milk samples were examined for standard plate count, coliforms, M.B. Reduction and phosphatase. The milk supplied by village vendors to the city was found to be of poorest quality. The raw milk collected by Government [Milk Scheme,], Poona, was not of standard quality, coliform being detected even in pasteurised milk samples.

Indian Standards Institution[1] has prescribed the bacteriological standards for both raw and pasteurised milk. Surveys of bacteriological quality of milk has

TABLE 1. QUALITY OF MILK SAMPLES COLLECTED FROM GOVERNMENT MILK SCHEME, POONA

Test	Raw milk			Pasteurised milk		
	Average	Minimum	Maximum	Average	Minimum	Maximum
SPC/ml.	126.66×10^5	80×10^5	300×10^5	36.62×10^3	7×10^3	80×10^3
Coliforms in 0.1 ml.	1223.56	6	1800	64.84	0	1600
MBR test	12 min	5 min	30 min	2.1/2 hr	45 min	> 4 hr
Phosphatase test		+ ve			—ve	

TABLE 2. QUALITY OF MILK SAMPLES COLLECTED FROM SHOP KEEPERS

Test	Raw milk			Boiled milk		
	Average	Minimum	Maximum	Average	Minimum	Maximum
SPC/ml	52.50×10^5	45×10^5	60×10^5	18×10^3	7×10^3	35×10^3
Coliforms in 0.1 ml	>1800	—	—	91	21	240
MBR test	45 min	30 min	1 hr	2.1/2 hr	2 hr	2.1/2 hr

been made in different cities of India by a number of workers[2-8].

In the present study, 170 samples of raw milk and 50 samples of pasteurised milk supplied to Poona city were collected and examined. Out of 170 raw milk samples, 50 were collected from the rural collection of Government Milk Scheme, Poona. 50 samples were obtained from retail vendors and 70 were collected from shop-keepers. The pasteurised samples were all obtained from Government Milk Scheme, Poona.

Samples of milk were collected carefully in sterile 500 ml capacity stoppered narrow mouth Mac-Cartny bottles.

Following standard procedures[9], the collected samples were examined immediately for standard plate count (SPC); coliforms, methylene blue reduction test and phosphatase test in case of pasteurised milk.

The results of bacteriological examination of milk samples collected from different sources are presented in Table 1, 2 and 3.

Government Milk Scheme, Poona: The average counts were much higher than minimum standards prescribed by I.S.I.[1] Phosphatase test was negative for all pasteurised samples, positive for raw milk.

The reason for raw milk not conforming to prescribed standards is that it is accepted at the collection centers in rural areas only on the basis of butterfat. The holding of milk at the collection centers in unclean containers at atmospheric temperature for considerably long time may probably increase the bacterial counts in the raw milk samples.

TABLE 3. QUALITY OF MILK SAMPLES COLLECTED FROM RETAIL VENDORS

Test	Raw milk		
	Average	Minimum	Maximum
Coliforms in 0.1 ml	>1800	17	>1800
MBR test	34 min	1 min	2 hr

Samples collected from retail vendors: The milk supplied by village retail vendors to the city was found to be of poorest quality. The average value for coliform count was more than 1800 in 0.1 ml of milk and average time of MBR was 34 min. The vendors bring milk in utensils which are not properly cleaned and this is largely responsible for the high bacterial counts.

The samples from shop-keepers were also of poor quality.

The authors are grateful to Dr V. N. Rao, Jt. Director of Health Services for permission to publish this paper.

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17 April 1972

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I, A. G. Mathew, hereby declare that the particulars given above are true to the best of my knowledge and belief.

A. G. Mathew

Signature of Publisher

Directory of Indian Processed Food and Allied Industries: Published by Central Food Technological Research Institute, Mysore, India, 1972, pp. 992. Price: Rs 50.

This is a Directory of Indian Processed Food and Allied Industries giving voluminous information on Central Government Departments, Research Organizations, Training Institutions, Export Promotion Agencies, Trade Associations, Processed Food, Milling and Allied Industries, Fabricators/Suppliers of Food Machinery and Equipment, Manufacturers/Formulators in Insecticides and Pesticides, Exporters, Indian Food Laws and Specifications and Indian Standards for Food and Allied Products. It is a very commendable and useful effort by the Central Food Technological Research Institute, Mysore and fills a long felt need of the food industry and the general public.

In a compilation work of this type some omissions are bound to be there and the compilers have rightly requested to provide information about these for inclusion in the subsequent editions. A few important omissions may be mentioned herein: Defence Food Research Laboratory, Mysore 10, under Research Organization, Protinules (Alembic), Provitex (TCF-Rallis), Protinex (Dumex), Uniprotein Briskies (Unichem), Probisk (Britannia), Protamin (MPF food by JB Protein Food Industries, Sitapur) under Processed Protein Foods and Bedekar's under Condiments and Masala Powders.

Information on slaughter houses, and poultry dressing plants could have been included. It may be more useful if manufacturers of food grade chemicals and pesticides are indicated separately by putting an 'asterisk' (*) against one of these categories.

Binding, general get up and printing of the book are good. Appendices at the end covering CFTRI work, selected periodicals in Food Science and Technology and index to advertisers are useful.

The Directory will be a useful reference book for Food Industries, Government Departments, Research Organizations and Agricultural Universities.

B. S. BHATIA

Ice Cream: by W. S. ARBUCKLE. The AVI Publishing Co., Inc., Westport, Connecticut, 1972, pp. 474. Price: Domestic $19, Foreign $20.

With more and more advancement in the field of Food Technology, it has become necessary that one should get himself acquainted thoroughly with a particular type of product. The book under review would be helpful in serving this purpose for those who want to specialise in science and technology of ice cream and related products. This second edition is a complete revision of the first revised edition. Many unwanted and obsolete subjects have been removed and other portions have been expanded to include recent developments and additional information for the use of students, research workers, personnel in industry and prospective entrepreneurs.

By and large this is a complete book but for those who want to go for further details and background of the subject, a bibliography is given at the end of each chapter. For convenience, this publication can be divided into five broad sections. In the first Section chapters on (1) Development of the Ice Cream Industry; (2) Energy Value and Nutrients of Ice Cream; (3) Ice Cream and Related Product Classification; and (4) Composition and Properties can be included. This section gives preliminary knowledge about ice cream and related products—nutritive value, physico-chemical characteristics and product classification.

The second Section includes chapters on (1) Ice Cream Ingredients; (2) Stabilizers and Emulsifiers; (3) Flavouring and Colouring Materials; (4) Soft-frozen Dairy Products and Special Formula; (5) Sherbets and Ices; (6) Novelties, Fancy Molded Ice Creams and Specials. This enables one to develop suitable recipes using the right type of ingredients.

The third Section is meant for those who, having acquainted themselves with different aspects of ice cream and related products, want to enter the field of manufacturing them. It consists of chapters on (1) Calculation of Ice Cream Mixes; (2) Restandardising and Calculating Mixes; (3) Calculating Cost and Percentage of Overrun; (4) Mix-Processing; (5) The Freezing Process; (6) Packaging Hardening and Shipping; and (7) Refrigeration. This will be helpful in the selection of the plant, its operation and production of different types of products.

The fourth Section consists of chapters on (1) Defects, Scoring and Grading; (2) Sanitation and Quality Control; (3) Some Laboratory Tests Often Used in Ice Cream Plants; and (4) Formula and Industry Standards. This will be helpful in day to day quality control and product standardisation.

The publication has been made more complete by the inclusion of the fifth section consisting of chapters on Sales Outlets and Soda Fountain Recipes, which are important from the point of view of merchandising.

The appendix at the end includes History of Ice Cream Industry, Tables for aid in various calculations, Plant Inspection Form and Federal Standards for Frozen Desserts.

There is a need for rearranging the chapters in proper sequence. The Chapter on Calculation of Ice Cream Mixes is given just after describing various ingredients and additives without going into various types of products and their recipes. Likewise the Chapter on Refrigeration is given in between Chapters on Sanitation and Quality Control and Laboratory Tests. This should have been given before the Chapter on Freezing Process.

The author has not covered the subject of production and supply of ice cream mixes either as such or in powder form to the small manufacturers or for domestic use. Details of method of delactosing of milk solids (p. 330) for improving the texture of ice cream also should have been given.

The volume contains large number of excellent illustrations, particularly of equipment. The book will prove to be most useful to all interested in ice cream and related products.

V. K. MATHUR

Methods of Aflatoxin Analysis: B. D. JONES, Tropical Products Institutes, London, G. 70. 1972, pp. 58.

This report replaces the original TPI Report G. 13. In reviewing some of the methods available for the analysis of food and feeding stuffs for aflatoxin, the author has kept in view methods which do not require expensive apparatus, and which are suitable for use in laboratories in developing countries. However methods involving such instruments as spectrophotometer, and densitometer have also been referred to briefly.

The report is divided into eight sections, five appendices along with summary, introduction and literature references.

The introductory part of the report deals mainly with different types of aflatoxins which may commonly occur in commodities infected with *Aspergillus flavus* together with the general procedure involved in the chemical testing of aflatoxins. Sampling methods and preparation of sample for analysis, which is a crucial step in the procedure, has been dealt with in great detail in the first section of the report. The subsequent sections are devoted to different steps involved in the aflatoxin analysis such as (defatting,) methods of toxin extraction, clean up procedures and estimation of aflatoxin content in the extracts by thin layer chromatography. Confirmatory methods for aflatoxin B_1 have also been given in the report. Recommended sequences for the analysis of foods and feeding stuffs, and some practical points relevant to the analysis of products given in section G indeed serve as a useful guide in selecting sequences of analysis for different commodities. The last section of the report deals mainly with calculation of the aflatoxin content of the samples, giving model problems. Appendices given consist of apparatus and chemicals required for analysis, suppliers list of aflatoxin standards, bulk sampling methods, and determination of purity of aflatoxin samples.

All the sections dealing with different steps of the selected methods have been dealt with exhaustively and the report serves as a useful references to all those concerned with aflatoxin analysis.

C. T. DWARAKANATH

Pesticide Residues in Food: Report of the 1971 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. *WHO Technical Report Series*, No. 502; 1972, pp. 46 Price: 40 p, $1.00, Sw. fr. 4. (Available through WHO Regional Office, Indraprastha Estate, Ring Road, New Delhi.)

The report stresses that, as far as possible, dairy products should be free from the residues of pesticides used to control various parasites of dairy cows. In areas where parasites such as lice and ticks seriously affect the health of lactating cattle and hence the production of food, it is hardly avoidable to use such pesticides, but it is improbable that the levels of residues reaching the consumer attain the maximum values observed in supervised trials on individual cows.

Increasingly selective methods of analysing fumigant residues are being used to follow changes in the amount of unchanged fumigants in certain foods after fumigation. The report points out that, although these residues dwindle progressively during storage, handling, and processing, small amounts of them may occasionally subsist in food. It is thus desirable to reduce such residues to a minimum by adopting good practices in handling food after fumigation. The report recommends the levels of five fumigants that should not be exceeded if these good practices are followed.

Since the herbicide 2, 4-D was last evaluated, in 1971, the results of studies in rats and dogs have become available. Although the data indicate that there may be a slight increase in the incidence of tumours in rats fed on 2, 4-D for up to two years, the carcinogenicity of this herbicide has not been substantiated. An acceptable daily intake for man, calculated on the basis of this feeding study in rats, is shown in the report.

Another study reviewed in this report is one in which calculations were made of theoretical intakes of 35 pesticides in four countries from three regions of the world for which average food consumption data had been compiled by FAO. The results of the study showed that, for 20 of these compounds, there was not even a theoretical possibility that the acceptable daily intake might be exceeded. Several others, including malathion, are borderline cases warranting further study. There is a significant theoretical possibility that the acceptable daily intakes of DDT, dieldrin, fenthion, omethoate, and piperonyl butoxide might be exceeded. Processing and cooking considerably reduce the potential intake from food of carbaryl, DDT, and particularly malathion.

IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Volume 1, Lyon, International Agency for Research on Cancer, 1972, pp. 184. Price: 40p, $1.00, Sw. fr. 4., Fr. fr. 5. (Available through WHO Regional Office, Indraprastha Estate, Ring Road, New Delhi.)

Both in industry and in the environment in general there are a growing number and quantity of chemicals that may carry a risk for human health and for cancer in particular. Governments have become increasingly aware of the importance of this problem and will have to take stronger measures, based on existing biological evidence, to control or ban the use of potentially carcinogenic compounds. The risk to man may come from chemicals used in industry, from substances added to food preparations, from pharmaceutical products or from uncontrolled pollution in the environment. Naturally occurring substances like aflatoxin must also be taken into account.

Many hundreds of compounds have been shown to be carcinogenic in experimental animals but this does not necessarily mean that they are carcinogenic in man. The International Agency for Research on Cancer has begun compiling a compendium of monographs on potentially carcinogenic substances in which the existing experimental data are evaluated and an estimate made of the magnitude of the carcinogenic risk for man. The first volume of these monographs has recently been published. It contains 19 monographs, which were finalized by a group of experts from research centres in a number of countries.

The monographs summarize only those data considered to be strictly relevant to the evaluation and are not intended as a comprehensive review of the subject. Nor do they contain recommendations as to practical measures to be taken for prevention of carcinogenic risks; such decisions have to be taken by national and international authorities on the basis of a risk/benefit assessment.

Sweet Potatoes: Production, Processing and Marketing: by J. B. EDMOND AND G. R. AMMERMAN, AVI Publishing Company Inc. Westport Connecticut, 1971, pp. 334, Price: Not mentioned.

Sweet potato is a widely grown crop in tropical and some parts of temperate regions of Africa, India, China, Japan, the Pacific Islands, tropical America and Southern United States. In USA the cultivation of the crop is concentrated mainly in the states of Louisiana, North Carolina, Virginia, New Jersey, Texas and Georgia. The data provided upto 1960 show that there was a steady fall in the production and *per capita* consumption of this root tuber in USA since the great depression of 1930. However the position of USA cannot be viewed in terms of world production as the relevant data are not provided.

The book covers wide range of topics like the description of the plant, including its physiology and morphology in two chapters, research and development in agricultural production aspects in five chapters, technology of processing in four chapters and marketing of produce in fresh form in one chapter.

The developments in agricultural production technology discussed cover the research done in the sweet potato producing areas of USA and it is hoped that the presentation of the research findings in a consolidated form will help to reduce the cost of cultivation and increase the yield of this tuber at a time when it is gradually loosing its importance as a food crop. The set back in consumption has been attributed mainly to the preference for animal foods which contain high essential proteins and moderately high stored energy. The publication brings under one cover the research achievements accomplished after the third decade of the present century; the earlier three publications on this title cover the period from 1890 to about 1930.

The research work undertaken on the morphology and anotomy of the plant will help to understand the different aspects of the plant better although it did not

help much to classify the varieties and even today classification suggested by Boswell (1950) exists as the widely accepted one. A thorough understanding of the physiology, biochemistry and ecology of the plant and the fleshy root was made possible by the latest research accomplishments and this has resulted in a marked increase in yield per unit area with low cost of production. Sweet potato should not be regarded only as a source of starch, it is valued equally for its supply of carotene and vitamin C, which are so essential in human nutrition.

Basic investigations carried out on the mineral requirement of the plant will help the growers to apply the essential elements in proper time at suitable doses as per the requirement of the plant; but prediction of yield based on the mineral content of leaf blade has not reached that stage where it can be applied directly to commercial varieties on large scale. The discovery that the fleshy roots often produce lower quality plants by mutation thus demanding a careful and rigid seed stock selection to maintain the quality, and the induction of functional flowers and production of viable seeds are some of the achievements made in crop improvement. The work done on the selection of seed stock, local studies conducted on handling and artificial curing of seed stocks and the production of plants with artificial heating are some of the topics useful for the extension workers.

Experiments conducted with the choice of soils and the commercial fertilizers are still insufficient to give any definite guideline to the cultivators and the areas where further research will prove useful have been pointed out by the author. Control of weeds and checkings of growth cracks in the fleshy root to maintain the quality are the problems faced in the field production operations. Fungi, viruses, nematodes and insects are the major pests and these have been treated separately by pooling the latest results of research specially those of destructive fungi which are so varied and cause not only extensive damage to the plant and the tuber but also lower the market quality of the produce. The time of harvest and the improvement made in harvesting equipment including the vine cutters and the development of walking plows are some of the topics of interest to the producer. Continued research on the curing, post-curing operation and storage requirements along with the basic study on the principles underlying these has helped in reducing losses in weight and decay and aided in prolonging the storage life. Efforts are on the way to mechanise the entire curing and storing operations. Major part of the tuber is sold as fresh product and preparation of the produce for the market, as per the choice of the consumer, grading, transporting and distribution are some of the associated problems and these have been covered under the marketing of fresh product.

The trend in consumption is gradually shifting from the fresh product to the processed convenience foods which include canned, dehydrated and frozen sweet potatoes. After reviewing the developments made in different processing steps involved in the canning, the author feels that further research on methods of maintaining firmness and other quality attributes in the canned product besides exploring the avenues for developing new products offering convenience and interest to the consumer will be useful. Sweet potato drying technology is an ancient process dating back to 1899; much progress has been made in recent years especially in unit operations in the manufacture of precooked dehydrated potato flakes. After reviewing the research achievements on freezing of sweet potatoes the author is of the view that further research is needed on the quality of sweet potatoes and their products as related to the speed of freezing, packaging materials, and storage temperatures with reference to time of storage. The last chapter deals with the industrial uses of sweet potatoes in the preparation of starch, syrup, alcohol etc., as also its use as feed for farm animals.

The monograph brings together under one cover the results of present research and those of immediate past carried out in USA and also indicates wherever further research is needed to fill the gap in our knowledge of sweet potato production and consumption. In this way the book will be useful to those engaged in the cultivation of this tuber, to country agents and farm advisers of all producing districts in USA, to all extension workers and to those engaged in sweet potato research. The information provided may also prove useful to those engaged in research on sweet potatoes outside USA as they can know the trend of research going on in one of the sweet potato producing countries of the world.

The printing and get up of the publication are good, and it will prove useful to the agricultural scientists, food technologists and institutions engaged in similar type of work.

K. A. RANGANATH

New Publication

The First Asian Congress of Nutrition was held in Hyderabad last year under the auspices of the International Union of Nutritional Sciences. The Congress was jointly sponsored by the Nutrition Society of India and the Indian National Science Academy.

The Proceedings of the Congress are now published in the form of a book. The publication runs to over 900 pages and it contains the proceedings of ten symposia, seventeen special reports, three special lectures and abstracts of a large number of research communications presented at the Congress. The publication will be found immensely useful by all those interested in the science of nutrition. Price U.S. $15; Indian Rs 100.

New Journal

A new Journal, *History of Agriculture*, provides the channel of communication for a world wide net work of people interested in establishing an agricultural historiography of a high standard of scholarship. It aims to integrate the research of historians of agriculture on an international plane and thereby to contribute towards a better understanding of the development of world wide agriculture.

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History of Agriculture also publishes information concerning the most important events of international agrico-historical activity. A special 'News and Notes' section illuminates projects such as the establishment of new museums, agrico-historical institutes and societies and the proceedings of national or international congresses, etc. The Journal is published quarterly in the third week of February, May, August and November. The subscription rate in U.S. $25.00 a year in U.S.A. and Canada and £10.00 in Europe and elsewhere. The publishers are K. K. Roy (Private) Ltd., 55 Gariahat Road, P.O. Box 10210, Calcutta 19, India.

AMI News Bulletin

The Association of Microbiologists of India publishes from its Central Office a news bulletin titled 'AMI News Bulletin'. While serving as a house-journal containing news about its members and on organisational activities of its various constituent units and Central Office, information of general interest to microbiologists and scientists in related areas is also included.

The News Letter will be covering a wide variety of organisational and scientific activities carried out by AMI and other fraternal organisations in microbiology and allied disciplines. It is also planned to cover information on (*i*) some of the most recent major scientific advances; (*ii*) awards and distinctions conferred on members and other distinguished scientists; (*iii*) forthcoming visits to our country by distinguished scientists from abroad; (*iv*) particulars on forthcoming conferences, meetings, symposia, workshops, etc., both at the National and International level; and (*v*) recent publications of books, monographs, etc., on microbiology and related areas.

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IS:6406-1971 Brilliant Blue, FCF, Food Grade Rs 3.50

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(*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.

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

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